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Basic Medical Science Center, Semmelweis University
Tűzoltó utca 37-47, 1094 Budapest, Hungary

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SCIENTIFIC PROGRAMME

27 August, 2014

10:00-19:00 **REGISTRATION**

SZENT-GYÖRGYI ALBERT ROOM

13:00-16:30
S1-A **FEPS European Young Physiologists Symposium**
Chair: **Andrea Tamás,** Hungary

13:00
S1-A1 **Cell type-specific subcellular distribution of ion channels in the central nervous system**
Andrea Lőrincz, Invited speaker
Laboratory of Cellular Neurophysiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary

13:25 S1-A2 **Rapid generation of human neurons for modeling neuropsychiatric disorders**
Tamás Dankó
Department of Molecular and Cellular Physiology, Institute for Stem Cell Biology and Regenerative Medicine, Department of Pathology, Stanford University School of Medicine, Stanford, USA

13:40 S1-A3 **Descending effect on spinal nociception by amygdaloid glutamate varies with the submodality of noxious test stimulation**
Nora Bourbia, B. Sagalajev, A. Pertovaara
Institute of Biomedicine/Physiology, University of Helsinki, Finland

13:55 S1-A4 **The effect of NOS inhibitors on radical signaling and antioxidant response in Wistar rats**
Miroslava Majzúnová, Z. Pakanová, P. Bališ, M. Drobná, I. Dovinová
Institute of normal and pathologigal physiology Slovak Academy of Sciences, Bratislava, Slovakia

14:10 S1-A5 **Quantitative pilomotor axon-reflex test to assess autonomic dysfunction in Parkinson's disease**
Timo Siepmann, E. Frenz, W. Kirch, B. Min-Woo Illigens
Department of Neurology, Carl Gustav Carus University Hospital, Dresden University of Technology, Dresden, Germany

14:25 **BREAK**

14:50
S1-A6 **Reprograming of liver metabolism by alternative p38-mediated modulation of neutrophil migration**
Guadalupe Sabio, Invited speaker
Stress kinases in Diabetes, Cancer and Cardiovascular Disease Department Vascular Biology and Inflammation Centro Nacional de Investigaciones Cardiovasculares Carlos III, Madrid, Spain

15:15 S1-A7 **A hypermuscular mouse model to study SOCE and muscle fatigue**
Mónika Sztretye
Department of Physiology, University of Debrecen, Debrecen, Hungary

15:30
S1-A8 **MiRNA-24 antagonism prevents renal ischemia reperfusion injury**
J .M. Lorenzen, **Tamás Kaucsár** , C. Schauerte, R. Schmitt, S. Rong, A. Hübner, K. Scherf, J. Fiedler, F. Martino, K. Regalla, Malte Kölling, I. Sörensen, H. Hinz, J. Heineke, E. v. Rooij, H. Haller, T. Thum
Institute of Pathophysiology, Semmelweis University, Budapest, Hungary

15:45 S1-A9 **Elevated adipose and liver IRAP/oxytocinase activity may account for increased oxytocin degradation in obesity. Effect of IRAP blockade on obese phenotype**
Lucia Gajdosechova, K. Krskova, M. Suski, S.Y. Chai, R. Olszanecki, S. Zorad
Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia

16:00 S1-A10 **The A+-helix of PCI, which is removed by testisin cleavage, is a cell penetrating peptide and responsible for internalization of PCI by Jurkat cells**
Hanjiang Yang
Department of Vascular Biology and Thrombosis Research, Medical University of Vienna, Austria

16:15 S1-A11 **In vitro studies on the mechanisms involved in chemoprevention using Calluna vulgaris on vascular endothelial cells exposed to UVB**
Elena Diana Olteanu, A. Filip, S. Clichici, B. Ioana, P. Bolfa, C. Mihai, A. Muresan
Department of Physiology, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

16:30 **BREAK**

17:00 **OPENING CEREMONY**

17:30 **PLENARY LECTURE**
FEPS Keynote Lecture
Chair: **David Eisner,** UK
Vascular connexins: cell communication and beyond
Ulrich Pohl, Germany

AULA

18:30 **WELCOME RECEPTION**

HEVESY GYÖRGY ROOM

14:00-16:20
S1-B **History of European Physiological Sciences, Physiology - Origin of Prizes**
Chairs: **Osmo Hänninen,** Finland and **Emil Monos,** Hungary

14:00 S1-B1 **Robert Tigerstedt, Alfred Nobel and The Prizes**
Osmo Hänninen, F. Fyhrquist
Department of Physiology, University of Eastern Finland, Helsinki, Finland

14:20 S1-B2 **From Ivan Pavlov to space Physiology**
Alexander Meigal
Institute of Advanced Biomedical Technologies, Petrozavodsk State University, Petrozavodsk, Russia

14:40 S1-B3 **Female healers in early modern England: medical care for man and beast?**
Louise Hill Curth
University of Winchester,Great Britain

15:00 **BREAK**

15:20 S1-B4 **History of Dutch-Hungarian research platforms emerged from physiological sciences**
Csaba Nyakas, P. GM Luiten
Brain Physiology Research Unit, Semmelweis University, Budapest, Hungary

15:40 S1-B5 **Hungarian heritage in physiological sciences**
Emil Monos, L. Szollár
Institute of Human Physiology Semmelweis University, Budapest, Hungary

16:00 **PANEL DISCUSSION**
OWARDS VIRTUAL EUROPEAN MUSEUM OF PHYSIOLOGY AND MEDICINE: STEPS OF PROGRESS

16:20 **BREAK**

BÉKÉSY GYÖRGY ROOM

14:00-16:30

S1-C **FEPS Teaching Physiology Symposium**
Teaching Physiology in the Medical Curriculum: Traditional vs. Problem- based
Chairs: **Tamás Ivanics,** Hungary and **Ger J. van der Vusse,** The Netherlands

14:00 S1-C1 **Traditional medical curriculum in Semmelweis University**
Levente Kiss
Semmelweis University, Institute of Human Physiology and Clinical Experimental Research, Budapest, Hungary

14:20 S1-C2 **Problem-based learning in the medical curriculum at Maastricht University**
Mirjam G.A. oude Egbrink
Department of Physiology and Institute for Education at FHML, Maastricht University, Maastricht, The Netherlands

14:40 S1-C3 **Physiology in the medical curriculum, is it really necessary?**
Roger J.M.W. Rennenberg
Department of Internal Medicine Maastricht University Medical Centre and coordinator master in medicine Faculty of Health, Medicine and Life Sciences, Maastricht University, The Netherlands

15:00 **BREAK**

15:20 S1-C4 **Physiology teaching in the traditional curriculum of Semmelweis University**
Zsuzsanna Miklós
Institute of Human Physiology and Clinical Experimental Research, Semmelweis University, Budapest, Hungary

15:45 S1-C5 **Physiology teaching is a fully integrated Medical PBL curriculum in Maastricht**
Ger J. van der Vusse
Department of Physiology, Maastricht University, Maastricht, The Netherlands

16:10 **DISCUSSION**

16:30 **BREAK**

28 August, 2014

SZENT-GYÖRGYİ ALBERT ROOM

9:00 **PLENARY LECTURE**
Chair: **László Csernoch,** Hungary
Molecular Phenotyping in Personalised and Public Healthcare
Jeremy K Nicholson, UK

9:45 **BREAK**

10:15-12:15
S2-A **Acta Review Symposium, Electrical Propagation in Smooth Muscle organs**
Chairs: **Wim J Lammers**, United Arab Emirates and **Ger J. van der Vusse,** The Netherlands

10:15 S2-A1 **Slow wave propagation in the stomach: Advances in experimental and modelling techniques**
Leo Cheng
University of Auckland, New Zealand

10:40 S2-A2 **Normal and abnormal electrical propagation in the small intestine**
Wim J. Lammers
College of Medicine & Health Sciences, United Arab Emirates University, UAE

11:05 S2-A3 **Electrical propagation in the uterine muscle during pregnancy and labor**
Chiara Rabotti, M. Mischi
Eindhoven University of Technology, Eindhoven, The Netherlands

11:25 S2-A4 **Electrical propagation in the renal pelvis, ureter and bladder**
Fayez T. Hammad
United Arab Emirates University, UAE

11:45 S2-A5 **Electrical propagation in the various sites of the pulmonary veins of mammalian**
Vladislav Kuzmin, Y.V. Egorov
Institute of experimental cardiology, Russian Cardiological Research and Production Complex, Moscow, Russia

12:00 S2-A6 **Effects of methane inhalation on the nitrergic myenteric neurons and intestinal myoelectrical activity during mesenteric ischemia-reperfusion in rats**
Marietta Zita Poles, N. Bódi, P. Talapka, G. Varga, A. Pál, M. Bagyánszki, R. Gáspár, J. Kaszaki, É. Fekete, M. Boros
Institute of Surgical Research, School of Medicine; University of Szeged, Hungary

12:15 **BREAK**

AULA & GALERY

13:00-14:30 **POSTER SESSION**

P1; P 16 uneven numbers: P2; - P3; - P4; - P5; - P6; - P8; - P9; - P10; - P12
Detailed programme of the session see below.

SZENT-GYÖRGYİ ALBERT ROOM

14:30-16:30

S3-A **Non-canonical Functions of the Endogenous Opioid Peptides**
Chair: **Oleg Krishtal,** Ukraine and **Erika Pintér**, Hungary

14:30 S3-A1 **Pathophysiological consequences of endogenous opioids in injury and disease**
Kurt F. Hauser
Virginia Commonwealth University, Medical College of Virginia Campus, Richmond, Virginia, USA

14:55 S3-A2 **Glutamate mimicking mutant Dynorphin A causes spinocerebellar ataxia type 23**
Cleo J.L.M. Smeets
University Medical Center Groningen, The Netherlands

15:20 S3-A3 **Opioid neuropeptides make pores in plasma membrane: possible mechanism of signal transduction**
Oleg Krishtal, O. Maximyuk, V. Khmyz, C. Lindskog, V. Vukojević, T. Ivanova, A. Rajnisz, J. Solecka, A. Lipkowski, K. Hauser, G. Bakalkin
Bogomoletz Institute of Physiology Kiev, Ukraine

15:40 S3-A4 **The left-right neurohormonal regulation in the brain: Lateralized endogenous opioid system**
Georgy Bakalkin
Uppsala University, Uppsala, Sweden

16:00 S3-A5 **Investigation of possible interactions of pain modification actions of endogenous enkephalinergic and noradrenergic systems in Zymosan-induced chronic inflammatory pain model**
Ahmet Ayar, A. Kurt, S. Canpolat
Department of Physiology, Karadeniz Technical University, Faculty of Medicine, Trabzon, Turkey

16:15 S3-A6 **Hemokinin-1 is a potent inflammatory and pro-nociceptive peptide in acute and chronic mouse arthritis models**
Éva Borbély, K. Bölcskei, K. Békefi, A. Berger, C. J. Paige, J.J. McDougall, M. Attila, T. Németh, M. Kovács, E. Pintér, J. Szolcsányi, Zs. Helyes
Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Pécs, Pécs, Hungary

16:30 **BREAK**

17:00 **PLENARY LECTURE**
Chair: **Attila Mócsai,** Hungary
Yeast genetics in mammalian stem cells
Josef Penninger, Austria

17:45 **BREAK**

HEVESY GYÖRGY ROOM

10:15-12:15

S2-B **Nutrition and Cardiovascular Health: New Perspectives in Prevention and Therapy**

Chairs: **Grant N. Pierce,** Canada and **Dragan Djuric,** Serbia

10:15 S2-B1 **Homocysteine and homocysteine-thiolactone induce cardiac and vascular damage: interplay with oxygen consumption, oxidative stress, and gasotransmitters**

Dragan Djuric, V. Zivkovic, M. Radenkovic, M. Stanic, D. Krstic, O.Stanojlovic, J. Jakovljevic, V. Jakovljevic

University of Belgrade, Serbia

10:40 S2-B2 **Epigenetic modulation of cardioprotection with plant compounds**

Vincenzo Lionetti

Institute of Life Sciences, Scuola Superiore Sant' Anna, Pisa, Italy

11:05 S2-B3 **Dietary factors for favorable modulation of platelet function**

Judit Barta

UDMHSC Institute of Cardiology, Debrecen, Hungary

11:25 S2-B4 **The use of Dietary Flaxseed to promote Cardiovascular Health**

Grant N. Pierce, A.L. Edel, R. LaVallee, S. Caligiuri, H. Aukema, A. Ravandi, R. Guzman, D.R. Leyva,M. Aliani

Canadian Centre for Agri-food Research in Health and Medicine (CCARM), St. Boniface Hospital, Canada

11:45 S2-B5 **Interaction of clopidogrel and statins in secondary prevention after ischemic stroke**

Timo Siepmann, D. Heinke, J. Kepplinger, K. Barlinn, S. Gehrisch,X. Grählert, U. Schwanebeck, H. Reichmann, V. Puetz, U. Bodechtel, G. Gahn , J. Kepplinger, K. Barlinn, S. Gehrisch,X. Grählert, U. Schwanebeck, H. Reichmann, V. Puetz, U. Bodechtel, G. Gahn

Department of Neurology, Carl Gustav Carus University Hospital, Dresden University of Technology, Dresden, Germany

12:00 S2-B6 **Effects of L-arginine, vitamin C and folic acid on coronary hemodynamics, oxidative stress markers and NO system in isolated rat heart**

Vladimir Jakovljevic, A. Vusanovic, V. Zivkovic, I. Srejovic, N. Barudzic, D. Djuric

Department of Physiology, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia

12:15 **BREAK**

14:30-16:30

S3-B **Signalling at Membrane Contact Sites**

Chair: **Nicolas Demaurex,** Switzerland and **Péter Várnai,** Hungary

14:30 S3-B1 **Coupling acidic organelles and the endoplasmic reticulum through Ca2+. A role for membrane contact sites?**

Sandip Patel

UCL Research Department of Cell and Developmental Biology, London, UK

14:55 S3-B2 **Calcium and ROS signaling at the ER-mitochondrial interface**
Gyorgy Hajnoczky
MitoCare Center, Thomas Jefferson University, Philadelphia, USA

15:20 S3-B3 **Broadband connections within the cell: How the mitochondria talk to the endomembrane?**
Benoît Kornmann
ETH Zürich- Institute of Biochemstry, Zürich, Switzerland

15:45 S3-B4 **Investigating membrane contact sites between the endoplasmic reticulum and phagosomes**
Paula Nunes
University of Geneva, Switzerland

16:10 S3-B5 **Differential impact of 5-phosphatase-mediated and phospholipase C-induced plasma membrane PtdIns(4,5)P2 depletion on G protein-coupled receptor endocytosis**
Dániel J. Tóth, J.T. Tóth, B. Tallósy, L. Hunyady, P. Várnai
Department of Physiology, Semmelweis University, Budapest, Hungary

16:20 S3-B6 **Glycine modulates membrane potential, cell volume, and phagocytosis in murine microglia**
Marlena Beyreis, B. Komm, M. Kittl, M. Jakab, M. Ritter, H. Kerschbaum
Institute of Physiology and Pathophysiology, Paracelsus Medical University, Salzburg, Austria

16:30 **BREAK**

BÉKÉSY GYÖRGY ROOM

10:15-12:15

S2-C **New Therapeutical Targets in Acute Pancreatitis**
Chairs: **Ole H. Petersen,** UK and **Péter Hegyi,** Hungary

10:15 Opening remarks
Péter Hegyi, Hungary

10:20 S2-C1 **Characterization of pancreatic acinar Ca2+ influx pathway leads topotential new therapy for pancreatitis**
Ole H. Petersen
Cardiff University, Cardiff, UK

10:40 S2-C2 **How to target the inflammatory response in pancreatitis**
Julia Mayerle
Department of Medicine A, University Medicine, Ernst-Moritz-Arndt-University Greifswald, Germany

11:00 S2-C3 **Insulin protects pancreatic acinar cells from pancreatitis-inducing agents**
Jason Bruce,
A. Samad, P.Mankad, J. Whitehouse, W. Patel, M. Alves-Simoes, A. K.Siriwardena Faculty of Life Sciences, The University of Manchester, UK

11:20 S2-C4 **Inhibition of CFTR function is critical in the development of pancreatitis**
József Maléth
University of Szeged, Hungary

11:40 S2-C5 **ER-PM junctions in pancreatic acinar and pancreatic cancer cells: from structure to function**
Alexei Tepikin
The University of Liverpool, UK

12:00 S2-C6 **The effect of taurocholic acid on ryanodine receptor and SR calcium pump activity**
János Almássy, N. Geyer, Gy. Diszházi, I. Jóna
University of Debrecen, Faculty of Medicine, Department of Physiology, Hungary

12:15 **BREAK**

14:30-16:30
S3-C **Epithelial Function and Repair**
Chairs: **Gábor Varga,** Hungary and **Zoltán Rakonczay**, Hungary

14:30 S3-C1 **Epithelial fluid and HCO3- secretion**
Shmuel Muallem
National Institutes of Health/ NIDCR/NIH, Beersheba, Israel

14:55 S3-C2 **HCO3- secretory function of pulmonary epithelial cells**
Mike Gray
Epithelial Research Group, Institute for Cell & Molecular Biosciences, Newcastle University Medical, UK

15:20 S3-C3 **Physiological and pathophysiological roles of pancreatic ducts**
Zoltán Rakonczay Jr.
University of Szeged, First Department of Medicine, Szeged, Hungary

15:40 S3-C4 **Epithelial transport processes of ameloblasts leading to dental enamel formation**
Gábor Varga
Department of Oral Biology, Semmelweis University, Budapest, Hungary

16:00 S3-C5 **Aquaporin-3 (AQP3) in cell proliferation, a potential target for therapeutic drugs**
Miriam Echevarría, A. Galán-Cobo, A. Serna, R.R. Lorca, I.S. Gomar, J.J. Toledo-Aral
Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío/CSIC/Universidad, Seville, Spain

16:15 S3-C6 **Basal ciliary activity depends on ATP release in respiratory epithelium of mouse trachea**
Manuel J. Villalón, K. Droguett, N. Barrera
Pontificia Universidad Católica de Chile, Santiago, Chile

16:30 **BREAK**

HÁRI PÁL ROOM

10:15-12:15
S2-D **Lipid Homeostasis: What We Learnt from Sex Hormone Estrogens**
Chairs: **Maria Marino,** Italy and **Raquel Marin**, Spain

10:15 S2-D1 **Estrogen more than a sex hormone**
Filippo Acconcia, V. Pallottini, M. Marino
University Roma TRE, Italy

10:40 S2-D2 **Estrogen suppresses lipid synthesis: Role of membrane estrogen receptors**
Ellis R. Levin
University of California, Irvine, USA

11:05 S2-D3 **Complex interplay between estrogens and polyunsaturated fatty acids in hippocampal lipid homeostasis: Relevance for Alzheimer's Disease**
Mario Díaz
University of La Laguna, Tenerife, Spain

11:30 S2-D4 **Bisphenol-A as a potential environmental factor that alters the development**
Alfonso Abizaid
Carleton University, Department of Neuroscience, Ottawa, Ontario, Canada

11:55 S2-D5 **Cholesterol homeostasis in the brain: A sex and age viewpoint**
Valentina Pallottini, M. Segatto, F. Acconcia, M. Marino
University Roma Tre, Italy

12:05 S2-D6 **The determination of sodium salicylate effect to body weights and fatty acid values in the frontal lobe of brain on the immobilized rats**
Alpaslan Dayangaç, S. Citil, M. Bahsi, T. Aktas, O. Yilmaz
Ahi Evran University, Faculty of Art & Science, Department of Biology, Kırşehir, Turkey

12:15 **BREAK**

14:30-16:30
S3-D **Novel Mechanisms Contributing to Aging**
Chairs: **Ákos Koller,** Hungary and **Alexander Bürkle**, Germany

14:30 S3-D1 **Functional, morphological and molecular changes in arteries as a function of age**
Ákos Koller
Department of Pathophysiology and Gerontology, Medical School, and Szentágothai Res. Center, University of Pécs and Department of Physiology, Hungary; New York Medical College, NY, USA

14:50 S3-D2 **Mechanisms of extension of cognitive health span with IGF-1**
William E. Sonntag
University of Oklahoma Health Sciences Center, Oklahoma City, USA

15:15 S3-D3 **Novel model of age-related cognitive impairment and molecular mechanism of synaptic failure**
Ferenc Deák, S. Logan, N. Szarka, A. Orock, C. Giles, M.C. Mitschelen, J. Wren, Á. Koller, W.E. Sonntag
Dept. Geriatric Medicine, Univ. Oklahoma, USA

15:35 S3-D4 **Determination of biological age in humans: Results from the EU FP7 MARK-AGE project**
Alexander Bürkle
University of Konstanz, Germany

16:00 S3-D5 **Age-related changes in response to ischemia and adaptation in male rat hearts: Potential molecular mechanisms behind**
Tanya Ravingerova, L. Griecsova, V. Ledvenyiova, Vinoth KM Khandelwal, I. Gablovsky, I. Bernatova, Z. Tatarkova
Institute for Heart Research, Slovak Academy of Sciences, Bratislava, Slovakia

16:15 S3-D6 **Aging dependent GDNF induction by hypoxia in Carotid Body: Implications for antiparkinsonian cell therapy**
Juan J. Toledo-Aral, A.B. Muñoz-Manchado, R. Ramirez-Lorca, S. Romo-Madero, N. Suárez-Luna, A. Bermejo-Navas, M. Olivares, M. Oliver, M. Echevarría, J. López-Barneo, J. Villadiego
Dep. Physiology. Instituto de Biomedicina de Sevilla. HUVR/CSIC/US, Sevilla, Spain

16:30 **BREAK**

BEZNÁK ALADÁR ROOM

10:15-12:15
S2-E **Regulation of Mitochondrial Function in Heart Failure: From Health to Dysfunction**
Chairs: **Ger JM Stienen**, The Netherlands and **Rob CI Wüst,** The Netherlands

10:15 S2-E1 **Alternations in mitochondrial structure and function in rat myocardium in chronic heart failure**
Rob Cl Wüst, G. JM Stienen
Department of Physiology, VU Medical Center Amsterdam, Amsterdam, The Netherlands

10:35 S2-E2 **Diffusion obstacles shape the environment surrounding mitochondria in heart**
Marko Vendelin, N. Jepihhina, P. Simson, M. Laasmaa, P. Peterson
Institute of Cybernetics at Tallinn University of Technology, Tallinn, Estonia

10:55 S2-E3 **Myocardial mitochondrial respiration in human heart failure**
Flemming Dela, N. Stride, L.B. Christensen, T. Yokota
Center for Healthy Aging, University of Copenhagen, Denmark

11:20 S2-E4 **Ca2+ microdomains and interaction between mitochondria and ER/SR: Open questions**
Marta Giacomello
Venetian Institute of Molecular Medicine, Padova, Italy

11:45 S2-E5 **The effects of Simvastatin on skeletal muscle treated with LPS in rats**
A S. Tamer, **Elif Ozkok** , H. Yorulmaz, G. Ates, P. Oflazer
Istanbul University, Deparment of Neuroscience, The Institute for Experimental Medicine, Istanbul, Turkey

12:00 S2-E6 **Exercise performed before and during sub-chronic Doxorubicin treatment mitigates cardiac mitochondrial alteration**
A. Ascensao, **Inês Marques Aleixo** , E.S. Alves, D.R. Roca, A.I. Padrão, J.R. Torrella, G. Viscor, R. Ferreira, P.J. Oliveira, J. Magalhaes
CIAFEL-Research Centre in Physical Activity, Health and Leisure, Faculty of Sport, University of Portugal, Porto, Portugal

12:15 **BREAK**

14:30-16:30

S3-E **Current Trends in Respiratory Physiology: From Lung Function Towards System Biology Approaches**
Chairs: **Ildikó Horváth,** Hungary and **Peter J. Sterk,** The Netherlands

14:30 S3-E1 **The links between lung function and cardiovascular diseases**
Ildikó Horváth
Semmelweis University, Department of Pulmonology, Budapest, Hungary

14:55 S3-E2 **Oxidative stress pathways as new therapeutic opportunities: from infection to lung ageing**
Kazuhiro Ito
NHLI, Imperial College, London, UK

15:20 S3-E3 **Mast cell biology in the human lung**
Hans Jürgen Hoffmann
Aarhus University , Denmark

15:45 S3- E4 **Systems medicine and big data to phenotype and treat chronic airway diseases**
Peter J. Sterk
Dept. Respiratory Medicine Academic Medical Centre, University of Amsterdam, The Netherlands

16:10 S3- E5 **Bronchoconstriction and alveolar derecruitment following extracorporeal circulation: good by(e)pass?**
Ferenc Peták, Á.L. Balogh, K. Névery, J. Tolnai, B. Babik
University of Szeged, Department of Medical Physics and Informatics, Szeged, Hungary

16:20 S3- E6 **Metabolic risk factors in insulin resistant vs. insulin sensitive asthma patients**
Krisztián Pák, Z. Képes, T. Erdei, M. Bombicz, D. Priksz, B. Varga, B. Juhasz, A. Fodor, M. Szilasi, J. Zsuga, R. Gesztelyi
Department of Pharmacology, Faculty of Pharmacy, University of Debrecen, Hungary

16:30 **BREAK**

POSTER SESSION

AULA & GALERY

13:00-14:30

P1 **Teaching & History of Physiology**
Chair: **Tamás Ivanics,** Hungary

P1.1 **Replacement of animal use in medical physiology**
Beti Dejanova , D.Dewhurst, S. Petrovska, V. Antevska,S. Mancevska, J. Pluncevic Dewhurst, Petrovska, Antevska, Mancevska, Pluncevic
Institute of Physiology, Medical Faculty, University of Skopje, Macedonia

P1.2 **Ivan Petrovitsh Pavlov - the Nobel laureate for Physiology or Medicine**
Elena Chugunova
Faculty of Cell Biology. Division of Cellular and Molecular Neurobiology. University of Salzburg, Salzburg, Austria

P1.3 **Physiology in context of moral and political philosophy: Support of UNO-agenda 21**
Eva Neu, M.Ch. Michailov, L.-P.-Yorck Zebuhr, F. Braun, H. Walsch, A.R. Oswald, S. Molnar, M. Holler, G. Weber
Institute Umweltmedizin c/o ICSD/IAS e.V. Postfach 340316, 80100 München, Germany

P1.4 **On integrative Physiology in education and research (Part II): Summary of systematic investigations**
Eva Neu, M.Ch. Michailov, D. Martin, V. Foltin, U. Welscher, E. Gornik, W. Seidenbusch, H.W. Bauer, A. Hofstetter, G. Staehler
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P1.5 **On integrative Physiology (Part II): Regular congress participation and reports**
Michael Ch. Michailov, U. Welscher, E. Neu, J. Foltinova, G. Werner, G. Weber, M. Schratz
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P2 **Molecular and Cell Physiology**
Chair: **Tibor Zelles,** Hungary

P2.1 **Regulation of a human stem cell specific microRNA cluster C19MC**
Ábel Fóthi, A. Schamberger, Zs. Erdei, Á. Apáti, T.I. Orbán
Institute of Enzymology, RCNS, HAS, Budapest, Hungary

P2.3 **Endometrial oestrogen and progesterone receptors localization in the fat sand rat, Psammomys obesus, a diurnal gerbil**
Amina Boubekri, T.G. Spychalowicz, F. Khammar, E. Jean Marie
USTHB-FSB, Algiers, Algeria

P2.5 **Arrestin binding of the beta2-adrenergic receptor is regulated via heterodimerization with the angiotensin type 1A receptor**
András Tóth, P. Gyombolai, B. Szalai, P. Várnai, L. Hunyady
Physiology Department, Semmelweis University, Budapest, Hungary

P2.7 **The effects of Endothelin-1 on the level of redox proteins in H9c2 cells**
Anikó Barta, A. Czegledi, A. Czompa, A. Tosaki, I. Lekli
University of Debrecen, Faculty of Pharmacy, Department of Pharmacology, Debrecen, Hungary

P2.9 **Physiological effects of ophiobolins on inward rectifier ion channels comparing KAT1 channel in plants to Kir2.x channels in animals**
Balázs Kovács, V. Szuts, O. Bencsik, A. Szekeres, D. Borcsok, M. Horvath, F. Otvos, A. Kovacs, Cs. Vagvolgyi, K. Halasy, I. Tari, A. Ördög
Department of Plant Biology, Faculty of Science and Informatics, University of Szeged, Hungary

P2.11 **Improved methodical approach for quantitative BRET analysis of G protein coupled receptor dimerization**
Bence Szalai, P. Hoffmann, S. Prokop, P. Várnai, L. Hunyady
Semmelweis University and MTA-SE Laboratory of Molecular Physiology, Budapest, Hungary

P2.13 **GPCR-induced paracrine transactivation of CB1 cannabinoid receptors in vascular smooth muscle cells modulates calcium signaling and ERK pathways**
Eszter Soltész-Katona, M. Szekeres, D. Laczkó, A. Tóth, G. Turu, L. Hunyady
Department of Physiology, Semmelweis University, Budapest, Hungary

P2.15 **Endocannabinoid-mediated modulation of GPCR signaling-induced vasoconstriction and hypertension**
Mária Szekeres, Gy. Nádasy, E. Soltész-Katona, Z. Benyó, Zs. E. Tóth, L. Hunyady
Semmelweis University, Department of Physiology, Budapest, Hungary
Chair: **Péter Enyedi,** Hungary

P2.17 **Following the inositol lipid changes upon stimulation of EGF receptor in human HEK 293 fibroblasts**
József T. Tóth, G. Gulyás, D. J. Tóth, L. Hunyady, P. Várnai
Semmelweis University, Budapest, Hungary

P2.19 **Characterization of the inherited I130N substitution in V2 vasopressin receptor revealed a gain-of- function mutation leading to NSIAD**
László Sándor Erdélyi, W.A. Mann, A. Balla, L. Hunyady
MTA-SE Laboratory of Molecular Physiology, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary

P2.21 **Effects of apocynin, NADPH oxidase inhibitor, on levels of ADMA, MPO, iNOS and TLR4 induced by myocardial ischemia reperfusion**
Mete Ozcan, A. Uysal, I.M. Ozguler, O. Burma, N. Ilhan, E. Sahna
Firat University, Faculty of Medicine, Department of Biophysics, Elazig, Turkey

P2.23 **Human serum albumin suppresses the angiotensin-converting enzyme activities in human**
Miklós Fagyas, K. Úri, G.Á. Fülöp, V. Csató, I.E. Szentkirályi, T. Maros, T. Szerafin, I. Édes, Z. Papp, A. Tóth
Division of Clinical Physiology, Institute of Cardiology, University of Debrecen, Debrecen, Hungary

P2.25 **The bile acid, taurocholic acid activates ryanodine receptor and inhibits SERCA activity**
Nikolett Geyer, Gy. Diszházi, I. Jóna, J. Almássy
University of Debrecen, Faculty of Medicine, Department of Physilogy, Debrecen, Hungary

P2.27 **Determination of antitumor properties of synthesized chalcone-phosphazenes containing dioxybiphenyl groups against PC-3 and LNCaP cell lines**
Suat Tekin, K. Koran, F. Ozen, S. Sandal, A.O. Gorgulu

Inonu University, Faculty of Medicine, Department of Physiology, Malatya, Turkey

P2.29 **Function of RasGRP3 in the formation and progression of human breast cancer**
Zsuzsanna Nagy
University of Debrecen Department of Physiology, Debrecen, Hungary

P3 **Skeletal, Smooth and Cardiac Muscle Physiology**
Chair: **Zoltán Benyó**, Hungary

P3.1 **The effect of SERCA 1b shRNA on the differentiation of C2C12 skeletal muscle cells**
Adrienn Tóth, J. Fodor, J. Vincze, T. Oláh, T. Juhász, E. Zádor, L. Csernoch
University of Debrecen, Faculty of Medicine, Department of Physiology, Debrecen, Hungary

P3.3 **Neurokinin A induced contraction of the urinary bladder smooth muscle**
Bernadett Faragó, B. Dér, É. Ruisanchez, P. Őrsy, S. Offermanns, Z. Benyó I nstitute of Human Physiology and Clinical Experimental Research, Semmelweis University, Budapest, Hungary

P3.5 **Different characteristics of diabetic cardiomyopathy in rat models**
Csaba Mátyás, S. Korkmaz, A. Olah, B.T. Nemeth, L. Hidi, E. Birtalan, M. Torok, L. Szabo, M. Ruppert, G. Merkely, D. Kellermayer, A. Meltzer, B. Merkely, G. Szabo, T. Radovits
Heart and Vascular Center, Semmelweis University, Budapest, Hungary

P3.7 **Effects of methionine-enriched diet on the rat heart and aorta**
Dragan Djuric, O. Stanojlovic, D. Hrncic, N. Puskas, A. Rasic - Markovic, M. Colovic, D. Krstic, J.M. Bjekic, Z. Grubac, N. Sutulovic, V. Susic
Belgrade University Faculty of Medicine, Belgrade, Serbia

P3.9 **Impact of ion currents on beat-to-beat variability of action potential duration in canine myocytes**
Kornél Kistamás, F. Ruzsnavszky, B. Hegyi, K. Váczi, B. Horváth, N. Szentandrássy, T. Bányász, P.P. Nánási, J. Magyar
University of Debrecen, Department of Physiology, Debrecen, Hungary

P3.11 **The short term beat-to-beat variability of action potential duration depends on the the length of action potential and intracellular calcium**
Krisztina Váczi, B. Hegyi, F. Ruzsnavszky, K. Kistamás, B. Horváth, N. Szentandrássy, T. Bányász, P.P. Nánási, J. Magyar
Department of Physiology, University of Debrecen, Debrecen, Hungary

P3.13 **Characteristic of ischemic preconditioning under conditions of simulated hyperglycemia hyperglycemia**
Marek Zálešák, P. Blažíček, I. Gablovský, V. Ledvényiová, M. Barteková, A. Ziegelhoffer, T. Ravingerová
Institut e of Heart Research, Slovac Academy of Science, Centre of Excelence SAS NOREG, Bratislava, Slovakia

P3.15 **Selective Na+/Ca2+ exchanger inhibition prevents Ca2+ overload induced triggered arrhythmias**

Norbert Nagy, A. Kormos, Zs. Kohajda, Á. Szebeni, P. Pollesello, J. Levijoki, K. Acsai, L. Virág, P.P. Nánási, J.Gy. Papp, A. Varró, A. Tóth
MTA-SZTE Research Group of Cardiovascular Pharmacology, Hungarian Academy of Sciences, Szeged, Hungary

P3.17 **Cannabinoids and muscle weakness – Investigating the function of CB1 receptors in mammalian skeletal muscle**
Tamás Oláh, D. Bodnár, A. Tóth, J. Fodor, A. Kovács, A. Farkas, B. Nádró, P. Szentesi, L. Csernoch
University of Debrecen, Faculty of Medicine, Department of Physiology, Hungary

P4 Cardiovascular Physiology
Chair: **Eszter Horváth**, Hungary

P4.1 **Poly (ADP-ribose) polymerase (PARP) activation in chronic heart failure correlates with the level of cardiac dysfunction**
Andrea Simon, R. Benkő, G. Szabó, A. Oláh, K.V. Nagy, Cs. Mátyás, Á. Hajas, A. Kosztin, M. Pólos, I. Hartyánszky, E. Zima, T. Radovits, B. Merkely, E.M. Horváth
The Heart and Vascular Center of Semmelweis University, Budapest, Hungary

P4.3 **Angiogenic and positive inotropic effects of apelin fragments on human pluripotein stem cell-derived cardivascular cells**
Annamaria Kosztin, L. Polgár, L. Kőhidai, P. Várnai, E. Gara, J. Skopál, S. Harding, B. Merkely, G. Földes
Semmelweis University, Heart Center, Budapest, Hungary

P4.5 **Carbon monoxide pollution induces heme oxygenase-1 in ischaemic rat heart**
Attila Czompa, G. Meyer, C. Reboul, A. Motko, A. Holup, A. Tosaki, I. Lekli
University Debrecen, Faculty of Pharmacy, Department of Pharmacology, Debrecen, Hungary

P4.7 **Free radicals in civilization diseases – Friends or foe of endogenous protective processes in the myocardium**
A. Ziegelhoffer, M. Ferko, I. Waczulíková, T. Ravingerová, S. Pastoreková, I. Kancirová, M. Jašová, **Martina Muráriková**
Institute for Heart Research, Slovak Academy of Sciences, Centre of Excellence SAS NOREG, Bratislava, Slovakia

P4.9 **Interaction of Ca-sensitiser levosimendan and different catecholamines in chronic heart failure: experimental studies**
Balázs Sax, K.V. Nagy, E.M. Végh, A. Kosztin, G. Szucs, E. Zima, N. Turi-Kovacs, V. Kekesi, B. Merkely
Semmelweis University Heart and Vascular Center, Budapest, Hungary

P4.11 **Using the pulse transit time for calculation of systolic and diastolic pressure**
Calin Corciova, D. Matei, F. Corciova
Department Medical Science, University of Medicine and Pharmacy Grigore T. Popa Iasi, Romania

P4.13 **Role of store operated calcium and L-type calcium channels in coronary artery hypercontraction after ischemia and reperfusion process**
Eva M. Calderón-Sánchez, P. Callejo-García, J. Ávila-Medina, T. Smani-Hajami, A. Ordóñez-Fernández
Institute of Biomedicine of Seville (IBiS), Spain

P4.15 **Cardiovascular effects of beta-carotene are lost when it was applied at high concentration**
Evelin Csepanyi, A. Czompa, I. Lekli, A. Tosaki, I. Bak
University of Debrecen, Faculty of Pharmacy, Department of Pharmacology, Debrecen, Hungary
Chair: **Levente Kiss,** Hungary

P4.17 **Combined effects of chronic partial occlusion and gravitational load on saphenous vein: a new venous varicosity model in rat**
Gabriella Dörnyei, O. Sevcsik, M. Jäckel, E. Monos, Gy.L. Nádasy
Department of Morphology and Physiology, Institute of Basic Health Sciences, Semmelweis University, Budapest, Hungary

P4.19 **Interactions between the enzymes matrix metalloproteinases 2 and 9 and regulatory T-cell immunity in the pathogenesis of atherosclerosis**
Ines Mrakovcic-Sutic, V. Micovic, A. Lekic, A. Bulog, I. Sutic, V. Pavisic, G. Laskarin, M. Kovacevic
Department of Physiology and Immunology, Medical Faculty, University of Rijeka, Croatia

P4.21 **Autophagy and ventricular fibrillation in isolated rat hearts**
István Lekli, A. Czeglédi, A. Gyöngyösi, A. Czompa, Á. Tósaki
Department of Pharmacology and Pharmacodynamics, School of Pharmacy, University of Debrecen, Debrecen, Hungary

P4.23 **Role of the transient receptor potential vanilloid-1 (TRPV1) in the development of hydrogen chloride (HCl)-induced vasomotor response in isolated rodent carotid arteries**
Ivan Ivic, E. Pakai, M.Solymar, A. Koller, A. Garami
Medical School Pécs, Departmeent of Patophysiology and Gerontology, Pécs, Hungary

P4.25 **Functional crosstalk between L-type Ca2+ and Orai1 channels and their regulation of vascular tone**
Javier Avila-Medina, P. Gonzalez, J.A. Rosado, A. Castellano-Orozco, A. Ordoñez-Fernandez, T. Smani
Institute of Biomedicine of Seville (IBiS), Spain

P4.27 **Cardiovascular manifestations of complement activation-related pseudoallergy following administration of liposomal nanomedicines**
László Dézsi, R. Urbanics, T. Mészáros, Cs. Vázsonyi, T. Fülöp, E. Őrfi, L. Rosivall, J. Szebeni, G. Szénási
Nanomedicine Research and Education Center, Semmelweis University, Budapest, Hungary

P4.29 **Beneficial effects of Allium ursinum herbal extract (AUHE) on hypercholesterolemic hearts**
Mariann Bombicz, D. Priksz, B. Varga, R. Gesztelyi, K. Pák, A. Kertész, B. Juhász, Á. Tósaki

University of Debrecen, Faculty of Pharmacy, Department of Pharmacology, Debrecen, Hungary

P4.31 **Comparison of ishemic and omeprazole preconditioning on oxidative stress in isolated rat heart**
Nevena Barudzic, V. Jakovljevic, V. Zivkovic, I. Srejovic, D. Djuric
Department of Physiology, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia
Chair: **Mihály Boros,** Hungary

P4.33 **The effects of Tarantula cubensis extract on renal ischemia/reperfusion injury in the rats**
Nurettin Aydogdu, E. Tastekin, Z. Cukur, M.D. Poyraz, O. Y. Yavuz, O. Kaya
University Faculty of Medicine Dept. of Physiology, Edirne, Turkey

P4.35 **Pituitary adenylate cyclase-activating polypeptide (PACAP) induces location- and age-related relaxations of isolated arteries**
Péter Cséplő, Z. Vamos, I. Ivic, G. Toth, A. Tamas, D. Reglodi, A. Koller
Univ Pécs Medical School Dept Pathophysiology and Gerontology and PAMOK KAITO Győr, Hungary

P4.37 **Effect of swimming exercise on CO pathway of resistance and conduit arteries in chronic NOS inhibition induced hypertensive rats**
Seher Ülker, G. Koçer, Ü. K. Şentürk
Akdeniz University Faculty of Medicine, Antalya, Turkey

P4.39 **Cardiovascular target-organ damage in women during menopause**
Sunchica Petrovska, B. Dejanova, M. Papazova, S. Mancevska, J. Pluncevic-Gligorovska, V. Antevska
Medical faculty, Department Institute of Physiology, Skopje, Republic of Macedonia

P4.41 **Distinct effect of crowding stress on cardiac ischemic tolerance in borderline and spontaneously hypertensive male and female rats**
Veronika Ledvenyiova, I. Bernatova, P. Slezak, I. Gablovsky, S. Carnicka, M. Bartekova, T. Ravingerova
Institute for Heart Research, Slovak Academy of Sciences, Centre of Excellence SAS NOREG, Bratislava, Slovakia

P4.43 **Conduction of excitation in the rat atria and pulmonary veins under normal condition and after octanol application**
Viktoriya Karimova, V.S. Kuzmin
Biological Department, Moscow State University, Moscow, Russian

P4.45 **The effect of curcumin on mechanical function and monophasic action potential in isolated rat hearts**
Ziya Kaygisiz, B. Kaygisiz, O. Kutlay
Eskisehir Osmangazi University, Medical Faculty, Department of Physiology, Eskisehir, Turkey

P4.47 **Sphingomyelinase induced vasorelaxations in db/db mice depend on nitric oxide and hydrogen sulfide signaling**
Zsuzsanna Straky, D. Korda, A. Párkányi, É. Ruisanchez, Z. Benyó, L. Kiss

Semmelweis University, Institute of Human Physiology and Clinical Experimental Research, Budapest, Hungary

P5 **Respiratory Physiology**
Chair: **Ildikó Horváth,** Hungary

P5.1 **Physiological, pulmonary and immunological effects of negatively charged Waterfall-Nanoaerosol**
Arnulf Hartl, M. Winklmayr, J. Prossegger, C. Grafetstätter, P. Hahne, H. Braunschmid, C. Pichler, M. Ritter
Institute of Physiology and Pathophysiology, Paracelsus Medical University, Salzburg, Austria

P5.3 **Anti-inflammatory effect of apelin/ APJ receptor system on ovalbumin induced allergic lung disease**
Bogdan Gurzu, I.L. Gurzu, L. Gorgan, D. Ungureanu
"GRIGORE T. POPA" University of Medicine and Pharmacy IASI, Romania

P5.5 **N-acetylcysteine effectively diminished meconium-induced oxidative stress**
Daniela Mokra, A. Drgova, P. Mikolka, M. Petras, J. Mokry, A. Calkovska
Dep. of Physiology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Slovakia

P5.7 **Questions in differential diagnosis of bronchial asthma, chronic obstructive pulmonary disease and overlap syndrome**
Gábor Tajti, Cs. Papp, K. Bíró, K. Pák, Z. Képes, T. Erdei, R. Gesztelyi, M. Szilasi, J. Zsuga
Faculty of Pharmacy, University of Debrecen, Hungary

P5.9 **The involvement of local renin angiotensin system in obesity augmentation of pulmonary allergenic disease**
Irina Luciana Gurzu, F.E. Zugun, B. Gurzu
Grigore T. Popa University of Medicine and Pharmacy, Iasi, Romania

P5.11 **Cigarette smoke-induced upregulation of the Transient Receptor Potential Ankyrin 1 ion channel in the mouse lung and in a human pulmonary tissue 3-dimensional model**
József Kun, D. Feller, I. Szitter, Zs.Hajna, Á. Kemény, A. Perkecz, V. Csöngei, D. Ernszt, T. Kovács, J. Pongrácz, Zs. Helyes
Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, Hungary

P5.13 **MDR1 C3435T allele and genotype frequency in chronic obstructive pulmonary disease**
Maja Milojkovic, J. Radovic, N. Milacic
Medical Faculty in Nis, Universitiy in Nis, Serbia

P5.15 **Meconium-induced oxidative damage and surfactant/budesonide therapy in experimental meconium aspiration syndrome**
Pavol Mikolka, J. Kopincova, D. Mokra, L. Tomcikova, A. Calkovska
Deparment of Physiology, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia

P5.17 **Gene polymorphisms of surfactant protein B are associated with respiratory distress in neonates**

Silvia Smolárová, V. Holubeková, A. Štanclová, P. Lukáč, M. Škereňová, M. Zibolen, K. Maťašová, Z. Lasabová, A. Čalkovská
Department of Physiology, Jessenius Faculty of Medicine, Comenius University (JFM CU), Department, Martin, Slovakia

P6 **Gastrointestinal Physiology**
Chair: **Gábor Varga,** Hungary

P6.1 **The possible role of Apelin on formation and healing mechanisms of ischemia reperfusion (I/R) induced mucosal lesions in rats**

Burcu Gemici, İ. Eker, V.N. İzgüt-Uysal, M. Aslan
Near East University, Nicosia-TRNC, Turkey

P6.3 **Effect of chronic systemic ozone treatment on endogen level of Nesfatin-1 in intestinal ischemia-reperfusion created rat**

Ceylan Ayada, O. Genç, Ü. Toru, R. Akcılar, S. Şahin, G. Erken, H.A. Erken, G. Turgut, S. Turgut
Dumlupınar University, Medical Faculty, Department of Physiology, Kütahya, Turkey

P6.5 **Involvement of interleukin-24 in the pathogenesis of inflammatory bowel disease**

Erna Sziksz, A. Ónody, D. Pap, L. Himer, A. Veres-Székely, V. Ruszinkó, A. Fekete, G. Veres, A. Arató, T. Tulassay, A. Szabó, Á.Vannay
MTA-SE, Pediatrics and Nephrology Research Group, and 1st Department of Pediatrics, Budapest, Hungary

P6.7 **The Transient Receptor Potential Vanilloid 1,4 (TRPV1, TRPV4) and Ankyrin 1 (TRPA1) receptor mRNAs are expressed in the human gastric mucosa**

Kata Csekő, B. Szalontai, K. Pohóczky, I. Hegedűs, A. Perkecz, A. Illés, Á. Vincze, J. Czimmer, I. Szabó, Zs. Helyes
Department of Pharmacology and Pharmacotherapy, Szentagothai Research Centre, University of Pécs, Hungary

P6.9 **Antizyme (AZ) regulates intestinal cell growth independently of polyamines**

Leonard R. Johnson, R.M. Ray
Department of Physiology, University of Tennessee Health Science Center, USA

P6.11 **Stress-induced modulation of ileal motility in Capsici fructus-intake female rats**

Mari Kimoto, J. L. Zeredo, Z. Nihei, M. S Ota, H. Yamashita, K. Kaida, K. Toda
Japan Women's University, Tokyo, Japan

P6.13 **A novel laparoscopic device for quantifying gastric slow wave activity**

Rachel Berry, N. Paskaranandavadivel, P. Du, G. O'Grady, M.L. Trew, J.A. Windsor, L.K. Cheng
Auckland Bioengineering Institute, University of Auckland, New Zealand

P6.15 **The role of the ICC myenteric plexus network in the anisotropic propagation of intestinal slow wave activity**
Shameer Sathar, M.L. Trew, L.K. Cheng
Auckland Bioengineering Institute, University of Auckland, New Zealand

P6.17 **Possible activation of immunity by chronic peripheral Ozone and Nesfatin-1 application in ischemia-reperfusion**
Ümran Toru, C. Ayada, O. Genç, Ü. Toru, R. Akcılar, S. Şahin, G. Erken, H.A. Erken, G. Turgut, S. Turgut
Dumlupınar University, Medical Faculty, Department of Thoracic Medicine, Kütahya, Turkey

P8 **Physiology of the Immune System**
Chair: **Attila Mócsai,** Hungary

P8.1 **Preventive effects of resveratrol against Schistosoma mansoni-induced liver fibrosis in mice**
Abderrhman Ismeil
Alexandaria University - Faculty of Medicine- Physiology Department, Alexandria, Egypt

P8.3 **Detection of different gene expression in human residual Epithelial cells of anterior lens capsule after manual and femtosecond laser performed capsulorhexis**
Andrea Krisztina Süközd, J. Rapp, D. Feller, J.E. Pongrácz, A. Kerek, B. Gáspár, Zs. Biró
Department of Ophtalmology, Clinical Centre, The Medical School, University of Pécs, Hungary

P8.5 **Detailed characterization of the antibacterial effect of human neutrophilic granulocyte derived extracellular vesicles**
Csaba I. Timár, A. Mak, A. Lorincz, E. Ligeti
Semmelweis Egyetem, Élettani Intézet, Budapest, Hungary

P8.7 **Release of Diphtheria Toxin Fragment A (FA) from early endosomes into the cytosol**
Ebru Hacıosmanoğlu, B. Varol, B.Ö. Edis, M. Bektaş
Istanbul Bilim University, Faculty of Medicine, Department of Physiology, Istanbul, Turkey

P8.9 **Contribution of CD40L/Mac-1 interaction to visceral adipose tissue inflammation in mouse model of high fat diet-induced obesity and obesity-related nephropathy**
Éva Nóra Bukosza, T. Kaucsar, G. Szenasi, D. Wolf, A. Zirlik, P.Hamar
Institute of Pathophysiology, Semmelweis University Budapest, Hungary

P8.11 **Nuclear envelope circularity is related to chromatin textural variance in Feulgen-stained medullar thymocytes: application in apoptosis research**
Igor Pantic, M. Basailovic, J. Paunovic M . Basailovic, J. Paunovic
Institute of Medical Physiology, School of Medicine, University of Belgrade, Serbia

P8.13 **Tadalafil (PDE5 inhibitor) suppreeses inflammation after ovalbumin sensitization in guinea pigs**

Juraj Mokry, I. Medvedova, M. Prso, A. Eichlerova, P. Mikolka, P. Kosutova, A. Fulmekova, D. Mokra
Department of Pharmacology, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia

P8.15 **In the long run hyperbaric oxygen therapy attenuates pro-inflammatory processes in streptozotocin induced diabetes in rats**
Rita Benkő, V. Ágoston, K. Ihionvien, M. Szabó, N.J. Béres, Zs. Benkő, Cs. Répás, B. Bakk-Nurdisány, M. Szepes, L. Kiss, Z. Nagy, E.M. Horváth
Semmelweis University, Institute of Human Physiology and Clinical Experimental Research, Budapest, Hungary

P8.17 **Staphylococcus enterotoxin B and thymic stromal lymphopoietin treatment of keratinocytes as a model for atopic dermatitis**
Tamás Bíró, Á. Angyal, A.G. Szöllősi, N. Vasas, E. Lisztes, A. Oláh
DE-MTA "Lendület" Cellular Physiology Research Group, Department of Physiology, University of Debrecen, Hungary

P9 Endocrinology and Metabolism
Chair: **Attila Patócs,** Hungary

P9.1 **The potential contribution of alarin to the regulation of energy balance in rats**
Alexandra Mikó, P. Balla, B. Aubrecht, N. Füredi, Sz. Soós, M. Székely, M. Balaskó, S. Brunner, B. Kofler, E.
Pétervári University of Pécs, Departement of Pathophysiology and Gerontology, Pécs, Hungary

P9.3 **Magnesium status and insulin resistance in subjects at risk for type 2 diabetes**
Ghouini Ahmed, D.E.H. Djoghlaf
Faculté de Médecine de Blida, Blida, Algeria

P9.5 **Academic stress effects food choice in health school students**
Gülsün Memi, Z.N.Ö. Kumral, N.H. Nogay
Kirklareli University, School of Health, Department of Physiology, Kirklareli, Turkey

P9.7 **Effect of different doses of Quercetin supplementation on element levels of brain tissue in diabetic rats**
Enver Ahmet Demir , B. Yazgan, M. Oz, M.I. Alp, H.S. Gergerlioglu, R. Mogulkoc, A.K. Baltaci
Selcuk University, Faculty of Medicine, Konya, Turkey

P9.9 **Age- and nutritional state-related catabolic effects of a central leptin infusion**
Ildikó Rostás, T. Rimai, E. Varga, J. Tenk, Sz. Soós, M. Székely, E. Pétervári, M. Balaskó
University of Pécs, Medical School, Department of Pathophysiology and Gerontology, Pécs, Hungary

P9.11 **The uterine and vascular actions of Estetrol delineate a distinctive profile of Estrogen Receptor ? modulation, uncoupling nuclear and membrane activation**

Jean Francois Arnal, A. Abot, C. Fontaine, J-M. Foidart, G. L. Geoffrey, F. Lenfant
INSERM U1048, Toulouse, France

P9.13 **The electron transport chain component NDUFB8 is required for glucose-stimulated insulin secretion in MIN6 cells**
Julia Parnis, P. L. Chabosseau, G. A. Rutter
Imperial College London, South Kensington Campus, London, UK

P9.15 **Ornithine transcarbamylase deficiency in a young boy with acute liver failure**
Lyece Yargui, D. Berhoune
Biochemistry Central Laboratory, Mustapha Hospital, Algiers, Algeria

P9.17 **Decreased insulin sensitivity in multiple sclerosis**
Miroslav Vlcek, A. Penesova, R. Imrich, L.Krizova, B. Kollar, P. Turcani, D. Jezova
Institute of Experimental Endocrinology & Center for Molecular Medicine, Slovak Academy of Sciences, Bratislava, Slovakia

P9.19 **Effects of cholesterol, FSH and LH on steroidogenic activity in cat granulosa cell culture**
Ozkan Simsek, S. Arikan
Department of Physiology, Faculty of Veterinary Medicine, University of Kirikkale, Turkey Chair: András Balla, Hungary

P9.21 **Asiatic acid improves vascular functions in mesenteric vascular beds isolated from high-carbohydrate, high-fat diets-induced metabolic syndrome rats**
Poungrat Pakdeechote, P. Maneesai, U. Kukongviriyapan, P. Prachaney, P. Tangsucharit
Department of Physiology, Faculty of Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

P9.23 **Correlation of visfatin expression in subcutaneous adipose tissue with anthropometrical measures and biochemical parameters of youth male**
Sanja Novak, D. Divkovic, A. Cosic, I. Drenjancevic, K. Selthofer-Relatic
Department of Physiology and Immunology, Faculty of Medicine Osijek, University of Josip Juraj Strossmayer Osijek, Croatia

P9.25 **Effects of application of chlorpyrifos ethyl and rose water on rat pancreas**
Serdal Öğüt
Health School, Turkey

P9.27 **Irisin level in response to the patients with metabolic impairments**
Sermin Algul, S.Ozcan, A. Barutcu, I. Serhatlioglu, S. Berilgen, O.Ozcelik
Fırat University Faculty of Medicine Department of Physiology, Elazig, Turkey

P9.29 **Effects of chronic central administration of irisin on food intake, body weight and body temperature in the rats**
Suat Tekin, Y. Erden, C. Colak, S. Sandal
Inonu University, Faculty of Medicine, Department of Physiology, Malatya, Turkey

P9.31 **Chronic intracerebroventricular apelin-13 infusion in rats increases daily food intake and body weight by reducing leptin levels**
Suleyman Sandal, S. Tekin, B. Yilmaz
Inonu University, Faculty of Medicine, Department of Physiology, Malatya, Turkey

P9.33 **Does apelin-13 affect the development of brown fat?**
Ümit Yılmaz, Y. Erden, S. Tekin, E. Etem, S. Sandal
Inonu University, Faculty of Medicine, Department of Physiology, Malatya, Turkey

P9.35 **Increasing selenium concentration in animal tissues by wheat agrofortification**
Zdenko Lončarić, I. Drenjančević, B. Popović, K. Karalić, V. Ivezić, S. Novak, A. Ćosić, B.R. Singh
Faculty of Agriculture, University of Josip Juraj Strossmayer in Osijek, Croatia

P9.37 **Role of the hypothalamic CRF and AVP in mediating the activation of the HPA axis in alcohol-treated and alcohol-deprived rats**
Zsolt Bagosi, M. Palotai, A. Buzás, P. Bokor, A. Jenei, K. Csabafi, M. Jászberényi, Gy. Telegdy, Gy. Szabó
Department of Pathophysiology, University of Szeged, Hungary

P10 **Neurophysiology**
Chair: **Gábor Pethő,** Hungary

P10.1 **Nociceptive role of hemokinin-I, the newest member of the tachykinin family, in chronic traumatic neuropathy of the mouse**
Ágnes Hunyady, T. Gubányi
University of Pécs, Hungary

P10.3 **Effects of intraamygdaloid microinjections of RFRP-1 on anxiety and positive reinforcement**
Anita Kovács, L. Kristóf, T. Ollmann, L. Péczely, O. Zagoracz, R. Gálosi, N. Bencze, L. Lénárd
Institute of Physiology, Pécs University Medical School, Pécs, Hungary

P10.5 **Role of capsaicin-sensitive nerve terminals in chronic restraint stress induced increase of nociception**
Bálint Scheich, P. Vincze, B. Gaszner, E. Pintér, J. Szolcsányi, Zs. Helyes
Department of Pharmacology and Pharmacotherapy, University of Pécs Medical School; János Szentágothai, Pécs, Hungary

P10.7 **IL-1ß modifies the taste reactivity in the cingulate cortex of the rat**
Bettina Cseténi, E. Hormay, B. Nagy, I. Szabó, M. Bajnok Góré, Z. Karádi
University of Pécs, Medical School, Institute of Physiology, Pécs, Hungary

P10.9 **Complex functional attributes of cingulate cortex glucose-monitoring neurons and their metabolic significance**
Edina Hormay, B. Cseténi, I. Szabó, B. Nagy, B. Hideg, M.B. Góré, Z. Karádi
University of Pécs, Medical School, Institute of Physiology, Pécs, Hungary

P10.11 **New semicarbazide-sensitive amine oxidase (SSAO) inhibitor as a dual antagonist of TRPA1 and TRPV1 ion channels**
Éva Sághy, M. Payrits, É. Szőke, T. Bagoly, P.Mátyus, D. Rúth, Zs. Helyes
Department of Pharmacology and Pharmacotherapy, Szentágothai Research Center, University of Pécs, Pécs, Hungary

P10.13 **Neuroprotective effect of early postnatal environmental enrichment in a rat model of Parkinson's disease**
Gábor Horváth, A. Jungling, Zs. N. Karadi, D.Cs. Farkas, G. Novogradecz, A. Kovacs, P. Kiss, B. Gaszner, D. Reglodi, A. Tamas
Department of Anatomy, University of Pecs Medical School, Pécs, Hungary

P10.15 **Kallikrein 8: a promising novel biomarker in brain tumors**
Gamze Turna, N. Kilic, G. Kurt, F. Dogulu
Ahi Evran University, Faculty of Medicine, Department of Medical Biochemistry, Kirsehir, Turkey

P10.17 **Anterior cingulate responses evoked by mechanical nociceptive stimulation in female rats**
Hiromi Yamashita, J. L. Zeredo, Z. Nihei, K. Kaida, M. Kimoto, M. Umeda, I. Asahina, K. Toda
Nagasaki University, Nagasaki, Japan
Chair: **Gyula Szabó,** Hungary

P10.19 **A possible way to decrease "crowdedness" through functional asymmetry in the hypothalamus**
István Tóth, D. Kiss, G. Jocsak, L. Frenyo, A. Zsarnovszky
SzIE, Faculty of Veterinary Sciences, Dept. of Physiology and Biochemistry, Budapest, Hungary

P10.21 **Surgical level of ketamine anesthesia induces EEG microstructure and respiratory pattern disturbances following pedunculopontine tegmental nucleus lesion in rat**
Katarina Lazic, J. Petrovic, A. Kalauzi, J. Saponjic
University of Belgrade, Department of Neurobiology, Institute for Biological Research - Sinisa Stank, Serbia

P10.23 **Long term consequences of early postnatal domoic acid administration on spontaneous behavior of Wistar rats**
Katerina Jandova, V. Riljak
Institute of Physiology, 1st Faculty of Medicine, Charles University in Prague, Czech Republic

P10.25 **The role of intraamygdaloid oxytocin in reinforcing mechanisms**
Kristóf László, A. Kovács, G.D. Lacy, T. Ollmann, L. Péczely, E. Kertes, Z. Karádi, L. Lénárd
Institute of Physiology, University of Pécs, Pécs, Hungary

P10.27 **Dopamine and serotonin in frog and turtle retina: an immunoflourescent study**
Liliya Vitanova
Desislava Zhekova Dept. Physiology, Medical University, Sofia 1431, Bulgaria

P10.29 **Effect of visual and auditive stimuli in amygdala neurons**

Maria del Pilar Montes Lourido, F.A. Vicente, A.M. Bermudez, M.C. Romero, R. Perez, F. Gonzalez
CIMUS (Department of Physiology), University of Santiago de Compostela, Av. Barcelona, Spain

P10.31 **Intracellular Fe2+ and 4-hydroxynonenal suppresses a swelling-activated chloride current in microglial cells**
Martin Jakab, J. Schmölzer, N. Bresgen, M. Ritter, H.H. Kerschbaum
Institute of Physiology and Pathophysiology, Paracelsus Medical University, Salzburg, Austria

P10.33 **Hedonic impact of sweet taste on food consumption and activation of reward related neurons in intrauterine undernourished rats**
Máté Durst, K. Könczöl, R. Matuska, R. Reichardt, Zs.E. Tóth
Neuroendocrine and In Situ Hybridization Laboratory, Department of Anatomy, Histology and Embryology, Semmelweis University, Budapest, Hungary

P10.35 **The role of the melanocortin system and neuropeptide Y in the regulation of energy homeostasis in SHR rats**
Nóra Füredi, B. Aubrecht, P. Balla, A. Mikó, Sz. Soós, M. Székely, M. Balaskó, B. Gaszner, E. Pétervári
University of Pécs, Departement of Pathophysiology and Gerontology, Pécs, Hungary
Chair: **Dóra Reglődi**, Hungary

P10.37 **CRT and LCD monitors in science**
Péter Csibri, A. Bognár, Gy. Sáry
University of Szeged, Faculty of Medicine Department of Physiology, Szeged, Hungary

P10.39 **Disruption of sensorimotor integration in writer's cramp**
N. Langbour, V. Michel, B. Dilharreguy, D. Guehl, M. Allard, Pierre Burbaud
CHU de Bordeaux, France

P10.41 **The treatment of orofacial pain by using theta burst rTMS stimulation**
Richard Rokyta, F. Jitka
Charles University in Prague, Third Faculty of Medicine, Department of Physiology, Prague, Czech Republic

P10.43 **The role of segmentation interval in detecting seizure from EEG series by using embedding entropy metrics**
Serap Aydin
Bahçeşehir University, Biomedical Engineering Department, Istanbul, Turkey

P10.45 **Complexity and coherence analysis on EEG of patients with obsessive compulsive disorder**
Serap Aydin, E. Ergül, N. Arıca, O. Tan
Bahçeşehir University, Biomedical Engineering Department, Istanbul, Turkey

P10.47 **A role for the neurokinin-1 receptor in endotoxin-induced fever in mice**
Valeria Tékus, E. Pákai, R. Mátics, R. Schipp, Á. Kemény, E. Pintér, A. Garami
Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, Hungary

P10.49 **Effect of antioxidants in preventing trimethyltin-induced neurodegeneration**

Veronika Stará, J. Navarová, P. Janega, N. Sedláčková, M. Mach, E. Ujhazy, Z. Gáspárová
Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Bratislava, Slovak Republic

P10.51 **Neuronal responses of the rat medial prefrontal cortex during appetitive classical conditioning**

Zoltán Petykó, A. Tóth, R. Gálosi, I. Szabó, K. Máté, I. Szabó, Z. Karádi, L. Lénárd
University of Pécs, Medical School, Institute of Physiology, and Szentágothai Research Centre, Pécs, Hungary

P11 **Exercise Physiology**
Chair: **Gábor Pavlik**, Hungary

P11.1 **The effect of aerobic training on performance and hormonal changes a mong prepubertal female handball players**

Alexandra Cselkó, É. Tékus, M. Váczi, G. Schuth, T. Kőszegi, M. Wilhelm
University of Pécs, Faculty of Health Sciences, Doctoral School of Health Sciences; Pécs, Hungary

P11.2 **Development and complete morphological and functional reversibility of athlete's heart in a rat model**

Attila Oláh, Á. Lux, B. T. Németh, Cs. Mátyás, D. Kellermayer, E. Birtalan, M. Ruppert, L. Szabó, L. Hidi, M. Török, G. Merkely, A. Meltzer, B. Merkely, T. Radovits
Semmelweis University, Heart and Vascular Center, Budapest, Hungary

P11.3 **Periodical changes in the characteristics of the athlete's heart**

Eszter Csajági , I. Szauder, Zs. Major, G. Pavlik
Semmelweis University, Department of Health Sciences and Sports Medicine, Hungary

P11.4 **The effect of exercise on blood plasma markers of skeletal muscle injuries**

Éva Tékus, M. Váczi, A. Cselkó, G. Pintér, T. Kőszegi, M. Wilhelm
Institute of Sport Sciences and Physical Education University of Pécs, Hungary

P11.5 **Amino acid levels, enzyme activity, and lipid peroxidation in smokers and non-smokers after a 6-week long ?-Alanine rich diet**

Gergő Pintér, M. Wilhelm, F. Gallyas Jr.
University of Pécs, Doctoral School of Health Scienses, Pécs, Hungary

P11.6 **Myocardial consequences of a treatment with prolyl-hydroxylase inhibitors used to improve exercise performance**

Gregory Meyer, B. Poncon, F. Favier, S. Gayrard, P. Obert, C. Reboul, G. Py
University of Avignon, Physiology and Physiopathology of Cardiovascular Adaptations to Exercise, Avignon, France

P11.7 **Effectiveness of constant load exercise test on critical power output estimation in sedentary male subjects**

Ihsan Serhatlioglu, S. Algul, B. Yilmaz, O. Ozcelik
Fırat University Faculty of Medicine Department of Biophysics, Elazig, Turkey

P11.8 **Carotid-radial pulse transit time compared to the pulse arrival time to the capillary bed of the finger tip during and after aerobic exercise in young healthy subjects**

N. Potočnik, **Helena Lenasi**
Institute of Physiology, Medical Faculty, University of Ljubljana, Slovenia

P11.9 **Effect of high protein diet and exercise on cardiac Aquaporin 7 expression**

Orkide Palabiyik, A. Karaca, S.A. Vardar, E. Tastekin, B.E. Yamasan, B. Tokuc, T. Sipahi
Trakya University Faculty of Medicine Department of Biophysics, Edirne Turkey

P11.10 **Effects of recreational physical exercise on metabolic and cardiovascular parameters in type 2 diabetic rat model**

Renáta Szabó, A. Pósa, A. Csonka, Z. Szalai, K. Kupai, A. Magyariné Berkó, Sz. Török, L. Daruka, Cs. Varga
Department of Physiology, Anatomy and Neuroscience, University of Szeged, Hungary

P11.11 **Clinical, functional and inflammatory factors associated with muscle fatigue and self-perceived fatigue in elderly community-dwelling women**

LS.M. Pereira, J.P. Silva, D.S. Pereira, L.P. Lustosa, B.Z. de Queiroz, N.M.B. Rosa, A.M. Assumpção, J.M.D. Dias, Ronaldo Luis Thomasini
Institute of Sciences and Technology, Federal University of Jequitinhonha and Mucuri Valleys, Diamantina, Brazil

P11.12 **Correlation between inflammatory mediators with muscular handgrip strength in community-dwelling elderly women**

D.C. Felício, D.S. Pereira, A. M. Assumpção, B.Z. de Queiroz, N.M. de B. Rosa, J.P. Silva, D.M. da C. dos Anjos, J.M.D. Dias, Ronaldo Luis Thomasini, LS.M. Pereira
Institute of Sciences and Technology, Federal University of Jequitinhonha and Mucuri Valleys, Diamantina, Brazil

P11.13 **Right ventricular adaptation of the athlete's heart**

Zsuzsanna Major , E. Csajági, T. Kováts, Zs. Kneffel, G. Pavlik
Semmelweis University, Faculty of Physical Education and Sport Sciences, Budapest, Hungary

P12 **From Cell Signalling to Bioenergetics and Cell Damage**
Chair: **Geiszt Miklós,** Hungary

P12.1 **The combined therapy with melatonine and hypothermia prevents apoptosis and improves oxidative stress in a neonatal rat model of hypoxic-ischemic encephalopathy**

Alina M. Toader, A. G. Filip, G. Dogaru, F. Tabaran, C. Anescu, L. Farcas, O. Grad, A. Muresan
University of Medicine and Pharmacy Iuliu Hatieganu Cluj-Napoca, Romania

P12.3 **The effect of hyperbaric therapy on the levels of oxidative stress**

Anita Ćosić, Z. Mihaljevic, D. Kibel, S. Novak, A. Cavka, I. Grizelj, M. Mihalj, I. Drenjancevic
Faculty of Medidine University of Osijek, Croatia

P12.5 **Possible anti-tumorigenic usage of angiostatin in oncotherapy**
Balint Gergely Szabo, J. Timar, A. Marton, Cs. Vizler, E. Tatrai, J. Tovari, L. Szilak
2nd Department of Pathology, Semmelweis University, Budapest, Hungary

P12.7 **Nitric oxide can serve as indicator for severity injury of polytrauma**
Dana Mikova
2nd Faculty of Medicine, Charles University in Prague, Department of Physiology, Prague, Czech Repub

P12.9 **Sphingosine-1-phosphate enhances the contractile responsiveness of vascular smooth muscle via distinct S1P2 receptor mediated pathways**
Dorottya Móré, É. Ruisanchez, P. Dancs, M. Kerék, S. Offermanns, Z. Benyó
Semmelweis University, Institute of Human Physiology and Clinical Experimental Research, Budapest, Hungary

P12.11 **Ruthenium red differentiates between closely related K2P channels**
Gabriella Braun, M. Lengyel, P. Enyedi, T. Hegedűs, G. Czirják
Semmelweis University, Institute of Physiology, Budapest, Hungary

P12.13 **UVB-induced apoptosis signaling cascade and changes of molecular markers expression in a human dermal fibroblast line**
Ioana Zinuca Pavel, C. Dehelean, O. Duicu, D. Muntean, F. Bojin
"Victor Babes" University of Medicine and Pharmacy Timisoara, Romania

P12.15 **Mapping the subcellular localization and activity of the Nox4-p22phox enzyme complex**
Melinda Zana
Department of Physiology, Semmelweis University, Budapest, Hungary

P12.17 **Time- and dose-dependent characteristics of endogenous protoporphyrin IX production from delta- aminolevulinic acid and its derivatives**
Tobias Kiesslich, L. Helander, R. Illig, C. Oberdanner, A. Wagner, H. Lettner, M. Jakab, K. Plaetzer
Department of Internal Medicine I, Paracelsus Medical University / Salzburger Landeskliniken (SALK), Salzburg, Austria

P16 **Aging**
Chair: **Ákos Koller,** Hungary

P16.1 **The evolution of K+-evoked spreading depolarization shortly after carotid occlusion in young and aged rats**
Ákos Menyhárt, B. Szepes, O.M. Tóth, P. Hertelendy, F. Bari, E. Farkas
Department of Medical Physics and Informatics, University of Szeged, Hungary

P16.2 **The effect of recreational physical exercise, caloric restriction and high triglyceride diet in experimental menopause**
Anikó Pósa, R. Szabó, A. Csonka, L. Daruka, Sz. Török, M. Veszelka, A. Magyariné Berkó, K. Kupai, Cs. Varga
GLP Toxicology Lab, Department of Physiology, Anatomy and Neuroscience, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

P16.3 **Comparison of exfoliated human mammary cells count with demographical and nutritional parameters of lactating mothers**

S. Agus, S.E. Dinc, A. Apaydin, S. Sandal, A. Vitrinel, Bayram Yilmaz
Yeditepe University, Faculty of Medicine, Department of Physiology, Istanbul, Turkey

P16.4 **Quercetin treatment reverse endothelial dysfunction and oxidative stress in patients with rheumatoid arthritis**

Doina Baltaru, A. Mureşan, I.C. Chiş
Constantin Papilian Military Emergency Hospital, Cluj Napoca, Romania

P16.5 **The effects of body mass on CMRgluc-related metabolic activity in mouse joints investigated by in vivo dynamic PET/MRI**

Mariann Semjéni
CROmed Ltd, Hungary

P16.6 **Exposure to static magnetic field induces decrease of antioxidant oligoelements in aging heart**

Marija Stankovic, SR. De Luka, S. Jankovic, S. Stefanovic, DM. Djordjevich, ID. Milovanovich, AM. Trbovich
University of Belgrade, Faculty of Medicine, Institute of Pathophysiology, Belgrade, Serbia

P16.7 **The projected increase of rheumatoid diseases due to an aging population in Austria from 2012 to 2050**

Markus Ritter, A.Moder, W. Hitzl, M. Gaisberger, H. Dobias
Institut of Physiology and Pathophysiology, Gastein Research Institute; Paracelsus Medical University, Salzburg, Austria

P16.8 **Beta-herpesviruses related to aging and frailty**

Ronaldo Luis Thomasini, D.S. Pereira, F.S.M. Pereira, L.S.M. Pereira, M.M. Teixeira, A.L. Teixeira-Jr
Federal University of Jequitinhonha and Mucuri Valleys, Diamantina, MG, Brazil

P16.9 **Poor correlation between handgrip strength and isokinetic performance of knee flexor and extensor muscles in community-dwelling elderly women**

D.C. Felicio, D.S. Pereira, A.M. Assumpção, F.R. de Jesus-Moraleida, B.Z. de Queiroz, J.P. da Silva, N. M.B. Rosa, J.M.D. Dias, Ronaldo Luis Thomasini, L.S.M. Pereira
Institute of Sciences and Technology, Federal University of Jequitinhonha and Mucuri Valleys, Diamantina, Brazil

P16.10 **Aging exacerbates hypertension-induced intracerebral microhemorrhages in mice**

Stefano Tarantini, P. Toth, Zs. Springo, D. Sosnowska, T. Gautam, Zs. Tucsek, C. Giles, J.D. Wren, A. Koller, W.E. Sonntag, A. Csiszar, Z. Ungvari
Reynolds Oklahoma Center on AgingDepartment of Geriatric MedicineUniversity of Oklahoma Health Scien, Oklahoma City, USA

P16.11 **Changes in norepinephrine induced vasomotor response and vascular ?1-receptor expression as a function of age**

Zoltán Vámos, P. Cséplő, I. Ivan, R. Mátics, Á. Koller
Department of Pathophysiology and Gerontology, University of Pécs, Hungary

P16.12 **Age-related impairment of myogenic adaptation to pulsatile pressure in cerebral arteries of Mus musculus**

Zsolt Springo, P. Toth, S. Tarantini, Zs. Tucsek, P. Cseplo, A. Koller, W.E. Sonntag, A. Csiszar, Z. Ungvari

Department of Pathophysiology & Gerontology, Szentagothai Res. Ctr, University of Pécs, Pécs, Hungary

29 August, 2014

SZENT-GYÖRGYİ ALBERT ROOM

9:00 **PLENARY LECTURE**
Chair: **Ole Petersen**, UK
MicroRNAs in pancreatic beta-cell physiology
Lena Eliasson, Sweden

10:00 **BREAK**

10:15-12:15
S4-A **Sodium Signalling in Astroglia**
Chair: **Alexei Verkhratsky,** UK and **Csaba Fekete**, Hungary

10:15 S4- A1 **The mitochondrial 3Na+/Ca2+ exchanger NCLX is a hub for cellular and mitochondrial Ca2+ signaling in astrocytes. or Na+**
Israel Sekler
Ben Gurion University, Beer Sheva, Israel

10:40 S4- A2 **Exocytotic glutamate release from astrocytes: Intracellular Ca2+ and Na+ dynamics**
Vladimir Parpura
Department of Neurobiology, University of Alabama at Birmingham, Birmingham, USA

11:05 S4- A3 **Astrocytic Na+ influences extracellular GABA/glutamate balance in the neocortex**
Sergei Kirischuk
Institute of Physiology, University Medical Center Mainz, Mainz, Germany

11:30 S4- A4 **Sodium signalling in astroglia**
Alexei Verkhratsky
The University of Manchester, UK

11:55 S4- A5 **A putative counter-apoptotic role of the non-gastric H+/K+-ATPASE ATP1AL1 (ATP12A)**
Markus Ritter, N. Ketterl, D. Streif, M. Beyreis, J. Fürst, M. Jakab
Institute of Physiology and Pathophysiology, Gastein Research Institute, Paracelsus Medical University, Salzburg, Austria

12:15 **BREAK**

AULA & GALERY

13:00-14:30 POSTER SESSION
P7; P 11 ; P 13; P 14; P 15
even numbers: P2 ; - P3; - P4; - P5; - P6; - P8 , - P9; - P10; - P12
Detailed programme of the session see below.

SZENT-GYÖRGYİ ALBERT ROOM

14:30-16:30
S5-A **From Macro- to Microvessels: Function, Structure and Molecular Mechanisms**
Chairs: **Ines Drenjančević,** Croatia and **Jozef Dulak,** Poland

14:30 S5- A1 **Role of metabolites of arachidonic acid in regulation of vascular function**
Ines Drenjančević
Department of Physiology and Immunology, School of Medicine Osijek, Josip Juraj Strossmayer University of Osijek, Croatia

14:50 S5- A2 **Angiotensin II and leukocyte trafficking: New insights for an old vascular mediator**
Maria-Jesus Sanz, M-J. Sanz
Department of Pharmacology, Faculty of Medicine, University of Valencia, Research Institute INCLIVA, Valencia, Spain

15:10 S5- A3 **Microvascular mechanisms of age-related cognitive decline**
Zoltan Ungvári, P. Toth, Zs. Tucsek, D. Sosnowska, T. Gautam, M. Mitschelen, S. Tarantini, F. Deak, A. Koller, W. Sonntag, A. Csiszar
Reynolds Oklahoma Center on Aging, Department of Geriatric Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, USA

15:35 S5- A4 **Role of antioxidant genes and microRNAs in revascularisation after hind limb ischemia**
Jozef Dulak
Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland

16:00 S5- A5 **A new functional role of Ca2+ sensitization mechanisms in the regulation of vascular smooth muscle contraction**
María del Carmen González-Montelongo, C. Porras-González, A. Castellano, J. Ureña
Institute of Biomedicine of Seville (IBiS) and Department of Medical Physiology and Biophysical, Spain

16:15 S5- A6 **Myeloperoxidase promotes the vasoconstrictive effects of hydrogen-peroxide**
Viktória Csató, A. Pető, G.Á. Fülöp, E. Pásztorné Tóth, I. Édes, A. Tóth, Z. Papp
University of Debrecen, Institute of Cardiology, Division of Clinical Physiology, Hungary

16:30 **BREAK**

17:00 **PLENARY LECTURE**
Chair: **Gyula Sáry**, Hungary
Rhythmic re-distribution of inhibition on pyramidal cells in the hippocampus
Peter Somogyi, UK

17:45 **BREAK**

20:30-23:30 **GALA DİNNER**
Venue: Europe River Cruise

HEVESY GYÖRGY ROOM

10:15-12:15

S4-B **Lipid GPCRs in Physiology and Disease**
Chairs: **Zoltán Benyó,** Hungary and **Stefan Offermanns**, Germany

10:15 S4- B1 **Novel GPCRs for lysophosphatidylserine; their structure and function**
Junken Aoki
Tohoku University, Miyagi, Japan

10:40 S4- B2 **Cannabinoid type 1 receptor in noradrenergic/adrenergic cells and its role in metabolism and stress**
Beat Lutz
Institute of Physiological Chemistry, Mainz, Germany

11:05 S4- B3 **New functions for short chain fatty acid and prostanoid receptors**
Stefan Offermanns
Max Planck Institute for Heart and Lung Research and Goethe University Frankfurt, Germany

11:30 S4- B4 **Control of gastrointestinal epithelial integrity by lysophosphatidic acid GPCR**
Gábor Tigyi
Department of Physiology, University of Tennessee Health Science Center Memphis, USA

11:55 S4- B5 **Signaling pathways of thromboxane receptor-mediated vasoconstriction: Major role of phospholipase C epsilon**
Tamás Németh, É. Ruisanchez, L. Hricisák, A. Iring, B. Merkely, L. Hunyady, A.V. Smrcka, S. Offermanns, Z. Benyó
Semmelweis University Heart and Vascular Center, Budapest, Hungary

12:05 S4- B6 **Mutations in the conserved 'DRY' motif of the CB1 cannabinoid receptor result in functionally selective receptor conformations**
Pál Gyombolai, A.D. Tóth, G. Turu, L. Hunyady
Semmelweis University Department of Physiology; MTA-SE Laboratory of Molecular Physiology, Budapest, Hungary

12:15 **BREAK**

14:30-16:30

S5-B **Physiology and Regulation of K2P Channels**
Chair: **Péter Enyedi,** Hungary and **Florian Lesage**, France

14:30 S5- B1 **Excitability tuning by two-P-domain channels: from inhibitory potassium-selective channels to excitatory cationic channels**
Florian Lesage
Nice Sophia Antipolis University, France

14:55 S5- B2 **The intracellular traffic of the two-pore-domain potassium channel TASK-1**
Jürgen Daut
Institute of Physiology Marburg University, Marburg, Germany

15:15 S5- B3 **Pharmacological and genetic recovery of current through truncated and mutated K2P channels**
Alistair Mathie, E. Veale
University of Kent, UK

15:35 S5- B4 **TRESK background K+ channel is regulated by calcineurin and other interacting proteins**
Gábor Czirják, G. Braun, P. Enyedi
Department of Physiology, Semmelweis University, Budapest, Hungary

15:55 S5- B5 **Amusing functions of TWIK-1 in the brain**
Eun Mi Hwang
Korea Institute of Science and Technology, Seoul, Korea

16:15 S5- B6 **Stable gene silencing of TASK-3 channels in melanoma cells induce intrinsic apoptosis**
Dénes Nagy, M. Gönczi, Zs. Nagy, A. Tóth, B. Dienes, J. Fodor, G. Szücs, Z. Rusznák, Á. Szöőr, L. Csernoch
University of Debrecen, Faculty General Medicine, Department of Physiology, Debrecen, Hungary

16:30 **BREAK**

BÉKÉSY GYÖRGY ROOM

10:15-12:15
S4-C **Physiology and Pathophysiology of Bicarbonatesecretion in the Airways – Key to Therapy of Cystic Fibrosis**
Chair: **Ákos Zsembery,** Hungary and **Mike Gray**, UK

10:15 S4- C1 **Anion secretion by calcium-activated anoctamin chloride channels: A direct or indirect mechanism?**
Karl Kunzelmann
University of Regensburg, Germany

10:40 S4- C2 **HCO3-, fluid, mucus and the structure of small airways**
Paul M. Quinton, A. Shamsuddin, G. Flores
Pediatrics, UC San Diego School of Medicine, and Biomedical Sciences, UC Riverside School of Medicine, San Diego, USA

11:05 S4- C3 **The importance of bicarbonate and proteases for mucin secretion and mucus formation**
Gunnar C. Hansson
Department of Medical Biochemistry, University of Gothenburg, Gothenburg, Sweden

11:30 S4- C4 **Effects of bicarbonate and pH on bacterial growth and MIC of Erythromycin**
Ákos Zsembery
Institute of Human Physiology and Clinical Experimental Research, Semmelweis University, Budapest, Hungary

11:45 S4- C5 **HAT-7 cells, a new model to study the intracellular pH regulation and bicarbonate transport of ameloblasts**

Erzsébet Bori, P. D. Besten, H. Harada, M. Steward, A.L.J.J. Bronckers, G. Varga
Department of Oral Biology, Semmelweis University of Medicine, Budapest, Hungary

12:00 S4- C6 **The role of aquaporins in pancreatic ductal cells**
Viktória Venglovecz
University of Szeged, Department of Pharmacology and Pharmacotherapy, Szeged, Hungary

12:15 **BREAK**

14:30-16:30
S5-C **From Cell Signalling to Bioenergetics and Cell Damage**
Chair: **Alexei Tepikin,** UK and **András Spät,** Hungary

14:30 S5- C1 **Pathways to calcium mediated neuronal injury: Starvation in the midst of plenty**
Michael R. Duchen
Department of Cell and Developmental Biology and UCL Consortium for Mitochondrial Research, University College London, UK

14:55 S5- C2 **The crucial role of mitochondrial damage and consequent breakdown of bioenergetics in acute pancreatitis**
Péter Hegyi, V. Venglovecz, J. Maléth, Z. Rakonczay
First Department of Medicine, University of Szeged, Hungary

15:20 S5- C3 **The mitochondrial calcium uniporter: Molecular identity and physiological role**
Rosario Rizzuto
Department Biomedical Sciences, University of Padua, Italy

15:45 S5- C4 **Imaging incretin-regulated bioenergetics in intact pancreatic islets**
Guy A. Rutter
Imperial College London, UK

16:10 S5- C5 **Cardiac calsequestrin and heart failure**
Joachim Neumann, C. Fahrion, S. Fabian, U. Gergs
Medical Faculty Halle, Germany

16:20 **GENERAL DİSCUSSİON (10')**

16:30 **BREAK**

HÁRİ PÁL ROOM

10:15-12:15
S4-D **Revealing the Prominent Role of Neuroglia in Neurodegeneration**
Chair: **José Julio Rodríguez Arellano**, Spain and **László Lénárd,** Hungary

10:15 S4- D1 **Neuroglial morphological and metabolical alterations during the progression of Alzheimer´s disease and ageing**
José Julio Rodríguez Arellano
IKERBASQUE/University of the Basque Country (UPV/EHU), Spain

10:40 S4- D2 **Dysfunction of AMPA-type glutamate receptors in microglia may cause neurodegeneration**
Mami Noda, K. ABeppu, R. Sprengel
Kyushu University, Graduate School of Pharmaceutical Sciences, Fukuoka, Japan

11:05 S4- D3 **Does innate immunity contribute to the pathogenesis of Alzheimer's disease?**
Michael T. Heneka
Clinical Neuroscience, Dept. Of Neurology, University of Bonn, Bonn, Germany

11:25 S4- D4 **The response of NG2-glia (oligodendrocyte precursors) to aging in an animal model of Alzheimer's Disease**
Arthur M. Butt
University of Portsmouth, UK

11:45 S4- D5 **Release of 4- hydroxynonenal and 4-hydroxyhexenal-modified proteins in exosomes**
Florentina Kopp, N. Bresgen, M. Jakab, M. Ritter, H.H. Kerschbaum
Department of Cell Biology, University of Salzburg, Salzburg, Austria

12:00 S4- D6 **Galanin is a modulator for phagocytosis in microglial cells**
Julia K. Landrichinger, M. Beyreis, S. Wintersteller, B. Kofler, M. Ritter, H.H. Kerschbaum
Department of Cell Biology; University of Salzburg, Institute of Physiology and Pathophysiology, Gastein Research Institute, Paracelsus Medical University, Salzburg, Austria

12:15 **BREAK**

14:30-16:30
S5-D **Pulmonary Surfactant: From Molecule to Function**
Chair: **Andrea Calkovska,** Slovakia and **László Hunyady,** Hungary

14:30 S5- D1 **Exocytosis of the lamellar body, a calcium mobilizing secretory lysosome**
Paul Dietl
Institute of General Physiology, University of Ulm, Germany

14:55 S5- D2 **Misfolding of surfactant protein C and how it is solved by Nature and by rational design**
Jan Johansson
Karolinska Institutet, Stockholm, Sweden

15:20 S5- D3 **The role of surfactant in host defence**
Egbert Herting
Department of Paediatrics University of Lübeck, Germany

15:40 S5- D4 **Surfactant inhibition and its reversal**
Andrea Calkovska
Department of Physiology, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia

16:00 S5-D5 **Lymphatic function is required prenatally for lung inflation at birth**
Zoltán Jakus, J.P. Gleghorn, D.R. Enis, A. Sen, S. Chia, X. Liu, D.R. Rawnsley, Y. Yang, P.R. Hess, Z. Zou, J. Yang, S.H. Guttentag, C.M. Nelson, M.L. Kahn
University of Pennsylvania, USA; Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary

16:15 S5- D6 **The N-terminal domain of spider silk proteins for synthetic surfactant production**
Anna Rising
Karolinska Institute, Stockholm, Sweden

16:30 **BREAK**

BEZNÁK ALADÁR ROOM

10:15-12:15
S4-E **Cardiovascular Exercise Physiology**
Chair: **Gábor Pavlik,** Hungary and **Attila Tóth,** Hungary

10:15 S4-E1 **Use of virtual patients in teaching veterinary physiology at the Faculty of Veterinary Science, Szent István University, Budapest**
Mira Mándoki, G. Jócsák, V. Somogyi, D.S. Kiss, I. Tóth, T. Bartha
Department of Pathology, Faculty of veterinary Science, Szent István University, Budapest Hungary

10:30 S4-E2 **The effect of detraining on the characteristics of the athlete's heart**
Gábor Pavlik
Semmelweis University, Fac. Physical Education and Spports Sciences, Budapest, Hungary

10:45 S4-E3 **Effects of Darbepoetin-alpha treatment and TNF-alpha blockage on cardiovascular parameters, blood cCells, and body and kidney weights in L-NAME induced hypertensive rats**
Mete Özkurt, K. Uzuner, N. Erkasap, G. Kus, R. Özyurt, Ö. Kutlay
Physiology Department of Eskisehir Osmangazi University Medical Faculty, Eskisehir, Turkey

11:00 S4-E4 **The effects of provinol on cardiodynamics and coronary flow in isolated rat heart**
Vladimir Zivkovic, V. Jakovljevic, I. Srejovic, N. Barudzic, D. Djuric, O. Pechanova
Department of Physiology, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia

11:15 S4-E5 **Evolution of cerebrocortical spreading depolarizations in a rat microembolic stroke model**
Eszter Farkas, Zs. Bere, G. Kozák, F. Bari
Department of Medical Physics and Informatics, University of Szeged, Hungary

11:30 S4-E6 **Reduced dietary zinc and selenium levels impairs vascular function via oxidative stress in Sprague- Dawley rats aortas**
Ana Cavka, S. Novak, Z. Mihaljevic, I. Grizelj, A. Cosic, Z. Loncaric, B. Popovic, I. Drenjancevic

Department of Physiology and Immunology Faculty of Medicine University of Osijek, Osijek, Croatia

11:45 S4-E7 **Different expression and localization pattern of MT1 melatonin receptor between conduit and resistant arteries can be involved in positive effects of melatonin on blood pressure control**
Lubos Molcan, P. Svitok, K. Stebelova, A. Vesela, I. Ellinger, M. Zeman
Department of Animal Physiology and Ethology, Faculty of Sciences, Comenius University, Bratislava, Slovak Republic

12:00 S4-E8 **Angiogenic properties of human pluripotent stem cell-derived arterial and venous endothelial cells**
Edit Gara, J. Skopal, B. Merkely, S.E. Harding, G. Foldes
Heart and Vasculat Center, Budapest, Hungary

12:15 **BREAK**

14:30-16:30
S5-E **MicroRNA Networks and Potential Clinical Implications in Cancer, Cardiovascular and Renal Diseases**
Chair: **Péter Hamar**, Hungary and **Dontscho Kerjaschki,** Austria

14:30 S5- E1 **MiRNAs in renal glomerular disease: novel insights into pathogenic Mechanisms and clues for treatment**
Dontscho Kerjaschki
Institute of Pathology, Medical University of Vienna, Austria

14:50 S5- E2 **MicroRNAs in ischemia/reperfusion injury and cardioprotection by ischemic conditioning: ProtectomiRs**
Z.V. Varga; Á. Zvara; N. Faragó; G.F. Kocsis; M. Pipicz; R. Gáspár; P. Bencsik; A. Görbe; Cs. Csonka; L.G. Puskás; T. Thum; T. Csont; **Péter Ferdinandy**
Institute of Pharmacology, Semmelweis University, Budapest, Hungary

15:10 S5- E3 **MicroRNA-25 regulates NOX4 in the hypercholesterolemic heart**
Zoltán Varga
Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary

15:25 S5- E4 **miR-200 in extracellular vesicles promotes metastasis of breast cancer cells**
M.T.N. Le, **Peter Hamar**, J. Lieberman
Institute of Pathophysiology, Semmelweis University, Budapest, Hungary

15:45 S5- E5 **Targeting basal-like Triple Negative Breast Cancers and epithelial tumor-initiating cells with aptamer-siRNA chimeras**
Judy Lieberman, A. Gilboa-Geffen, P. Hamar, L.A. Wheeler, A. Wittrup, F. Petrocca
Institute of Immune Diseases, Harvard Medical School, Boston, MA. USA

16:10 S5- E6 **MicroRNA expression might predict prognosis of epithelial hepatoblastoma and sorafenib treated hepatocellular carcinoma**
András Kiss, B. Gyöngyösi, M. Gyugos, G. Lendvai, J. Halász, M. Fassan, É. Végh, B. Járay, E. Székely, Gy. Bodoky, Zs. Jakab, M. Garami, Zs. Schaff
Institute of Pathology, Semmelweis University, Budapest, Hungary

16:30 S5- E7 **Exiqon A/S Symposia Exosomal microRNA in biofluids - Robust biomarkers for disease**
Michael Hansen, A.R. Thomsen, T. Blondal, P. Mouritzen, D. Andreasen, M.W. Teilum, N. Tolstrup, J. Stenvang, C.L. Andersen, H.J. Nielsen, N. Brünner
Exiqon A/S, Vedbaek, Denmark

16:50 **BREAK**

POSTER SESSION
AULA & GALERY

13:00-14:30

P2 **Molecular and Cell Physiology**
Chair: **Norbert Szentandrássy,** Hungary

P2.2 **Beneficial effects of hydrogen sulphide treatment of human adipose derived stem cells in a cell- based model of cell therapy**
Ágnes Csizmazia, E. Dongó, Zs. Benkő, G. Marosi, U. Schumacher, L. Kiss
Semmelweis University, Institute of Human Physiology and Clinical Experimental Research, Budapest, Hungary

P2.4 **The effects of angiotensin II on autophagy pathways in H9c2 cells**
András Czeglédi, K. Szőke, A. Barta, Á. Tósak, I. Lekli
University of Debrecen, Hungary

P2.6 **Examination of pituitary adenylate cyclase activating polypeptide (PACAP)-like immunoreactivity in different pathological clinical samples**
Andrea Tamás, A. Javorhazy, P.D. Sarlos, Zs. Sarszegi, I. Zapf, Z. Szanto, B. Faludi, T. Molnar, J. Nemeth, Gy. Reman, Zs. Nagy, Zs. Szabo, A. Kovacs, D. Banyai, D. Reglodi
Department of Anatomy, PTE-MTA Lendulet PACAP Research Group, University of Pécs, Pécs, Hungary

P2.8 **Role of intracellular signalling pathways in the control of transient receptor potential melastatin 3 (TRPM3) channel activity**
Balázs István Tóth, J. Vriens, D. Ghosh, T. Voets
Laboratory of Ion Channel Research, Department of Cellular and Molecular Medicine, KU Leuven, Belgium

P2.10 **NGF-induced neurodifferentiation of PC12 cells is not influencing the expression of Na,K-ATPase genes**
Barbora Kaločayová, J. Vlkovičová, L. Lichvárová, Ľ. Lacinová, N. Vrbjar
Institute of Heart Research, Slovak Academy of Science, Slovakia

P2.12 **Effects of adenosine on human hair follicls and hair follicle deirved outer root sheath keratinocytes**
Erika Lisztes, E. Shitrit, I. L. Szabó, A.G. Szöllősi, A. Oláh, Á. Angyal, E. Hollósi, T. Bíró
DE-MTA "Lendület" Cellular Physiology Research Group, Department of Physiology, University of Debrecen, Hungary

P2.14 **Role of inositol lipids in the localization of peripheral membrane proteins in mammalian cells**

Glória Radvánszki , G. Gulyás, L. Hunyady, P. Várnai
Department of Physiology, Faculty of Medicine, Semmelweis University, Budapest, Hungary
Chair: **Péter Várnai,** Hungary

P2.16 **Frequency of specific methods for the detection of EGFR in lung tumors**

Jasmina Obradovic, V. Jurisic
Univeristy of Kragujevac, Faculty of Sciences, Kragujevac, Serbia

P2.18 **Screening chimique et activité antioxydante des extraits d'écorces de tronc de 3 esp?ces de plantes utilisées par les bonobos, pan paniscus ? Lui-Kotale en R.D.Congo: Massularia acuminata (G. Don) B ullock ex Hoyl (Rubiaceae), Enantia olivacea Robyns & Ghesq (Annonaceae) et Garcinia punctata oliv. (Clusiaceae)**

Kunyima Wa Kunyima Papy, N. Mbomba, J. Lami, P. Mpiana, M. Muganza
Unit of Physiology, Faculty of Medicine, University of Kinshasa, Lemba, D.R. Congo

P2.20 **Infuence of γ-irradiation on properties of Na,K-ATPase in cardiac sarcolemma**

Lucia Mézešová, B. Kaločayová, V. Jendruchová, J. Vlkovičová, M. Barančík, M. Fulop, J. Slezák, P. Babál, P. Janega, N. Vrbjar
Heart Research Institute, Slovak Academy of Science, Slovakia

P2.22 **Regulatory proteins of myocardium in evaluation of cardiotoxicity**

Michaela Adamcova, O. Popelova-Lenčova, E. Jirkovsky, Y. Mazurova, V. Geršl, M. Štěrba
Department of Physiology, Faculty of Medicine in Hradec Kralove, Charles University in Prague, Czech Republic

P2.24 **Investigation of metabolic processes in cultured melanoma cell lines**

Mónika Gönczi, Zs. Nagy, D. Nagy, P. Bai, L. Csernoch
Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

P2.26 **Spatiotemporal analysis of miR-17 and miR-21 during murine kidney ischemia-reperfusion injury**

Tamás Kaucsár, J. Lorenzen, Cs. Révész, M. Godó, C. Schauerte, M. Albert, T. Krenács, G. Szénási, T. Thum, P. Hamar
Institute of Pathophysiology, Semmelweis University, Budapest, Hungary

P2.28 **New synthesized phosphazenes containing chalcone on human prostate cancer cell lines: An in vitro study**

Suat Tekin, K. Koran, F. Ozen, S. Sandal, E. Cil, A.O. Gorgulu
Inonu University, Faculty of Medicine, Department of Physiology, Malatya, Turkey

P2.30

Investigation of thefate of type I angiotensin receptor after biased activation

Gyöngyi Szakadáti, A. Balla, L. Hunyady
Department of Physiology, Semmelweis University, Budapest, Hungary

P3 **Skeletal, Smooth and Cardiac Muscle Physiology**
Chair: **János Magyar,** Hungary

P3.2 **Vascular smooth muscle cell functional network. The difficulties and usefulness of graphic representation**
Angela Madalina Lázár
University of Medicine and Pharmacy Carol Davila, Department of Physiology, Bucharest, Romania

P3.4 **Morphological and molecular changes after application of ionizing radiation on the rat myocardium**
Branislav Kura, Cs. Viczenczová, K. Frimmel, T. Ravingerová, N. Tribulová, Ľ. Okruhlicová, A. Lazou, R.C. Kukreja, M. Fulop, J. Slezák
Heart Research Institute, Slovak Academy of Sciences, Slovakia

P3.6 **Beneficial effects of Allium ursinum herbal extract on hyperthrophic hearts**
Daniel Priksz, M. Bombicz, A. Kertész, B. Varga, R. Gesztelyi, K. Pák, Á. Tósaki, B. Juhász
University of Debrecen, Department of Pharmacodynamics, Debrecen, Hungary

P3.8 **Contribution of carbon monoxide on vascular tonus in different vascular beds and segments. A descriptive study**
Günnur Koçer, S. Ülker, Ü.K. Şentürk
Near East University, Lefkoşa, North Cyprus

P3.10 **Expession and estrogen-dependent up-regulation of Transient Receptor Potential Ankyrin 1 (TRPA1) and Vanilloid 1 (TRPV1) ion channels in the rat endometrium**
Krisztina Pohóczky, J. Kun, B. Szalontai, K. Kovács, J. Garai, A. Garami, A. Perkecz, Zs. Helyes
University of Pécs, Medical School, Department of Pharmacology and Pharmacotherapy, Pécs, Hungary

P3.12 **Exogenous nicotinamide adenine dinucleotide (NAD+): effects and mechanisms of action on the mammalian heart**
Ksenia B. Poustovit, V.S. Kuzmin, D.V.Abramochkin
Biological Separtment of Moscow State University, Moskow, Russia

P3.14 **Nicotinic acetylcholine receptors containing the ?7-like subunit mediate contractions of muscles responsible for space positioning of the snail tentacle**
Nóra Krajcs, Zs. Pirger, L. Hernádi, T. Kiss
Balaton Limnological Institute Centre for Ecological Research MTA, Tihany, Hungary

P3.16 **C hronic L-DOPA administration decreases relaxation responses of corpus cavernosum tissue of rabbit**
Şeniz Sırma Yıldırım, G.S. Ozturk Fincan, F. Isli, S. Ercan, Y. Sarioglu
Kırıkkale University Medical Faculty, Department of Medical Pharmacology, Kırıkkale, Turkey

P4 **Cardiovascular Physiology**
Chair: **Violetta Kékesi**, Hungary

P4.2 **Remodelling of coronary artery network during quercetin supplementation**
Anna Monori-Kiss, G. Pásti, E. Monos, Gy.L. Nádasy
Semmelweis Universtiy, Institute of Human Physiology and Clinical Experimental Research, Budapest, Hungary

P4.4 **Consumer investigation and toxicological analysis of sour cherry seed kernel extract**
Attila Czompa, A. Nagy, I. Bak, Z. Hendrik, I. Lekli, Z. Csiki, A. Tosaki
University Debrecen, Faculty of Pharmacy, Department of Pharmacology, Debrecen, Hungary

P4.6 **Angiotensin converting enzyme-2 as biomarker of human hypertension and systolic heart failure**
Attila Tóth, K. Úri, M. Fagyas, I. Mányiné Siket, A. Kertész, Z. Csanádi, G. Sándorfi, M. Clemens, R. Fedor, Z. Papp, I. Édes, E. Lizanecz
Division of Clinical Physiology, Institute of Cardiology, University of Debrecen, Debrecen, Hungary

P4.8 **Beta-adrenergic stimulation reverses the IKr-IKs dominant pattern during the cardiac action potential**
Balazs Horvath, T. Banyasz, Z. Jian, L.T. Izu, Y. Chen-Izu
University of Debrecen, Faculty of Medicine, Department of Physiology, Debrecen, Hungary

P4.10 **Two sides of the same coin: integrative role of the calcium-activated chloride channels in the ventricular myocardium**
Bence Hegyi, K. Váczi, F. Ruzsnavszky, K. Kistamás, M. Gönczi, B. Horváth, T. Bányász, J. Magyar, P.P. Nánási, N. Szentandrássy
Department of Physiology, University of Debrecen, Debrecen, Hungary

P4.12 **In vitro effect of apelin on contractions and endothelial-independent relaxation in the human internal mammary artery**
Emine Kacar, O. Burma, N. Ulker, A. Yardimci, A. Uysal, H. Kelestimur
Firat University, Faculty of Medicine, Department of Physiology, Elazig, Turkey

P4.14 **Vasoactive actions of lysophosphatidic acid**
Éva Ruisanchez, P. Dancs, M. Kerék, T. Németh, B. Faragó, R. Panta, A. Balogh, G. Tigyi, Z. Benyó
Semmelweis University, Institute of Human Physiology and Clinical Experimental Research, Budapest, Hungary
Chair: **Árpád Tósaki**, Hungary

P4.16 **Effects of Apelin-13 on arterial blood pressure in the epileptic male rats**
G. Gurol , Fatma Burcu Şeker, M. S. Ethemoglu, B. Yılmaz
Yeditepe University, Faculty of Medicine, Department of Physiology, Turkey

P4.18 **PPAR-gamma agonists treatment affected radical and cell signaling, antioxidant response and blood pressure of hypertensive rats**
Ima Dovinova, M. Kvandová, M. Barancik, M. Majzunova, L. Gresova, P. Bališ, L. Gajdosechova, S. Zorad

Institute of Normal and Pathol Physiol, Slovak Acad Sci, Bratislava, Slovakia

P4.20 **Protective effects of Quercetin from oxidative/nitrosative stress under intermittent hypobaric hypoxia exposure in rat heart**
Irina Camelia Chis, D. Baltaru, A. Dumitrovici, A. Coşeriu, B. C. Radu, R. Moldovan, A. Mureşan
"Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj Napoca, Romania

P4.22 **Dietary supplementation of zinc increases acethylcholine induced relaxations of isolated rat carotid arteries**
Ivan Ivic, A. Cavka, I. Grizelj, Z. Mihaljevic, Z. Loncaric, A. Koller, I. Drenjancevic
Medical School Pécs, Departmeent of Patophysiology and Gerontology, Pécs, Hungary

P4.24 **The effects of NMDA receptors modulation on cardiodynamic parameters in isolated rat heart**
Ivan Srejovic, V. Jakovljevic, V. Zivkovic, N. Barudzic, D. Djuric
Department of Physiology, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia

P4.26 **Shift in Na+/Ca2+ exchange balance modulates the inotropic consequences of the NCX inhibition**
Károly Acsai, K. Oravecz, A. Kormos, Z. Márton, J.Gy. Papp, A. Varró
Department of Pharmacology and Pharmacotherapy, University of Szeged, Szeged, Hungary

P4.28 **Autonomic function and cold-induced vasoconstriction in nicotine dependent young people**
Ludmila Gerasimova, T. Guseva, E. Uhanova , A. Fedosova
Petrozavodsk State University, Petrozavodsk, Russia

P4.30 **Innovation of education of cardiovascular physiology with respect to clinical practice**
Michaela Adamcova
Department of Physiology, Faculty of Medicine in Hradec Kralove, Charles University in Prague, Czech Republic
Chair: **Attila Tóth**, Hungary

P4.32 **Asynchronous activation of calcium and potassium currents by beta-adrenergic stimulation in mammalian ventricular myocardium**
Norbert Szentandrássy, B. Hegyi, K. Váczi, K. Kistamás, F. Ruzsnavszky, B. Horváth, T. Bányász, J. Magyar, P.P. Nánási
Department of Dental Physiology and Pharmacology, University of Debrecen, Debrecen, Hungary

P4.34 **Ellagic acid prevents cardiac fibrosis and attenuates high blood pressure in chronic nitric oxide-deficient hypertensive rats**
Parichat Prachaney, P. Boonprom , T. Berkban, P.Pakdeechote, J. Umka Welbat, V. Kukongviriyapan, U. Kukongviriyapan, P. Sretarugsa
Department of Anatomy, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

P4.36 **Combined modulation of IK, ATP and IKr to reduce reverse use dependency and repolarization heterogeneity**
Richárd Varga, T. Hornyik, Z. Husti, J.Gy. Papp, A. Varró, I. Baczkó
Department of Pharmacology and Pharmacotherapy, University of Szeged, Szeged, Hungary

P4.38 **Hemodynamic effects of Isatin on isolated perfused heart**
Selma Arzu Vardar, Z. Guksu, S.A. Vardar, O. Palabıyık, A. Karaca, E. Taştekin, N. Sut
Department of Physiology, Trakya University Medical Faculty, Edirne, Turkey

P4.40 **Evaluation of Urotensin-II and Urocortin as biomarkers of myocardial damage in an animal model of acute myocardial infarction**
Tarik Smani, I. Diaz, A. Dominguez-Rodriguez, E. Calderon-Sanchez, A. Ordoñez
Institute of Biomedicine of Seville, Spain

P4.42 **Impaired baroreflex-function is not related to deteriorated carotid elasticity in schizophrenic patients**
Viktor László Lakatos, B. Mersich, D. Cseh, A. Sárközi, M.Kollai, A. Pintér
Institute of Human Physiology and Clinical Experimental Research, Budapest, Hungary

P4.44 **Assessment of myocardial protection with new biochemical markers during on-pump coronary bypass surgery**
Z. Işık Solak Görmüş, M.C. Çiçek, H. Solak, N. Görmüş, S. Kutlu
University of Necmettin Erbakan, Meram Medical School, Department of Physiology, Konya, Turkey

P4.46 **Estrogens prevent impairment of Ca2+-sequestration and efficiently improve ischemia tolerance of the diabetic heart**
Zsuzsanna Miklós, P.Paragi, G. Dunay, L. Sára, T. Rátkai, K. Takács, N. Ács, T. Ivanics
Institute of Human Physiology and Clinical Experimental Research, Semmelweis University, Budapest, Hungary

P5 **Respiratory Physiology**
Chairs: **Ákos Zsembery,** Hungary

P5.2 **The endocannabinoid system of human bronchial epithelium**
Attila G. Szöllősi, N. Vasas, M. Szilasi, Á. Angyal, E. Lisztes, A. Oláh, T. Bíró
DE-MTA "Lendület" Cellular Physiology Research Group, Department of Physiology, University of Debrecen, Hungary

P5.4 **Effect of radical stress on NO production in rats exposed to chronic hypoxia**
Dana Mikova, O. Vajnerova, V. Hampl, J. Herget
Charles University in Prague, Czech Republic

P5.6 **Administration of exogenous surfactant: global and regional lung functional changes in a rabbit model of surfactant deficiency**
Ferenc Peták, L. Porra, W. Habre, G. Albu, I. Malaspinas, C. Doras, S. Bayat
University of Szeged, Department of Medical Physics and Informatics, Szeged, Hungary

P5.8 **Respiratory effects of acute blood loss and subsequent fluid resuscitation with colloid or crystalloid solutions**

Gergely H. Fodor, B. Babik, D. Czövek, C.Doras, S. Bayat, W.Habre, F. Peták
Department of Medical Physics and Informatics, University of Szeged, Szeged, Hungary

P5.10 **Predictive mouse model of chronic cigarette smoke-induced pulmonary and cardiac pathophysiological alterations**

István Szitter, R. Halmosi, L. Deres, K. Erős, Z.V. Varga, P. Bencsik, K. Kiss, P. Ferdinandy, Zs. Helyes
Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Pécs, Pécs, Hungary

P5.12 **Impact of altered ventilation pattern on capnography phase III slope in patients undergoing elective heart surgery**

József Tolnai, F. Peták, B. Babik
University of Szeged, Department of Medical Physics and Informatics, Szeged, Hungary

P5.14 **Lipopolysaccharide-induced fever elicits changes in lung surfactant proteins**

Maroš Kolomazník, I. Zila, J. Kopincova, D.Mokra, A. Calkovska
Department of Physiology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovakia

P5.16 **Changes of pro-inflammatory and apoptotic markers in an experimental model of acute lung injury Petra Košútová,**

D. Mokra, P. Mikolka, S. Balentova, H. Pistekova, L. Tomcikova, A. Calkovska
Department of physiology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovakia

P6 **Gastrointestinal Physiology**
Chair: **Péter Hegyi**, Hungary

P6.2 **Effect of chronic systemic Nesfatin-1 treatment in intestinal ischemia/reperfusion**

Ceylan Ayada, Ü. Toru, R. Akcılar, S. Şahin, G. Erken, H.A. Erken, G. Turgut, S. Turgut, O. Genç
Dumlupınar University, Medical Faculty, Department of Physiology, Kütahya, Turkey

P6.4 **The dynamic of non-invasive predictive markers for incipient experimental liver fibrosis**

Cristian Cezar Login, A. Muresan, A. Nagy, S. Clichici
Iuliu Hatieganu University of Medicine and Pharmacy Cluj-Napoca, Department of Physiology, Cluj-Napoca, Romania

P6.6 **Local poly(ADP-ribose)polymerase activation in children with Crohn's disease**

Eszter M. Horváth, N.J. Béres, K. Borka, G. Szabó, Sz. Heininger, R. Benkő, G. Veres
Semmelweis University, Institue of Human Physiology and Clinical Experimental Research, Budapest, Hungary

P6.8 **Hydrogen sulfide confers protection in TNBS induced colitis in rat: role of heme oxigenase**

Krisztina Kupai, Z. Szalai, M. Korsós, Z. Baláth, Sz. Török, R. Szabó, A. Csonka, L. Daruka, A. Pósa, Cs. Varga
Dept. of Physiology, Anatomy and Neuroscience, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

P6.10 **Effects of dietary fatty acids on gut microbiota and IRAP activity**

Magdalena Martinez Cañamero, A. B.Segarra, M. Hidalgo, A.B.Villarejo, M.Ramírez, I. Prieto
University of Jaén, Jaén, Spain

P6.12 **How to use PET/MRI to observe metabolic and cellular effects of portal vein ligation in healthy rat liver**

Mariann Semjéni3
CROmed Ltd, Hungary

P6.14 **Contribution of Capsaicin-sensitive sensory nerves and nitric oxide to the protective action of Orexin-A against ischemia/reperfusion-induced gastric mucosal injury in rats**

Ruken Tan, B.Gemici, V.N. İzgüt-Uysal
Near East University Faculity of Medicine Department of Physiology, Nicosia/TRNC, Turkey

P6.16 **Effects of Silymarin on the initiation and progression of liver fibrosis in CCl4-induced experimental model**

Simona Clichici, D. Olteanu, A. Nagy, F. Adriana, M. Petru
Physiology Department, UMF Cluj-Napoca, Romania

P6.18 **Transparent, true 3D qualitative and quantitative microscopic investigation of orofacial histological structures**

Zsolt Lohinai, I. Nagy, M. Gyurkovics, B. Keremi, E. Komarek, Cs. Korom, G. Varga, I. Stuber
Department of Conservative Dentistry, Semmelweis University, Budapest, Hungary

P6.19 **The protective effect of microemulsion of sour cherry (prunus cerasus) kernel extract on carbon tetrachloride -induced hepatotoxicity in mice**

Kalantari Heibatullah , M. Eisa, S. Anayatollah, R. Anahita, G. Mehdi
Department of Pharmacology and Toxicology and Nanotechnology Research Center, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, IR Iran

P7 **Renal Physiology**
Chair: **Péter Hamar**, Hungary

P7.1 **Influence of crowding stress on properties of renal Na,K-ATPase in young male normotensive and spontaneously hypertensive rats**

Barbora Kaločayová, L. Mézešová, V. Jendruchová, A. Púzserová, P. Bališ, I. Bernátová, N. Vrbjar
Institute of Heart Research, Slovak Academy of Science, Slovakia

P7.2 **Metformin prevents renal ischemia-reperfusion injury: a biochemical and histopathological evaluation of experimental model**

Branislava Medic, D. Jovičić, Z. Todorović, K. Savić-Vujović, R. Stojanović, M. Prostran
Department of Pharmacology, Clinical Pharmacology and Toxicology, Belgrade, Serbia

P7.3 **Less inflammation and oxidative damage is responsible for the resistance of Rowett rats against focal segmental glomerulosclerosis**
Csaba Imre Szalay, G. Kökény, K. Erdélyi, E. Lajtár, M. Godó, M. Sárközy, T. Kaucsár, T.B. Csont, T. Krenács, G. Szénási, P. Pacher, P. Hamar
Institute of Pathophysiology, Semmelweis University, Budapest, Hungary

P7.4 **Knockout of the Tau T gene predisposes C57 BL/6 mice to a ST2-induced diabetic nephropathy**
X. Han, AB Patters, I. J Azuma, SW Schaffer, Russell W. Chesney
University of Tennessee Health Science Center, Memphis, TN USA

P8 Physiology of the Immune System
Chair: **Zsuzsa Helyes**, Hungary

P8.2 **Characterization of extracellular vesicles produced during spontaneous death of neutrophilic granulocytes**
Ákos Márton Lőrincz, M. Schütte, Cs. Timár, E. Ligeti
Department of Physiology, Semmelweis University, Budapest, Hungary

P8.4 **Evidence for the involvement of galanin receptor 3 in an inflammatory arthritis model of the mouse**
Bálint Botz, M. Kovács, T. Németh, A. Mócsai, S. Brunner, B. Kofler, E. Pintér, Zs. Helyes
University of Pécs, Medical School, Dept. of Pharmacology and Pharmacotherapy, University of Pécs, Pécs, Hungary

P8.6 **Direct inhibition of complement c5a has long-term anti-inflammatory effects after partial aortic occlusion**
Dániel Érces, G. Varga, A. Mészáros, Sz. Szűcs, T. Fischer-Szatmári, C. Cao, H. Okada, N. Okada, J. Kaszaki, M. Boros
University of Szeged, Institute of Surgical Research, Szeged, Hungary

P8.8 **The effects of treatment with Simvastatin on liver ghrelin, HIF-1 alpha and trace elements in endotoxemic rats**
Elif Ozkok, H. Yorulmaz, G. Demir, İ. E. Yalcın, G. Ates, A.S. Tamer
Istanbul University, Deparment of Neuroscience, The Institute for Experimental Medicine, Istanbul, Turkey

P8.10 **Effects of 6-hydroxydopamine on fractal complexity of lymphocyte chromatin organization**
Igor Pantic
Institute of Medical Physiology, School of Medicine, University of Belgrade, Serbia

P8.12 **Correlation between fractal and grey level co-occurrence matrix parameters in nuclear structure of toluidine blue - stained thymus cortical lymphocytes**
Igor Pantic, M. Basailovic, J. Paunovic M . Basailovic, J. Paunovic
Institute of Medical Physiology, School of Medicine, University of Belgrade, Serbia

P8.14 **Role of hypoxia inducible factor in cytokine secretion responses to cadmium of rat alveolar macrophages in normoxic and hypoxic conditions**
Nuray Yazihan, F. Sahin, E. Akcil,M. Kacar
Ankara University, Faculty of Medicine, Pathophysiology, Ankara, Turkey

P8.16 **Muscle fatigue index and lactate level in sedentary young and elderly women**
D.C. Felício, D.S. Pereira, D.B. Coelho, B.Z. de Queiroz, J.M.D. Dias, E.S. Garcia, Ronaldo Luis Thomasini , L. S. M. Pereira
Institute of Sciences and Technology, Federal University of Jequitinhonha and Mucuri Valleys, Diamantina, Brazil

P8.18 **CARD9 mediates autoantibody-induced autoimmune diseas by linking the syk tyrosine kinase to chemokine production**
Tamás Németh, K. Futosi, J. Weisinger, K. Csorba, C. Sitaru, J. Ruland, A. Mocsai
Semmelweis University Heart and Vascular Center, Budapest, Hungary

P9 Endocrinology and Metabolism
Chair: **Tibor Bartha**, Hungary

P9.2 **Endocrine distruptor effect of Bisphenol A on the developing cerebellum, through estrogen and thyroid hormone receptor expression level changes**
Gergely Jócsák, V. Somogyi, I. Tóth, G. Goszleth, T. Bartha, A. Zsarnovszky
University of Szeged, Department of Physiology and Biochemistry, Szeged, Hungary

P9.4 **Evaluation of spatial learning and memory, level of serum cholesterol and tryglyceride in caloric restriction applied adolescent female rats**
Gülay Üzüm, Z. Kaptan
Istanbul University, Medical faculty of Istanbul, Dept of Physiology, Istanbul, Turkey

P9.6 **Effects of Quercetin on depression-like behavior**
Hasan Serdar Gergerlioglu, E.A. Demir, M. Oz Selcuk
University, Faculty of Medicine, Konya, Turkey

P9.8 **Exercise and milk-protein supplements: effects on skeletal muscle sirtuins in rats with elevated risk factors for metabolic disorders**
Heikki Kainulainen, S. Lensu, S. Pekkala, A. Mäkinen, J.J. Hulmi, A. Turpeinen, U.M. Kujala, L.G. Koch, S.L. Britton
University of Jyväskylä, Finland

P9.10 **Adipocytokines and inflammation as a link between obesity and related endothelial dysfunction**
Ivana Grizelj, A. Čavka, Z. Ivanović, A. Ćosić, S. Novak, M. Mihalj, I. Drenjančević
Department of Physiology and Immunology, Faculty of Medicine University of Osijek, Osijek, Croatia

P9.12 **Peripheral CCK-1 receptors in age-related regulatory alterations affecting energy balance**
Judit Tenk, E. Varga, T. Rimai, I. Rostás, Sz. Soós, M. Székely, E. Pétervári, M. Balaskó

University of Pécs, Medical School, Department of Pathophysiology and Gerontology, Pécs, Hungary

P9.14 **The effect of obestatin on corticosterone secretion and anxiety behaviour**
Júlia Szakács, K. Csabafi, N. Lipták, K. Bene, B. Kincses, Gy. Szabó
Department of Pathophysiology; Faculty of Medicine; University of Szeged; Hungary

P9.16 **A novel kisspeptin antagonist peptide 234 prevents kisspeptin-induced pubertaladvancement in the female rats**
Mete Ozcan, Z. Sahin, S. Canpolat, B. Yilmaz, H. Kelestimur
Firat University, Faculty of Medicine, Department of Biophysics, Elazig, Turkey

P9.18 **The effects of hyperbaric oxygen therapy (HBOT) on blood viscosity and erythrocyte aggregation in diabetic patients**
Nesrin Zeynep Ertan, M. Sinan, B. Mirasoglu, O. Yalcin, N. Atac, A.S. Toklu
Istanbul University, Istanbul Faculty of Medicine, Dept of Physiology, Turkey
Chair: **Zoltán Rakonczay**, Hungary

P9.20 **Exposure of pregnant rats to angiotensin 2 leads to an increase in blood pressure in their adult male offspring**
Pavel Svitok , L. Molčan, P. Štefánik, A.Vesela, M. Zeman
Department of Animale Physiology and Ethology, Faculty of Natural Sciences, Comenius University in Bratislava, Slovakia

P9.22 **Examination of macrophage migration inhibitory factor(MIF) and pituitary adenylate cyclase- activating polypeptide(PACAP) in human breast milk samples**
Réka Anna Vass, D. Reglodi, J. Garai, A. Kovacs, K. Csanaky, L. Santik, Zs. Helyes, I. Tarcai, A. Tamas
Department of Anatomy, PTE-MTA Lendulet PACAP Research Team, University of Pécs, Hungary

P9.24 **Effect of boron on spontaneous and oxytocin induced contractions in rat myometrium**
Selim Kutlu, M. Akgunlu, H. Solak, Z.I. Solak Gormus, H. Uysal, N. Ergene
Necmettin Erbakan University, Meram Faculty of Medicine, Department of Physiology, Konya, Turkey

P9.26 **Nesfatin-1 levels in response to the patients with different glucose tolerance levels**
Sermin Algul, Y. Ozkan, İ. Serhatlioglu, O. Ozcelik
Fırat University Faculty of Medicine Department of Physiology, Elazig, Turkey,

P9.28 **Can Apelin-13 be a new actor in control of obesity?**
Suat Tekin, Y. Erden, E. Etem, S. Sandal, C. Colak
Inonu University, Faculty of Medicine, Department of Physiology, Malatya, Turkey

P9.30 **Estimation of the relationships between irisin concentration and food intake, body weight and body temperature using polynomial regression models in the rats**
Suat Tekin , C. Colak, Y. Erden, S. Sandal

Department of Physiology, Faculty of Medicine, University of Inonu, Turkey

P9.32 **Effects of intracerebroventricular infusion of apelin-13 on the metabolism rate and energy expenditure**
Suleyman Sandal, Y. Erden, S. Tekin, E. Etem
Inonu University, Faculty of Medicine, Department of Physiology, Malatya, Turkey

P9.34 **Changes os C-AMP level during oestrus cycle in normotensive and sponataneous hypertensive rats**
Vaska Antevska, B. Dejanova, S. Petrovska, O.Nikodijevic
Institute of Physiology, Medical Faculty Skopje, Macedonia

P9.36 **Glutathione-S-Transferase induction effect of red mud contaminated food**
Zoltán Attila Godó, Cs. Révész, D. Kocsis
University of Debrecen, Faculty of Informatics – Department of Information Technology, Debrecen, Hungary

P10 **Neurophysiology**
Chair: **Gábor Czirják**, Hungary

P10.2 **Crosstalk between CB1 and TRPV1 receptors in primary sensory neurons**
Ágnes Jenes, A. Varga, L. Csernoch, I. Nagy
University of Debrecen, Hungary

P10.4 **A maternally induced thalamic neuropeptide mediates the effect of suckling to hypothalamic centers of maternal motivation and lactation**
Árpád Dobolyi, É.R. Szabó, I. Bodnár, A. Lékó, M. Palkovits, Gy.M. Nagy, T.B. Usdin, M. Cservenák
MTA-ELTE NAP Laboratory of Molecular and Systems Neurobiology, Hungarian Academy of Sciences and ELTE; Human Brain Tissue Bank and Laboratory of Neuromorphology, Semmelweis University, Budapest, Hungary

P10.6 **How does maternal smoking influence the early neurobehavioral development of rat pups?**
Barbara Mammel, T. Kvárik, P. Kiss, J. Gyarmati, T. Ertl, Zs. Szabó, D. Reglődi
Department of Anatomy, University of Pécs, Hungary

P10.8 **Investigating the retinoprotective effects of PACAP eye-drop in ischemic retinopathy**
Dóra Werling, T. Kvarik, R. Varga, N. Nagy, F. Mayer, A. Vaczy, D. Reglodi, P. Kiss, A.Tamas, Zs. Biro, G. Toth, T. Atlasz
University of Pecs, Dept of Anatomy, Pécs, Hungary

P10.10 **Induction of amylin in the preoptic area of lactating dams depends on TIP39-containing posterior thalamic neurons**
Éva Rebeka Szabó, M. Cservenák, E. Udvari, Á. Dobolyi
Semmelweis University, Department of Anatomy, Histology and Embryology, Budapest, Hungary

P10.12 **Analysis of connexin 26 expression in hearing loss**
J.G. Kiss , J. Jarabin, A. Kovacs, F. Otvos, Hanna Kozak, Cs. Vagvolgyi, V. Szuts, L. Rovo

University of Szeged, Institute of Biochemistry, Biological Research Centre of HAS, Szeged, Hungary

P10.14 **Polysulfide compound dimethyl trisulfide is analgesic in heat-injury-induced hyperalgesia in mice**
Gábor Pozsgai, E. Steen, E. Pintér
Department of Pharmacology and Pharmacotherapy, University of Pécs, Hungary

P10.16 **Caffeine improves MK-801-induced learning and memory deficits**
Gülay Üzüm, A.S. Diler, Y.Z. Ziylan
Istanbul University, Medical faculty of Istanbul, Dept of Physiology, Istanbul, Turkey
Chair: **Gyula Sáry**, Hungary

P10.18 **Complex functional attributes of glucose-monitoring neurons in medial orbitofrontal cortex and their homeostatic significance**
István Szabó, E. Hormay, B. Csetényi, B. Nagy, M. Bajnok Góré, Z. Karádi
Institute of Physiology, Medical School, University of Pécs, and University of Pécs Szentágothai Research Centre, Pécs, Hungary

P10.20 **Putting the fission illusion into a new context**
Júlia Simon, P. Csibri, G. Csifcsák, A. Bognár, Gy. Sáry
University of Szeged, BSc biologist (III.), Department of Physiology, Szeged, Hungary

P10.22 **Synergism between NMDA receptor antagonists ketamine and magnesium in lowering body temperature in rats**
Katarina Savić Vujović, Savić Vujović S. Vuckovic, A. Vujovic, B. Medic, D. Srebro, R. Stojanovic, N. Divac, M. Prostran
Department of Pharmacology, Clinical Pharmacology and Toxicology, Faculty of Medicine, University of Belgrade, Serbia

P10.24 **Acupuncture modifies neuronal activities in the nucleus reticularis lateralis in rats**
Kazuo Toda, J.L. Zeredo, K. Moritaka, H. Yamashita, K. Kaida, M.S. Ota, M.Kimoto
Nagasaki University, Nagasaki, Japan

P10.26 **The effect of kisspeptin on cocaine-evoked behavioral changes**
Krisztina Csabafi, J. Szakács, B. Kincses, K. Bene, Zs. Bagosi, Gy. Telegdy, Gy. Szabó
Department of Pathophysiology, University of Szeged, Szeged, Hungary

P10.28 **Inhibition of transient receptor potential ion channels by endogenous lipid mediators**
Maja Payrits, É. Sághy, É. Szőke, T. Bagoly, Zs. Helyes, J. Szolcsányi
University of Pécs Medical School Department of Pharmacology and Pharmacotherapy, Pécs, Hungary

P10.30 **In vivo imaging of brain after cerebral ischaemia using SPECT/CT in mice**
Mariann Semjéni
CROmed Ltd, Hungary

P10.32 **Median raphe can establishes glutamatergic synapses in the mouse forebrain**
Márton Mayer
Institute of Experimental Medicine of the Hungarian Academy of Sciences, Budapest, Hungary
Chair: **Csaba Fekete**, Hungary

P10.34 **Effects of resveratrol and resveratrol delivered in liposome carrier system on penicillin-induced brain epileptic activity in male rats**
Muhsine Sinem Ethemoğlu, I. Arslan, F. B. Şeker, N. Ekimci, G. Duman, B.Yılmaz, E. Kılıç
Yeditepe University, Medical School, Turkey

P10.36 **Synapse-specific distribution of neuroligin-2 in the hippocampus**
Panna Hegedüs
Institute of Experimental Medicine of the Hungarian Academy of Sciences, Budapest, Hungary

P10.38 **Pharmacological manipulations of striatal interneurons induce a phenotype of dystonia in the monkey**
D. Guehl, E. Cuny, F. Lafourcade, Pierre Burbaud
CHU de Bordeaux, France

P10.40 **The voltage-dependent anion-channel (VDAC) is dephosphorylated by beta-amyloid peptide. Involvement in AD mechanisms of toxicity**
Raquel Marín, C. Fernández, A. Canerina-Amaro, M. Díaz, I. Ferrer
Laboratory of Cellular Neurobiology, School of Medicine, La Laguna 38320, Tenerife, Spain

P10.42 **Cannabinoid agonists evoke Ca2+ transients in spinal astrocytes**
Tamás Oláh, Z. Hegyi, J. Vincze, K. Holló, M. Antal, L. Csernoch
University of Debrecen, Faculty of Medicine, Department of Physiology, Hungary

P10.44 **Effect of intracerebroventricular irisin injection on the uncoupling protein expression in the rat brain**
Suat Tekin, Y. Erden, E. Etem, A. Tektemur, S. Kirbag, S. Sandal
Inonu University, Faculty of Medicine, Department of Physiology, Malatya, Turkey

P10.46 **The role of hemokinin-1 and substance P in acute pain in mice**
Tímea Gubanyi, A. Hunyady
University of Pécs, Medical School, Pécs, Hungary

P10.48 **Temporal characteristics of binocular visual information processing, a VEP study**
Vanda Nemes, G. Horváth, D. Fülöp, G. Jandó
Department of Physiology, Medical School, University of Pécs, Hungary

P10.50 **Protective effect of rasagiline in aminoglycoside ototoxicity**
Viktória Humli, G. Polony, R. Andó, M. Aller, T. Horváth, J. Szepesy, A. Harnos, L. Tamás, E.S. Vizi, T. Zelles
Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary

P12 **From Cell Signalling to Bioenergetics and Cell Damage**
Chair: **Bíró Tamás,** Hungary

P12.2 **Effect of intestinal cold preservation in PACAP-38 containing solution**
Andrea Ferenc, Gy. Szabó, D. Csukás, L. Seres, D. Fehér, J. Sándor, D. Reglődi, G. Jancsó, K. Kovács, Gy. Wéber
Semmelweis University Department of Surgical Research and Techniques, Budapest, Hungary

P12.4 **Pharmacological protections against retinal injuries**
Balázs Varga, M. Bombicz, D. Priksz, A. M. Szabó, D. Varga, Á. Kemény-Beke, R. Gesztelyi, Á. Tósaki, B. Juhász
University of Debrecen, Faculty of Pharmacy, Department of Pharmacology, Debrecen, Hungary

P12.6 **Evaluation of the cytotoxic effect of Diphtheria toxin on human umblical vein endothelial cells**
Başak Varol, B. Özerman, E. Hacıosmanoğlu, M. Bektaş, R. Nurten
İstanbul Faculty of Medicine, Biophysics Department, Istambul, Turkey

P12.8 **The effects of Src-family kinase inhibitors on osteoclast development**
Dániel Csete, D. Győri, B. Tél, T. Vántus, Gy. Kéri, Cs. SzántaI-Kis, A. Mócsai
Department of Physiology, Semmelweis University School of Medicine & MTA-SE, Budapest, Hungary

P12.10 **Depletion of 14-3-3? reduces the surface expression of Transient Receptor Potential Melastatin 4 (TRPM4b) channels and attenuates TRPM4b-mediated glutamate-induced neuronal cell death**
Eunju Kim, Y.-S. Lee, J.-Y. Park, E.M. Hwang
Korea Institute of Science and Technology, Seoul, Korea

P12.12 **Effects of methane inhalation on rat liver mitochondria following partial hepatic ischemia**
Gerda Strifler, P. Hartmann, A. Mészáros, E. Kaszonyi, C. Cao, J. Kaszaki, M. Boros
University of Szeged, Institute of Surgical Research, Szeged, Hungary

P12.14 **Antioxidant effects and cytotoxicity of compounds of natural origin**
István Bak, E. Csepanyi, I. Lekli, A. Tosaki
University of Debrecen, Hungary

P12.16 **Anti oxidative effect of Ozone on spinal cord injury**
O. Genç, R. Akcılar, Ceylan Ayada, H. Şimşek, S. Şahin, A. Koçak
Dumlupınar University, Medical Faculty, Department of Physiology, Kütahya, Turkey

P12.18 **Effect of L-alpha glycerylphosphorylcholine on mitochondrial dysfunction and increased endogenous methane production caused by chronic whisky consumption**
Tünde Tőkés, E. Tuboly, R. Molnár, R.N. Turányi, M. Boros
Institute of Surgical Research, University of Szeged, Szeged, Hungary

P13 **Microcirculation**
Chair: **Ferenc Bari** , Hungary

P13.1 **Application of local heat provocation test to assess vascular reactivity on healthy and inflammed human gingiva**
Eszter Molnár, A. Demeter, Zs. Bata, H. Parkonen, Zs. Lohinai, Zs. Tóth, J. Vág
Semmelweis Univerity Department of Conservative Dentistry, Budapest, Hungary

P13.2 **Hyperthyroidism reversibly impacts skin microvascular reactivity**
Helena Lenasi, N. Bedernjak, S. Gaberšček, K. Zaletel
Institute of Physiology, Medical Faculty, University of Ljubljana, Slovenia

P13.3 **Multimodal action of 5'adenosine monophosphate-activated protein kinase (AMPK) in reducing vascular tone of resistance arteries: effects on calcium stores and membrane potential**
Holger Schneider , S. Blodow, K.-M. Schubert, S. Erdogmus, M.M.Schnitzler, T. Gudermann, U. Pohl
Walter Brendel Centre of Experimental Medicine, LMU Munich, Germany

P13.4 **NO-donating oximes relax corpora cavernosa through mechanisms other than those involved in arterial relaxation**
Johan Van de Voorde, B. Pauwels, C. Boydens, K. Decaluwé
Department of Pharmacology, Ghent University, Belgium

P13.5 **Mechanisms involved in resveratrol-induced relaxation of isolated mice corpora cavernosa**
Johan Van de Voorde, C. Boydens, B. Pauwels, K. Decaluwé
Department of Pharmacology, Ghent University, Belgium

P13.6 **Foxo1 subcellular dynamic and its impact on redox homeostasis in endothelial cells**
Omar Porras, J.P.Benitez
Universidad de Chile, Santiago, Chile

P13.7 **Effect of systemic medical Ozone application on oxidative parameters in intestinal ischemia- reperfusion**
O. Genç, Ceylan Ayada , Ü. Toru, R. Akcılar, S. Şahin, G. Erken, H.A. Erken, G. Turgut, S. Turgut
Dumlupınar University, Medical Faculty, Department of Physiology, Kütahya, Turkey

P13.8 **Model-based assessment of blood substitute-induced vasoactivity and red blood cell aggregation**
Péter Mukli, István Portörő, Dario Caccia, Michele Perella, Luca Ronda, Andrea Mozzarelli, Andras Eke
Institute of Human Physiology and Clinical Experimental Research, Budapest, Hungary
Department of Biomedical Science and Technology, University of Milan, Italy
Department of Pharmacy, University of Parma, Italy

P14 **Circadian Rhythm**
Chair: **Krisztina Káldi**, Hungary

P14.1 **Effect of metabolic changes on the circadian clock**
Anita Szőke, K. Káldi, N. Gyöngyösi
Department of Physiology, Semmelweis University, Budapest, Hungary

P14.2 **Social jetlag negatively affects academic performance in medical students**
Krisztina Ella, R. Á. Haraszti, T. Roenneberg, K. Káldi
Department of Physiology, Semmelweis University, Budapest, Hungary

P14.3 **Effects of Apelin-13 administration on food and water intake in different photoperiod in male rats**
Sinan Canpolat, S. Saral, E. Ozcelik, M. Alkanat, Ö. Saral
University of Firat, Faculty of Medicine Deparment of Physiology, Elazig, Turkey

P14.4 **Role of the Transient Receptor Potential Ankyrin 1 (TRPA1) ion channel in the acute and chronic inflammatory pain models using gene-deficient mice**
Valeria Tékus, Á. Horváth, B. Botz, J. Szolcsányi, E. Pintér, Zs. Helyes
Dept. of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, Hungary

P15 **Systems Biology**
Chair: **Miklós Cserző**, Hungary

P15.1 **Insulin-like growth factor binding protein 3 in the brain of mother rats**
András Lékó, Á. Dobolyi
MTA-ELTE Laboratory of Molecular and Systems Neurobiology, Department of Anatomy, Histology and Embryology, Budapest, Hungary

P15.2 **Lifetsyle, hypertension and cancer- a modern reconsidering**
Angela Madalina Lázár
University of Medicine and Pharmacy Carol Davila, Department of Physiology, Bucharest, Romania

P15.3 **Alterations in gene expression patterns of atopic dermatitis patients-derived lesional and non-lesional keratinocytes**
Attila Oláh, N. Vasas, A. G. Szöllősi, E. Lisztes, Á. Angyal, R. Papp, R. Paus, T. Bíró
DE-MTA “Lendület” Cellular Physiology Research Group, Department of Physiology, University of Debrecen, Debrecen, Hungary

P15.4 **Screening of differentially expressed microRNAs in TNBS induced colitis in rat colon**
Csaba Varga, K. Kupai, Sz. Török, Z. Szalai, Z. Baráth, L. Nagy, L.G. Puskás, A. Pósa
Department of Physiology, Anatomy and Neuroscience, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

30 August, 2014

SZENT-GYÖRGYİ ALBERT ROOM

9:00 **PLENARY LECTURE**
Chair: **László Rosivall**, Hungary
Will it one day be possible to engineer a complete kidney? Problems and perspectives
Giuseppe Remuzzi, Italy

9:45 **BREAK**

10:15-12:15
S6-A **The Calcifying Vessel: New Genetic Findings for Future Patho-physiological Avenues**
Chairs: **Georges Lefthériotis,** France and **András Váradi**, Hungary

10:15 S6- A1 **Mechanisms of arterial calcification: Lessons learned from rare monogenic disorders**
Yvonne Nitschke
Department of General Pediatrics, Münster University Children's Hospital, Münster, Germany

10:40 S6- A2 **The role of ABCC6 in chronic and acute calcification, a tale of 3 diseases**
O. Le Saux, **Chris Brampton**
University of Hawaii, John A. Burns School of Medicine, Honolulu, HI

11:05 S6- A3 **Tissue-wide mineralization: What can we learn from the vasculature**
Olivier M. Vanakker
Center for Medical Genetics, Ghent University Hospital, Belgium

11:30 S6- A4 **Arterial calcifications and cardiovascular diseases: Clinical and therapeutic issues**
Georges Lefthériotis, Prunier, Kauffenstein, Omarjee, Abraham, Willoteau, Martin
Lab Vascular Invest - CHU Angers & UMR CNRS 6214 Inserm 1083, Angers, France

11:55 S6- A5 **Conformation correction therapy in arterial calcification disorders, PXE and GACI**
Viola Pomozi, C.N. Brampton, K. Fülöp, A. Apana, H. Gyergyák, N. Tőkési, O. Le Saux, A. Váradi
Institute of Enzymology, RCNS, Hungarian Academy of Sciences, Budapest, Hungary

12:15 **BREAK**

12:30 **PLENARY LECTURE**
Chair: **Alex Verkhratsky**, UK
Neutrophils: versatile cells of innate immunity
Erzsébet Ligeti , Hungary

13:15 **CLOSING CEREMONY**

HEVESY GYÖRGY ROOM

10:15-12:15

S6-B **Physiology of Interaction Between RAS, IRAP and Glucose Metabolism**
Chairs: **Stefan Zorad,** Slovakia and **Manuel Ramírez-Sánchez,** Spain

10:15 **OPENİNG REMARKS**
Stefan Zorad, Slovakia

10:20 S6-B1 **Does insulin-regulated aminopeptidase play a role in regulating glucose uptake in neurones?**
Siew Yeen Chai
Monash University, Clayton, Australia

10:50 S6-B2 **Presence, regulation and function of insulin-regulated aminopeptidase in macrophages**
Patrick Vanderheyden
Free University Brussels, Belgium

11:10 S6-B3 **Ex vivo assessment of tissue angiotensin metabolism. Focus on Ang I/Ang IV/IRAP axis in various kinds of fat tissue in rat model of obesity and insulin resistance**
Rafał Olszanecki, B. Bujak-Giżycka, M. Suski, L. Gajdosechova, K. Krskova, S. Zorad, R. Korbut
Jagiellonian University Medical College, Department of Pharmacology, Krakow, Poland

11:30 S6-B4 **Activity assays for proteolytic enzymes in complex biological samples - Technical aspects and novel methods for measuring angiotensinase and oxytocinase activities**
Marko Poglitsch
Attoquant Diagnostics, Vienna, Austria

11:45 S6-B5 **Effect of high fat diets on angiotensinase and IRA activities. Their role in blood pressure and glucose homeostasis control**
Isabel Prieto, A. B. Segarra, A.B. Villarejo, F.T. Pérez, L. Gajdosechova, M. Martínez-Cañamero, M.Ramírez
Jaén University, Jaén, Spain

12:00 S6-B6 **Chronic treatment of rats with oxytocin upregulates renin and (pro)renin receptor expression in kidney**
Katarina Krskova, L. Gajdosechova, S. Zorad, D. Jezova, R. Olszanecki
Institute of Experimental Endocrinology SAS, Bratislava, Slovakia

12:15 **BREAK**

BÉKÉSY GYÖRGY ROOM

10:15-12:15

S6-C **Basic Research Meets Clinical Endocrinology**
Chairs: **Károly Rácz,** Hungary and **Attila Patócs,** Hungary

10:15 S6-C1 **Organogenesis in a petridish - How to generate functional thyroid tissue from mouse embryonic stem cells**
Robert Opitz
IRIBHM, ULB Brussels, Belgium

10:40 S6-C2 **Central regulation of hypothalamic-pituitary-thyroid axis under physiological and pathophysiological conditions**
Csaba Fekete
Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary

11:00 S6-C3 **Regulation of cell cycle by microRNAs and its implication in the pathogenesis of endocrine tumors**
Attila Patócs Semmelweis University, Budapest and MTA-SE „Lendulet" Hereditary Endocrine Tumors Research Group, Hungarian Academy of Sciences, Hungary
MTA-SE „Lendulet" Hereditary Endocrine Tumors Research Group, Hungarian Academy of Sciences, Hungary

11:20 S6-C4 **The roll of microRNAs in rabbit preimplantation embryos and pluripotent stem cells**
Elen Gócza, P. Maraghechi, B.Bontovics, K. Németh, Zs. Bosze
NARIC, ABC, Gödöllő, Hungary

11:40 S6-C5 **Novel insight in the regulation of the brainrenin-angiotensin system and itsconnectionwith hypertension**
Y. Marc, J. Gao, F. Balavoine, M. Azizi, B. Roques, **Catherine Llorens-Cortès**
INSERM U1050, Collège de France, Paris, France

12:00 S6-C6 **Mutation of the palmitoylation site of Estrogen Receptor ER? in vivo reveals tissue-specific roles for membrane versus nuclear actions**
Francoise Lenfant, M. Adlanmerini, R. Solinhac, A. Abot, A. Fabre, I. Raymond-Letron, F. Boudou, C. Fontaine, A. Krust, P. Chambon, J. Katzenellenbogen, P. Gourdy, P. Shaul, D. Henrion, J-F. Arnal
INSERM U1048, I2MC, Toulouse, France

12:15 BREAK

S1-A

FEPS European Young Physiologists Symposium

S1-A1
Cell type-specific subcellular distribution of ion channels in the central nervous system

A. Lőrincz
Laboratory of Cellular Neurophysiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary

Neuronal excitability is underlined by active conductances generated by voltage-gated ion channels. The functional impact of a voltage-gated ion channel is highly affected by its precise location in the axo-somato-dendritic domains of nerve cells.

In this presentation, I will demonstrate that the organizing rules governing the subcellular distribution of voltage-gated Na+ (Nav) and K+ (Kv) channels are more complicated than previously believed.

In the axon initial segment (AIS) of neurons, high density of Nav channels ensure low threshold for action potential initiation and Kv channels are key regulators of action potential repolarization, firing rate and pattern. Highly sensitive immunohistochemical methods allowed us to reveal heterogeneity in the density and distribution of distinct Nav and Kv channel subunits within the AIS of different types of nerve cells. This precise arrangement is likely to contribute to the diversity of firing properties observed among central neurons.

In dendrites, active invasion of action potentials generated in the axon is essential for associative synaptic plasticity and neuronal ensemble formation. In pyramidal cells this action potential backpropagation is supported by dendritic Nav channels. Employing the high resolution SDS-digested freeze-fracture replica-labelling method allowed us to reveal quantitative differences in the subcellular distribution of the predominant Nav channels in the hippocampal CA1 pyramidal cells.

Our results suggest that variability in the subcellular distribution of ion channels is a way of increasing neuronal diversity in the central nervous system.

S1-A2
Rapid generation of human neurons for modeling neuropsychiatric disorders

T. Danko
Department of Molecular and Cellular Physiology, Institute for Stem Cell Biology and Regenerative Medicine, Department of Pathology, Stanford University School of Medicine, Stanford, USA

Previous studies demonstrated the availability of various methods for differentiating human embryonic stem cells (ESCs) and induced pluripotent cells (iPSCs) into induced neurons (iN). Alternatively, human fibroblasts can also be directly converted into induced neuronal (iN) cells. These studies also described that with present techniques conversion is inefficient, synapse formation is limited, and only small amounts of neurons can be generated. However, with the advent of patient- derived, disease-specific iPSC technology, there is an enormous potential in iN cells for studying the pathogenesis of various, poorly understood neurological disorders, such as autism, schizophrenia or Alzheimer's disease. Furthermore, this technology also enables researchers to design drug screening systems, and to produce neurons for purposes of regenerative medicine. This requires the capability of large-scale production of human iN cells and with a high yield, and also necessitates the generation of iN cells that readily form synapses. Moreover, such goals could be facilitated by a high degree of reproducibility independent of the starting cell line and by production of a relatively homogeneous population of functional iN cells for experimental purposes.

We performed lentivirus-mediated exogenous over-expression of the transcription factor neurogenin 2 (Ngn2) in human ESCs and iPSCs to derive induced neurons. Potential differences in neuronal maturation, morphology, synapse formation and synaptic function was then quantified by various techniques including electrophysiology.

We show that human ESCs and iPSCs can be converted into functional iN cells with nearly 100% yield and purity in less than 2 weeks by forced expression of a single transcription factor. The resulting ES-iN or iPS-iN cells exhibit quantitatively reproducible properties independent of the cell line of origin, form mature pre- and postsynaptic specializations, and integrate into existing synaptic networks when transplanted into mouse brain.

Our approach enables large-scale studies of human neurons for goals such as analyses of human diseases, examination of human-specific genes, and drug screening.

S1-A3
Descending effect on spinal nociception by amygdaloid glutamate varies with the submodality of noxious test stimulation

N. Bourbia, B. Sagalajev, A. Pertovaara
Institute of Biomedicine/Physiology, University of Helsinki, Helsinki, Finland

Amygdala has an important role in the processing of primary emotions, such as fear. Additionally, amygdala is involved in processing and modulation of pain. While the amygdala, particularly its central nucleus (CeA), has been shown to contribute to pain control, the descending pain regulation by the CeA is still only partly characterized. Here heat and mechanical nociception was tested in both hind limbs of healthy rats with a chronic guide cannula for microinjection of glutamate into the CeA of the left or right hemisphere.

The aim was to assess whether the descending pain regulatory effect by glutamate in the amygdala varies with the

submodality or the body side of nociceptive testing, brain hemisphere or the amygdaloid glutamate receptor. Motor performance was assessed with the Rotarod test. Amygdaloid glutamate, independent of the treated hemisphere, produced a dose-related heat and mechanical antinociception that varied with the submodality of testing. Heat antinociception was short lasting (minutes), bilateral and not reversed by blocking the amygdaloid NMDA receptor with MK-801. In contrast, mechanical antinociception lasted longer (>20 min), was predominantly contralateral and reversed by blocking the amygdaloid NMDA receptor. At an antinociceptive dose, amygdaloid glutamate failed to influence motor performance. The results indicate that independent of the brain hemisphere, the spatial extent and duration of the descending antinociceptive effect induced by amygdaloid glutamate varies with the amygdaloid glutamate receptor and the submodality of pain.

S1-A4
The effect of NOS inhibitors on radical signaling and antioxidant response in Wistar rats

M. Majzúnová[1], Z. Pakanová[2], P. Bališ[1], M. Drobná[1], I. Dovinová[1]
[1]Institute of Normal and Pathologigal Physiology Slovak Academy of Sciences, Bratislava, SR,
[2]Institute of Chemistry Slovak Academy of Sciences, Bratislava, SR

Despite of many experimental studies, the effect of NOS inhibition on activation of radical pathway (AT1R-NADPH oxidase-superoxide) is not yet sufficiently clarified. In our study, we used two inhibitors of NO-synthase, specific inhibitor of nNOS: 7-nitroindazole (7-NI) and nonspecific inhibitor of NOS: NG-nitro-L-arginine-methyl ester (L-NAME). The aim of our study was to determine changes in free radical signaling, antioxidant and detoxification response in left ventricular of heart (LV) and brainstem (BS) of young and adult Wistar rats (WR) during NO-deficiency.

Young (4 weeks) and adult (10 weeks) WR were treated with 7-NI (10 mg/kg/day), L-NAME (50 mg/kg/day) or drinking water (Control) during 6 weeks. Systolic blood pressure was measured by non-invasive plethysmography. Level of superoxide was determined by Lucigenin-enhanced chemiluminescence. Expression of genes (AT1R, p22phox subunit of NADPH oxidase, SOD and NOS isoforms, HO-1, MDR1a and housekeeper GAPDH) were identified by real-time PCR. Activity of NOS was detected by conversion of [3H]-L-arginine on [3H]-L-citrulline and activity of SOD was measured by UV VIS spectroscopy.

Blood pressure was increased after L-NAME treatment in young and adult WR. Activity of NOS was decreased only after L-NAME application (young and adult rats) without changes in eNOS and nNOS mRNA. Both inhibitors decreased superoxide level in LV of young animals without changes in AT1R or p22phox mRNA expression, while in adult rats only L-NAME affected AT1R and p22phox mRNA elevation in BS. Increased SOD activity positively correlated with SOD3 and HO-1 mRNA elevation only in this group, where we found also MDR1a mRNA elevation. Increase in MDR1a was observed also in young rats in different tissues and different inhibitors: in LV of L-NAME group and BS of 7-NI group.

We can conclude that radical signaling and antioxidant response was mainly influenced in L-NAME group and this response was more pronounced in LV of young rats and in BS of adult WR rats.

Supported by SAS-NSC JRP 2010/1, APVV-0348-12, Ministry of Health - 2012/51-SAV-1, SR.

S1-A5
Quantitative pilomotor axon-reflex test to assess autonomic dysfunction in Parkinson's disease

T. Siepmann[1,2], E. Frenz[2], W. Kirch[1], B. Min-Woo Illigens[1,3]
[1]Institute of Clinical Pharmacology, Carl Gustav Carus University Hospital, Dresden University of Technology, Dresden, Germany;
[2]Department of Neurology, Carl Gustav Carus University Hospital, Dresden University of Technology, Dresden, Germany;
[3]Department of Neurology, Beth Israel Deaconess Medical Center Harvard Medical School, Boston, Massachusetts, U.S.A.

BACKGROUND: Early detection of Parkinson's disease (PD) contributes to treatment success but is limited by the lack of techniques to assess early PD symptoms such as autonomic neuropathy. We recently demonstrated that peripheral autonomic nerve function can be assessed through iontophoresis of phenylephrine to elicit axon- reflex mediated piloerection in an indirect skin region outside the region of iontophoretic stimulation using the quantitative pilomotor axon-reflex test (QPART). (1) AIM. This study aimed to assess the hypothesis that QPART is a valid measure of autonomic pilomotor nerve dysfunction in early PD.

METHODS: Six healthy subjects and six age matched PD patients (Hoehn and Yahr stages I-II) participated in a controlled study. Piloerection was stimulated by iontophoresis of 1% phenylephrine on the dorsal forearm. Silicone impressions of resulting goose bumps were digitally quantified by number and area. Comparative automomic measures included heart rate variability (HRV)-analysis, cutaneous vasomotor function assessment using laser Doppler flowmetry, and cutaneous sudomotor function measurement using skin conductance resoponses.

RESULTS: In PD patients, the number of impressions in the indirect region was lower compared with healthy subjects (19.0±7.9 PD vs. 27.3±15.5 subjects, mean±SD, $p < 0.05$). Sudomotor function was attenuated in PD patients compared with healthy controls ($p < 0.01$) whereas we observed non-significant trends toward decreased HRV and vasomotor function in PD patients (p=n.s.).

CONCLUSIONS: This study demonstrates that axon-reflex pilomotor responses are impaired in early PD stages compared with healthy controls, consistent with impaired sudomotor function, suggesting that QPART is a valid test to detect early autonomic nerve dysfunction in PD. (1) Siepmann T, Gibbons CH, Illigens BM, et al. Quantitative pilomotor axon reflex test: a novel test of pilomotor function. Arch Neurol. 2012;69(11):1488-92.

S1-A6
Reprograming of liver metabolism by alternative p38-mediated modulation of neutrophil migration

G. Sabio
Assistant Professor: Stress kinases in Diabetes, Cancer and Cardiovascular Disease Department Vascular Biology and Inflammation Centro Nacional de Investigaciones Cardiovasculares Carlos III C/ Melchor Fernández Almagro, 3 28029 Madrid (Spain)

Nonalcoholic fatty liver disease (NAFLD) is a major health problem and the main cause of liver disease in Western countries. Although NAFLD is strongly associated with obesity and insulin resistance, its pathogenesis remains poorly understood. The disease begins with an excessive accumulation of triglycerides in the liver, which stimulates an inflammatory response. Alternative p38 mitogen-activated kinases (p38γ and p38δ), have been shown to contribute to inflammation in different diseases. Here we demonstrate that p38δ is elevated in livers of patients with NAFLD and that mice lacking p38γ/δ in myeloid cells are resistant to diet-induced fatty liver, hepatic triglyceride accumulation and glucose intolerance. This protective effect is due to defective migration of p38γ/δ-deficient neutrophils to the damaged liver. We further show that neutrophil infiltration in wild-type mice contributes to steatosis development by means of inflammation and liver metabolic changes. Therefore, p38γ and p38δ in myeloid cells provide a potential therapeutic target for NAFLD treatment.

S1-A7
A hypermuscular mouse model to study SOCE and muscle fatigue

M. Sztretye
University of Debrecen, Department of Physiology, Debrecen, Hungary

In our mouse model, a naturally occuring 12-bp deletion in the myostatin gene is considered responsible for the compact phenotype (MstnCmpt-dl1Abc) labeled by a tremendous increase in body weight along with signs of muscle weakness and easier fatiguability. To characterize and better understand the mechanism underlying these alterations, we monitored store-operated Ca2+-entry (SOCE) and also measured the cytosolic [Ca2+] (rhod2) using whole cell voltage clamp technique.
Since STIM1 and Orai1 endogenous protein levels in the mutant flexor digitorum brevis (FDB) muscles were reduced by ~30% we hypothesized that SOCE, a potential candidate to assist in the longer–term maintenance of EC coupling may be consequently altered. Enzymatically isolated fluo-8 AM loaded FDB fibers were used. To elicit a massive SR Ca2+-release a RyR1 agonist (4-chloro-meta-cresol, 4-CmC) was applied in a Ca2+ free medium and in the presence of the SR Ca2+ pump inhibitor (thapsigargin, TG). The above cocktail triggered a deep transient store depletion which in turn switched on SOCE. The peak of this slow Ca2+ transient was normalized to the peak of the SR Ca2+ release transient (0.63±0.09, n=18 vs 0.32±0.08*, n=11, p≤0.02).
The voltage dependence of the normalized fluorescence of the calcium transients in response to 100 ms long membrane depolarizations ranging between -60 and +30 mV, with 10 mV increments were not statistically different and were well fitted with a Boltzmann distribution (V0.5: -23.22±1.35 mV vs -24.15±0.77 mV with respective k values of 6.14±1.15 vs 6.93±0.65). To both elicit the release response and substantially deplete the SR of calcium and induce muscle fatigue, the evolution of [Ca2+]i during a train of eight long-lasting depolarizations (200 ms) to a maximally activating voltage (+30 mV) were monitored. The release flux parameters and the amount of Ca2+ released during these pulses were decreased in the mutant muscles.
Though myostatin deficiency is an unwanted condition by itself, understanding the underlying mechanisms may help developing new safe strategies to cure muscle wasting diseases and alleviate symptoms of muscle weakness.

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S1-A8
MiRNA-24 antagonism prevents renal ischemia reperfusion injury

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[2]Department of Nephrology, Hanover Medical School, Hanover, Germany
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[4]Department of Cardiology and Angiology, Hanover Medical School, Hanover, Germany
[5]Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences, and University Medical Center Utrecht, Utrecht, The Netherlands
[6]National Heart and Lung Institute, Imperial College London, UK

Ischemia-reperfusion (I/R) injury of the kidney is one of the major causes of acute kidney injury. MicroRNAs are powerful regulators of various diseases. We investigated the role of apoptosis-associated miR-24 in renal I/R-injury. MiR-24 is up-regulated in the kidney following I/R-injury of mice (4.7 fold, p <0.05) and in patients after kidney transplantation (2.1 fold, p <0.01). Cell sorting experiments revealed a specific miR-24 enrichment in renal endothelial (1.7 fold, p=0.08) and tubular epithelial cells (2.0 fold, p <0.01) after I/R-induction.
In vitro, anoxia/hypoxia induced an enrichment of miR-24 in endothelial (2.3 fold, p <0.001) and tubular epithelial cells (1.3 fold, p <0.05). Enrichment of miR-24 induced apoptosis, whereas it's silencing ameliorated apoptotic responses. MiR-24 effects were mediated through regulation of H2A histone family, member X as well as heme oxygenase 1, which were experimentally validated as direct miR-24 targets through luciferase reporter assays. In vivo, silencing of miR-24 in mice following I/R-injury resulted in a significant improvement of survival (p <0.05) and kidney function (p <0.01), a reduction of apoptosis (p <0.05), improved histological tubular epithelial injury (p <0.01) and less infiltration of inflammatory cells (p <0.01). In vitro, adenoviral overexpression of miR-24 targets lacking miR-24 binding sites along with miR-24 precursors rescued various functional parameters in endothelial and tubular

epithelial cells. HO-1 and H2A.X were also found to be regulated by miR-24 in vivo ($p < 0.01$ and $p = 0.08$, respectively). Therefore, miR-24 inhibition is a promising future therapeutic option in the treatment of patients with ischemic acute kidney injury.

S1-A9

Elevated adipose and liver IRAP/oxytocinase activity may account for increased oxytocin degradation in obesity. Effect of IRAP blockade on obese phenotype

L. Gajdosechova[1], K. Krskova[1], M. Suski[2], S.Y. Chai[3], R. Olszanecki[2], S. Zorad[1]
[1]Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia,
[2]Chair of Pharmacology, Jagiellonian University Medical College, Krakow, Poland,
[3]Department of Physiology, Monash University, Clayton, Victoria, Australia

Insulin regulated aminopeptidase (IRAP) has been shown to be identical molecule to oxytocinase, an enzyme playing a pivotal role in oxytocin degradation during pregnancy. The oxytocinase regulates circulating oxytocin levels and its biological activity in tissues by hydrolysis of the peptide. Since oxytocin treatment in rodents exerts anti-obesity effects, we aimed to determine whether obesity itself is accompanied by changes in plasma oxytocin and if this change is a result of altered oxytocin production or degradation.

Zucker fatty rats represent a well established model of obesity and insulin resistance. Obesity was accompanied by marked reduction in plasma oxytocin levels in these rats. Hypothalamic expression of oxytocin and genes involved in its regulation and secretion were not affected by the obese phenotype. To the contrary, we noticed elevated liver and adipose tissue IRAP/oxytocinase activity in Zucker fatty rats. These results indicate increased oxytocin degradation rather than altered oxytocin production in obesity. In addition, chronic IRAP/oxytocinase inhibition by benzopyrane derivate HFI-419 via osmotic minipumps improved glucose tolerance in obese Zucker rats without affecting body weight or adipose tisse mass.

In conclusion, our study highlights the importance of IRAP/oxytocinase in pathogenesis of obesity and suggests IRAP/oxytocinase inhibition as candidate approach in the therapy of metabolic disease.

S1-A10

The A+-helix of PCI, which is removed by testisin cleavage, is a cell penetrating peptide and responsible for internalization of PCI by Jurkat cells

H. Yang
Medical University of Vienna, Austria

Background: Human protein C Inhibitor (hPCI) is a multi-specific serpin, which is expressed in various tissues and present in many body fluids. In contrast, PCI expression in rodents is restricted to the reproductive tract. Both, human and mouse PCI (mPCI) can permeate through cell membranes and translocate to the nucleus. In testis PCI and the membrane anchored serine protease testisin are localized to similar sites. Male PCI-/- mice exhibit a disrupted blood-testis barrier, produce malformed sperm, and are infertile. Since deficiency of testisin impairs sperm maturation in epididymis and fertilizing ability, we investigated the interaction of PCI with testisin.
Methods: Western blotting and Edman degradation were used to detect and to identify the testisin cleavage sites of the PCI molecule. Recombinant wt mPCI and a truncated mPCI (lacking the first 18 amino acids, ΔR1-A18 mPCI), purified from E. coli system, were applied to investigate the effect of testisin-cleavage on the internalization of PCI by cells. Cell membrane penetration of peptides corresponding to the peptides released by testisin cleavage from the N-terminus of human and mouse PCI was studied.
Results: Both, human and mouse PCI were cleaved by testisin at the reactive site and at an additional site close to the N-terminus. This cleavage resulted in the release of a peptide rich in basic amino acids. Recombinant wt mPCI was internalized by Jurkat T cells and detected in nuclear fractions. Testisin-cleaved mPCI and ΔR1-A18 mPCI were not internalized. We could also demonstrate internalization of synthetic peptides corresponding to the N-terminal peptides of human and mouse PCI released by testisin cleavage.
Conclusion: Our data suggest that the A+-helix of PCI is a cell penetrating peptide, which mediates the internalization of PCI through cell membranes. Testisin or other proteases, which release this N-terminal peptide, may thereby regulate PCI internalization.

S1-A11

In vitro studies on the mechanisms involved in chemoprevention using *Calluna vulgaris* on vascular endothelial cells exposed to UVB

E.-D. Olteanu[1], A. Filip[1], S. Clichici[1], B. Ioana[1], P. Bolfa[2], C. Mihai[3], A. Muresan[1]
[1]Department of Physiology, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania,
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[3]The Department of Veterinary Reproduction, Obstetrics and Gynecology, USAMV Cluj-Napoca

Aim and objectives: Skin exposure to ultraviolet radiation B (UVB) induces changes in the vascular endothelial cells from the dermis, changes that are important for the homeostasis of the cutaneous circulatory and immune systems. Most of the photochemopreventive agents that have been studied up to now focused on protecting the keratinocytes and fibroblasts without taking into account the role of the chromophores, found in the blood vessels, in mediating the photoinduced inflammation and neoangiogenesis. This study aims to investigate the mechanisms involved in the in vitro effect of

UVB on endothelial vascular cells (HUVECs) pretreated with the Calluna vulgaris (Cv) extract.
Materials and methods: Two concentrations of Cv, below the limit of cytotoxicity IC50, were used (2,5 and 7,5 mEq/ml) and two doses of UVB (50 and 100 mJ/cm2). The generation of reactive oxygen species at 24 h after irradiation was determined by quantifying the malondialdehyde (MDA), the ratio between reduced and oxidised glutathione (GSH/GSSG) and by measuring the activity of glutathione peroxidase (GPx). The apoptosis was evaluated using flowcitometry, confocal microscopy (with annexin V and PI) and by determining the activity of caspase-3 (ELISA). The level of the transcription factor NF-kB and γ-H2AX was determined using western blot, and its intracellular localisation by confocal microscopy.
Results: UVB exposure led to the formation of lipid peroxides in a dose dependent manner ($p < 0.001$), induced apoptosis, increased the γ-H2AX levels and the activation of NF-κB. Pretreatment with 2.5 µg GAE/ml Cv improvesd the antioxidant defense, protected against DNA lesions and was able to decrease cellular death at low dose of irradiation. The dose of 7.5 µg GAE/ml Cv was prooxidant, favored the formation of DNA lesions, amplified the NF-κB activation UVB-induced ($p < 0.01$) and led to high levels of cellular death. Both doses of Cv inhibited caspase-3 activation. The modulatory effect of Cv extract on endothelial cells exposed to UVB depend on the concentration ofCv used.
This study provides insides into the mechanisms triggered by UVB and antioxidants on skin endothelial cells.

S1-B

History of European Physiological Sciences, Physiology - Origin of Prizes

S1-B1
Robert Tigerstedt, Alfred Nobel and The Prizes

O. Hänninen[1], F. Fyhrquist[2]
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As one the first professors of the Academy of Turku represented medicine the medical education started in Finland 1640. Only some 200 years later and after the transfer of the University to new capital Helsinki, also the experimental medical research started. Robert Tigerstedt (1852-1923) was one of those to open this path. After his dissertation on muscle function he had to move to Stockholm for better circumstances. There he stayed two decades (1881-1900), and became professor of physiology. There he also did one of his major findings i.e. on the humoral factor present in kidneys and the regulation of blood pressure. He reported this renal hypertensive factor which he named renin in 1898 in the international congress organized in Moscow. The renin studies started to ramify only three decades later. At present it is one of the current hot areas of circulation research from genes and peptides to antihypertensive drugs. In his days Robert Tigerstedt became a leading international figure of physiology. He participated in the First International Congress of Physiology in 1898 in Basel, Switzerland and was elected to serve as President of the next international one, which was never held due to the First World War. Robert Tigerstedt was invited in 1901 back to his home country and became professor of physiology in at the University of Helsinki. He started several areas of research in circulation, nutrition and medicine. He is thus also called the father of clinical research in Finland. He was the author of several books in physiology, handbooks included which were widely used in several countries. He also published the first textbook of physiology in Finnish. It was one of the texts of basic medicine in Finnish language. Robert Tigerstedt was a close friend of Alfred Nobel. After the death of Alfred Nobel, Tigerstedt served in the group to realize Nobel's will i.e. the creation the Nobel foundation and formulated the regulations of the greatest annual scientific prizes in several fields of science, Physiology or Medicine included. The first Nobel Prize in Physiology was given to Ivan Pavlov in 1904. This structure of Prizes has lasted now more than a century and expanded to new areas, like Peace. Alfred Nobel's own innovations covered explosives, dynamite and like and an immense fortune was collected by the Nobel Foundation and now annually used to promote human benefits. Ragnar Granit, also a Finn, active within one of Robert Tigerstedts' own research areas, electrophysiology, has obtained the Nobel Prize in Physiology for work on color vision.

S1-B2
From Ivan Pavlov to space Physiology

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Brain and Space. These two phenomena are associated in the history of Russian science by physiologist Ivan Pavlov (1849-1936) and first human ever been to space Yuri Gagarin (1934-1968). Ivan Pavlov was awarded the Nobel Prize for the year 1904 in Physiology or Medicine "in recognition of his work on the physiology of digestion…". However, I. Pavlov is famous and admired mostly for inventing his pioneering approach to study the central nervous system – the "conditioned reflexes" (CR, also known as "Pavlovian" or "classic" CR). This approach became an extremely popular tool due to its simplicity and reproducibility. Eventually, Pavlovian reflexes were accomplished by the instrumental CR. The dog drooling saliva ("Pavlovian dog") by ring became a powerful iconic cultural appearance of science, like Schrodinger's cat or Darvin's evolution. Pavlov's 30-year profound studies of higher nervous activities and enormous organizing activity motivated strong interest to neurophysiology in the Soviet Union for many years ahead. This, in turn, put firm basis to diverse applied studies in

physiology the Soviet Union, including space physiology set by Pavlov's disciples in 1940-1950s (Leon Orbeli, 1882-1958) and further by Vasilii Parin (1903-1971) and Oleg Gazenko (1918-2007). Space at that time was a novel and enigmatic habitat for the man. Pavlov's experiments on dogs allowed steady progress in training first dogs for space flight (Laika, Belka, Strelka, Veterok, Ugolyok), and, further to physiologically and psychologically prepare humans for microgravity. The Conditioned Reflexes are still in use in learning and memory studies. Due to high scientific standards set by Ivan Pavlov the Russian space biomedical program has ever been and still is successful. Thus, one can imagine a timely bridge between Pavlovian dog to cosmonaut dogs, between Pavlov and Gagarin, though they never met.

S1-B3
Female healers in early modern England: medical care for man and beast?

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Women played a major role in the provision of medical care in early modern England. They were generally the first, and often the only person, to provide medical care for humans. The good 'huswife' was also responsible for ensuring that her family enjoyed what we now call a 'healthy lifestyle'. It has long been thought, however, that females only looked after the health of very young or small animals such as poultry. While women might have been expected to care for cows in their dairy, the primary responsibility for horses and larger, more important animals lay with men.

As with most types of lay-healers, women would have obtained their medical knowledge both from the oral and the print tradition. In most cases, this probably included watching and working alongside their mother, or senior female in their household. It seems likely that both female and male laypeople utilized the same types of medical publications that practitioners did, in addition to acquiring information orally or through manuscript sources including recipe or household books or correspondence. This paper will discuss both the types of 'healing' roles women played in England during the sixteenth – eighteenth centuries, as well as the ways in which they learned the 'art of physick' (i.e. the contemporary term for medicine').

S1-B4
History of Dutch-Hungarian research platforms emerged from physiological sciences

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In the sixties of last century based on two university institutions aroused a notable Hungarian-Dutch scientific connection and cooperation within Europe. These institutes (may be honoured to name also as Schools) were the Physiological Institute in Pécs and the Rudolf Magnus Institute (RMI) in Utrecht headed by Kálmán Lissák and David deWied respectively. Around a dozen of young Hungarian physiologists spent academic research periods in RMI starting already in the seventies and joined the neuropeptide research projects which facilitated cooperation between brain physiology and neuropharmacology. A Hungarian scientist Béla Bohus should be recalled here as one of the tutors catalysing also this development. Next to neuropharmacology research on brain and behaviour and neuroendocrinology also developed and reached scientific accolade. From the early eighties the University of Groningen on one side and the Medical Universities in Szeged and Budapest (named after Albert Szent-Györgyi and Semmelweis respectively) joined in order to extend the scientific cooperations between the two countries. Areas of research activities in the last 30 years included physiology of learning and memory, the function of cholinergic brain, neurodegeneration and neuroprotection, brain development and aging, nutrition and brain metabolism just to name some of the most important fields may be recalled here. Based on brain research profiles a number of Hungarian PhD students finished their studies in Groningen and for years regular undergraduate courses are supported by joined efforts. For the future respectable jointed research areas have been emerged, mainly to pursue studies on healthy brain aging and on the pharmacological and non-pharmacological therapeutic aids supporting this research goal.

S1-B5
Hungarian heritage in physiological sciences

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Major historical milestones of teaching and research in physiological sciences will be highlighted, with emphasis on human physiology and pathophysiology in Hungary. Notable leaders of the field include among others Sámuel Rácz (1744-1807; author of the first physiology textbook in Hungarian), Mihály Lenhossék (1773-1840), Kálmán Balogh (1835-1888), Jenő Jendrássik (1824-1891), Endre Hőgyes (1847-1906), Ernő Jendrássik (1858-1921), Aladár Beznák (1901-1959), István Went (1899-1963), Frigyes Verzár (1886-1979), Kálmán Lissák (1908-1982), Albert Szent-Györgyi (1893-1986; Nobel Prize 1937), László Hársing (1920-1995), Arisztid GB Kovách (1920-1996), Péter Bálint (1911-1998), Szilárd Donhoffer (1902-1999), Sándor Juhász-Nagy (1924-2007). To them we owe achievements that inspire generations to develop and enrich further their legacy. These achievements encompass influential scientific schools, renowned institutions of basic medicine, the first association of Hungarian physiologists as early as in 1891, then the Hungarian Physiological Society in 1931 (Albert Szent-Györgyi as the first secretary), foundation of Acta Physiologica Hungarica in

1950, and espousing an integrative approach in education of medical physiology and pathophysiology. These fathers of the Hungarian heritage also established the close nexus between teaching and scientific research, and between progressive theories and practical training. They assured the availability and use of the best domestic and foreign textbooks, and forged extensive international relationships and cooperation. Recent testimony to this heritage is afforded by hosting in Budapest the 28th World Congress of International Union of Physiological Sciences (IUPS-1980), the 4th World Congress of International Society for Pathophysiology (ISP-2002), and the present Joint Meeting of the Federation of European Physiological Societies and the Hungarian Physiological Society (FEPS-2014).

S1-C

FEPS Teaching Physiology Symposium
Teaching Physiology in the Medical Curriculum: Traditional vs. Problem-based

S1-C1
Traditional medical curriculum in Semmelweis University

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Since reforms initiated by Flexner's report in 1910 the so-called traditional undergraduate curriculum implies that students should first acquire an understanding of the structure and function of the body before they would consider pathology and clinical medicine. The traditional curriculum for medical doctors at the Semmelweis University involves a 12-semester-long training period that covers at least 6,000 hours of teaching and it is divided into two parts. The first part consists of a two-year preclinical study period dealing with the basic or foundational sciences while the second part is focused on clinical studies, and lasts for four years. The internship period takes place during the 11th and 12th semesters, and is generally spent at University clinics or hospitals. Upon completion of the six-year programme students must submit and defend their written thesis and take a final written test and oral exam before an examination board. Having successfully passed all examinations, the student is granted the diploma and title M.D. (Medical Doctor).

Since 1910 several challenges and considerable amount of criticism have arisen against this information-oriented, traditional approach of teaching. These include the need for early clinical experience of the students, the problem of exploding scientific knowledge leading to information overload and the possibilities provided by new learning technologies such as e-learning, among others. It is important to realize that some of these problems are affecting not only the traditional teaching systems but medical education as a whole.

These challenges necessitate improvements and while the curriculum remains traditional in the sense of its structure, careful steps are being taken to answer the abovementioned pressing challenges in order to improve the quality of medical doctors graduating at the Semmelweis University.

S1-C2
Problem-based learning in the medical curriculum at Maastricht University

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From its start in 1974, problem-based learning (PBL) was the hallmark of the new medical curriculum at Maastricht University. PBL is characterized by active, student-centered learning in a multidisciplinary setting. Real-life patient problems are the core of the curriculum, from the beginning to the end. Through PBL, findings of cognitive psychology are applied to educational practice. PBL stimulates activation of prior knowledge, to which new information can be linked; this way, it drives constructive learning and encourages critical thinking and understanding, rather than memorization. In addition, it is assumed to increase retention of knowledge, improve the students' general problem-solving skills, foster the development of self-directed learning skills, make them effective collaborators and strengthen their intrinsic motivation to learn.

Many studies have been performed to investigate whether PBL really pays off in terms of learning outcomes. Overall, comparisons of the knowledge levels of Maastricht students showed at least similar results. The noncognitive, generic skills of our students appear to be relatively well-developed. The small scale character of the curriculum is probably one of the most important factors in this respect. Students have to work together in small groups, in which communication skills and professional behavior are evaluated continuously. In addition, students appear to become independent learners with problem-solving skills. At the same time, other factors can have negative effects on knowledge and skills acquisition in a PBL curriculum. The relevance of basic science knowledge for clinical reasoning needs to be stressed and protected by careful integration of clinical and basic knowledge throughout the curriculum. Also, the way in which knowledge and skills are assessed clearly influences the effectiveness of a PBL curriculum. A well-designed assessment program is needed to stimulate longitudinal development of all relevant competences.

In the presentation, the current Maastricht medical curriculum with its underlying concepts will be presented, and both positive and negative experiences will be addressed.

S1-C3
Physiology in the medical curriculum, is it really necessary?

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Traditionally many of the medical curricula start with courses focused on anatomy, physiology, biochemistry etc etc. Students spent many hours studying basic knowledge in these fields. This anatomical knowledge is considered to be important in the surgical professions whereas basic physiological or biochemistry knowledge is thought to be indispensible in for example internal medicine. However in modern medicine many doctors work according to professional protocols based on scientific research and the best available evidence. The question is raised whether every doctor still benefits from basic knowledge of the forementioned basic subjects or should the medical curricula be changed to more practical medicine and epidemiology.
This presentation adresses this question and wil discuss the pro's and con's of incorporating physiology in the medical curriculum.

S1-C4
Physiology teaching in the traditional curriculum of Semmelweis University

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The main goal of teaching physiology traditionally as a separate course in the medical curriculum is to give medical students a firm basic comprehensive knowledge to the understanding of pathological disorders and pharmacology before the introduction of clinical courses.
The traditional physiology course in Hungary has a two-semester schedule and follows a topic by topic approach where the students firstly learn the normal functioning of the different organ systems from the subcellular level to their complex operation and regulation. As a next step, special care is taken to highlight how the different basic physiological processes integrate into the complex functioning of the human organism.
Structurally, the course consists of lectures, seminars and lab practices. The lectures are aimed to give an up-to-date overview on the theoretical background by an expert of the topic. The lecturer guides the students through the topic by synthetizing and supplementing the available textbooks, emphasizing the essence and setting the physiological facts into a clinical context. Seminars are given to smaller groups (45-50 students) where the teachers conduct an interactive discussion on the acquired material. The fundamental purpose of lab practices is to deepen the theoretical knowledge using a practical approach in small (15-18) groups. Besides, lab experiments are designed to develop several skills including cooperativity, manual skills and accuracy. During labs the students perform simple physiological measurements on each other, carry out short animal experiments either on living preparations or in computer simulated laboratories and discuss clinical case reports.
Each semester is completed by exams. However, students are encouraged to study continuously during the semester and their progress is checked by weekly tests. The results of these tests are incorporated into the exam grades.
In summary, the traditional physiology course relies on a repetition based knowledge transfer. Versatility in forms of communication and approaches to the subject maintains the curiosity and motivation of students. Thereby they attain firm understanding of human physiology.

S1-C5
Physiology teaching is a fully integrated Medical PBL curriculum in Maastricht

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The Medical curriculum in of the Faculty of Health, Medicine and Life Sciences (FHML), University Maastricht, lasting 6 years, is divided in a 3-years bachelor and 3-years master program. The bachelor program consists of a fully integrated Problem-based Learning curriculum, the first two years characterized by working groups consisting of 10 students and one tutor, studying case histories from block books of selected subjects and occasional lectures (at maximum two per week). Practical medical skills can be learned in the so-called Skillslab. The third year is bridging the gap between the bachelor and master program: the students combine studying basic and clinical disciplines in four blocks with subjects such as "Heart, Circulation and Respiration" with real life experiences with patients seen by them in the "student policlinics". The master program consists of five clerkships (10 to 20 weeks in duration) in hospitals and/or practices of family doctors, two elective clinical clerkships of 10 weeks and one "Scientific Research Participation" and one "Health Care Participation" each of 18 weeks.

As Physiology is considered a key discipline in the Medical curriculum, physiologists participate in the curriculum at almost all levels. The department supplies members to the Organization committees of most of the individual blocks (taking care of constructing case histories seducing the students to study Physiology and Pathophysiology, providing multiple choice questions for the integrated block examination, etc), tutors to the tutorial groups and lecturers in the first two years. The participation in the third year of the bachelor program consists of providing interactive lectures, mentoring student groups, etc. During the master program, one day each week or each two weeks all master students return to the FHML in Maastricht and are following in groups of 14 to 16 students a teaching program organized by the basic disciplines varying from psychology to biophysics. Members of the Dept of Physiology are playing an important role at these so-called "home coming days". They provide interactive lectures on subjects, relevant for the student's stay in the clinic, such as "Disturbances in acid-base balance in patients" and guide discussion groups of students who are discussing the (Patho) physiological aspects of clinical cases. The

subjects to be discussed depend on the type of clerkship and the type of disease of the patients, seen by the students during the preceding weeks of their clerkship. Master students are entitled to participate in research programmes of the Dept of Physiology during the "Scientific Research Participating" block.

S2-A

Acta Review Symposium, Electrical Propagation in Smooth Muscle organs

S2-A1
Slow wave propagation in the stomach: Advances in experimental and modelling techniques

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Slow wave activity in the stomach is initiated and propagated by a network of interstitial cells of Cajal (ICC), located within the stomach musculature. Slow wave activity is responsible for coordinating the mechanical contractions of the stomach, necessary for the breakdown of ingested food. Degradation of the ICC network and the corresponding disruption of organised slow wave activity has been associated with functional motility disorders such as gastroparesis (delayed emptying of the stomach).

Cardiac electrophysiology leads the clinical use of extracellular measurements in the form of cutaneous ECG, catheter based measurements and more recently, non-contact endocardial mapping systems. In contrast, extracellular recordings from human gastrointestinal smooth muscle have had limited clinical impact and remain largely limited to research studies.

Until relatively recently, most experimental studies have used a limited number of electrodes (typically 4-8 electrodes). However, their limited coverage has meant that analysis techniques were primarily restricted to frequency dynamics and they were largely unable to accurately capture spatial dynamics. The introduction of high-resolution extracellular recording techniques (employing up to 250 electrodes at a 5 mm inter-electrode spacing) has accorded an improved understanding of the dynamic propagation patterns of slow wave activity. These techniques have subsequently translated to human studies resulting in the observation of a variety of complex spatial dysrhythmia patterns in patients with gastroparesis.

Advances in mathematical modelling techniques provide an alternative approach of interpreting and augmenting experimental data. The development of biophysically-based models capable of accurately simulating the electrical activity of ICC and smooth muscle cells have enabled investigations into the structure-function relationships of ICC networks and to investigate the response to different electrical stimulation protocols.

These recent advances in both experimental and modelling techniques now provide the potential to bridge the current gap between laboratory research and clinical application.

S2-A2
Normal and abnormal electrical propagation in the small intestine

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As in other muscular organs, small intestinal motility is determined to a large degree by the electrical activities that occur in the smooth muscle layers of the small intestine. In recent decades, the interstitial cells of Cajal, located in the myenteric plexus, have been shown to be responsible for the generation and propagation of the electrical impulse: the slow wave. It was also known that the slow waves as such do not cause contraction, but that the action potentials ('spikes') that are generated by the slow waves are responsible for the contractions.

Recording from large number of extracellular electrodes simultaneously is one method to determine origin and pattern of propagation of these electrical signals. This review reports the characteristics of slow wave propagation through the intestinal tube, the occurrence of propagation blocks along its length, which explains the well-known decrease in frequency, and the specific propagation pattern of the spikes that follow the slow waves.

But the value of high-resolution mapping is highest in discovering and analyzing mechanisms of arrhythmias in the gut. Most recently, circus movements (also called 'reentries') have been described in the small intestine in several species. Moreover, several types of reentries have now been described, some similar to what may occur in the heart, such as functional reentries, but others more unique to the small intestine, such as circumferential reentry. These findings, also discussed in this review, seem to suggest the possibilities of hitherto unknown pathologies that may be present in the small intestine.

S2-A3
Electrical propagation in the uterine muscle during pregnancy and labor

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The uterine muscle plays its most evident role during pregnancy, when quiescence is required for adequate nourishment and development of the fetus, and during labor, when forceful contractions are needed to expel the fetus and

the other products of conception. The uterine muscle is composed of smooth muscle cells. Contraction is initiated by the spontaneous generation of electrical activity at the cell level in the form of action potentials. The mechanisms underlying uterine quiescence during pregnancy and electrical activation during labor remain largely unknown; as a consequence, the clinical management of preterm contractions during pregnancy and inefficient uterine contractility during labor remains suboptimal. In an effort to improve clinical management of uterine contractions, research has focused on understanding the propagation properties of the electrical activity of the uterine muscle. Different perspectives have been undertaken, from animal and in vitro experiments up to clinical studies and dedicated methods for noninvasive parameter estimation. A comparison of the results is not straight forward due to the wide range of different approaches reported in the literature. However, previous studies unanimously reveal a unique complexity in the pattern of uterine electrical activity propagation which necessarily needs to be taken into consideration for future studies to be conclusive.

The aim of this review is to structure current variegated knowledge on the properties of the uterus in terms of pacemaker position, pattern, direction, and speed of the electrical activity during pregnancy and labor.

S2-A4
Electrical propagation in the renal pelvis, ureter and bladder

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Under normal conditions, following the passage of urine from the collecting duct, the urine get stored briefly in the renal pelvis before being transported to the bladder via the ureter. In the bladder, the urine gets stored for a longer time (hours) before being voided through the urethra.

The transport of urine from the renal pelvis to the bladder occurs spontaneously due to contractions of the muscles in the wall of the pelvis and ureter. Spontaneous contractions also occur in the detrusor muscle and are responsible for maintaining the bladder shape during the filling phase. These muscle contractions occur as result of electrical impulses, which are generated and propagated through different parts of the urinary tract. The renal pelvis and the ureter differ from the bladder in relation to the origin, characteristics and propagation of these electrical impulses. In the ureter, the electrical impulses originate mainly at the proximal region of the renal pelvis and are transmitted antegradely down the length of the ureter. The electrical impulses in the bladder, on the other hand, originate at any location in the bladder wall and can be transmitted in different directions with the axial direction being the prominent one. In this presentation, an overview of the current research on the origin and propagation characteristics of these electrical impulses in the normal and pathological conditions will be provided.

S2-A5
Electrical propagation in the various sites of the pulmonary veins of mammalian

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OBJECTIVES. Pulmonary veins (PVs) are characterized by unique properties – interposition of the two types of electrical excitable tissues: smooth and cardiac muscle layers. Some mammalians demonstrate tight interaction of the broad areas of extremely thin cardiac layer and smooth muscle in PVs "tree". However, electrical contact and functional interaction between these tissues remains questionable. The aim of the present study is to investigate conduction of excitation in various sites of pulmonary veins tree and to determine possible influence of "smooth muscle factors" on the conduction.

METHODS. Multicellular preparations of left atrium (LA) or PVs of left lung lobe were dissected from rabbits (male, 2.6-3.2 kg, n=8), rats (male, 200-250 g, n=20) and guinea pigs (GP, male, 250-300 g, n=5). Preparations were perfused in standard conditions with Tirode solution. Conduction of excitation was estimated with use of optical mapping technique (potential sensitive dye - di-4-ANEPPS, CCD camera - WuTech Instruments, software – RedShirtImaging). Conduction parameters were separately defined in LA, LA and PV junction region (PVJ), in PVs on the level of bifurcations (PVB), intrapulmonary region of PV (IP). Also, action potentials (APs) were recorded in all sites of preparations with applying of standard microelectrode technique.

RESULTS. Rat and GP demonstrates excitability and conduction of excitation in PVJ, PVB, IP (in rat) regions of PVs. Conduction velocity, anisotropy of conduction were approximately similar in all PV sites and similar to those of the LA. Rabbit characterized by only small PVJ zone, which conduct the excitation. Removing of endothelium did not affect conduction or APs in any sites of rat PVs. Also, NO-donor application was unable to evoke significant conduction alteration. APs alteration in rat PVs sites, after NO-donor application, was indistinguishable from LA.

CONCLUSIONS. Thin layer of myocardium in rat and GP PVs tree, which is adjacent to smooth muscle, reveal conduction similar to LA. Probably, smooth muscle does not interact with or, at least, unable to significantly affect the bioelectrical activity of cardiac tissue in PVs.

S2-A6

Effects of methane inhalation on the nitrergic myenteric neurons and intestinal myoelectrical activity during mesenteric ischemia-reperfusion in rats

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We have recently demonstrated anti-inflammatory effects for exogenous methane after intestinal ischemia-reperfusion (IR). Gases in the gut lumen may influence gastrointestinal motility, but the effect methane (CH4) inhalation on the IR-related increase of nitrergic myenteric neuronal density and on the intestinal myoelectric activity have not been investigated yet. Therefore, our aim was to follow the consequences of CH4 inhalation on the quantitative parameters of myenteric neurons and on the intestinal myoelectrical activity changes in a rat model of experimental IR.

For the study control, IR, and methane-treated IR groups of male Sprague-Dawley rats (300-350g) were used (n=8-8). Ischemia was induced by the occlusion of superior mesenteric artery for 50 min, inhalation of normoxic artificial air with or without 2.2% CH4 was started in the last 5 min of ischemia and maintained in the first 10 min of reperfusion. The myoelectric activity was monitored during IR and at the end of 120-min reperfusion, tissue samples were collected from the duodenum, ileum and colon for quantitative immunhistochemistry (HuC/HuD, nNOS and eNOS).

During ischemia the myoelectric activity increased, than decreased gradually until the end of the reperfusion period. The total number of myenteric neurons did not change, but the density of nitrergic neurons increased significantly exclusively in the duodenum at the end of reperfusion. CH4 inhalation resulted in an increase in myoelectric activity in the early stages of reperfusion and prevented the IR-induced increase in nitrergic neuronal numbers.

Based on these results we hypothesize that due to the increased density of nitrergic myenteric neurons in IR the descending inhibition of intestinal peristalsis was enhanced. Normoxic CH4 inhalation has significant biological effects by preventing the IR-related increase in nitrergic neuronal density and through changing of intestinal myoelectrical activity influences the intestinal motility.

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S3-A

Non-canonical Functions of the Endogenous Opioid Peptides

S3-A1

Pathophysiological consequences of endogenous opioids in injury and disease

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Endogenous opioid peptides are involved in a variety of normal physiologic functions. Alternatively, evidence suggests that, under pathophysiologic conditions, neuronal and glial-derived opioids cause inflammation and excitotoxicity leading to neuronal dysfunction, injury, and potentially death. Importantly, both normative and aberrant actions of opioids are highly contextual, differing among cell types, and even within subsets of the same cell type. Dynorphins, endogenous opioid neuropeptides derived from the prodynorphin gene, are exemplary in this regard. Dynorphins are well established as regulating a variety of normative physiologic functions via opioid receptors. Besides neurotransmission, at physiological levels, dynorphins can reduce microglial activation, enhance the survival of neurons, and promote oligodendroglial survival during maturation. By contrast, under pathophysiological conditions in which dynorphin levels are substantially elevated, or when the balance of prodynorphin biosynthetic peptide derivatives is disrupted, dynorphins can be excitotoxic. Although the mechanisms are incompletely understood, dynorphin excitotoxicity appears to be partially meditated through glutamate receptors, and possibly via direct biophysical interactions with cell membranes. Increased prodynorphin gene expression and/or altered prodynorphin processing occurs pathophysiologically (e.g., with stress, drug abuse, neuropathic pain, neuroAIDS, and neurotrauma). In addition, spontaneous mutations in dynorphin enhance synaptodendritic damage and have been demonstrated to underlie the etiology of spinocerebellar ataxia 23. Because the excitotoxic actions of dynorphins occur at supraphysiological concentrations or prolonged tissue exposure, there may be little evolutionary pressure to ameliorate the maladaptive, "non-opioid" consequences of dynorphins. Emerging evidence suggests that a variety of CNS pathologies are accompanied by altered dynorphin biogenesis. Such alterations are likely maladaptive and contribute to neuroinflammation, neuronal injury, and the pathogenesis of disease.

S3-A2
Glutamate mimicking mutant Dynorphin A causes spinocerebellar ataxia type 23

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Spinocerebellar ataxia type 23 (SCA23) is caused by mutations in PDYN, which encodes the opioid neuropeptide precursor protein, prodynorphin (PDYN). PDYN is processed into the opioid peptides, alpha-neoendorphin, and dynorphin (Dyn) A and B peptides, which play a role in pain signaling and addiction via opioid-receptor mediated actions. However, pathologically elevated Dyn A can also elicit non-opioid neurodegenerative functions via N-methyl-D-aspartate (NMDA) receptor signaling, leading to neuronal cell loss. Until now, it was not known if Dyn A can induce glutamate excitotoxicity in the cerebellum and whether this would cause motor dysfunction. Here, we show that elevated Dyn A levels lead to altered expression of glutamate and NMDA signaling components causing Purkinje cell loss and motor dysfunction in a mouse model of spinocerebellar ataxia type 23. Heterozygous SCA23-mutant PDYN-R212W mice reproduced the clinical features of SCA23, with gait deficits at three months of age, progressive loss of motor coordination and balance, and Purkinje cell loss by the age of 12 months. This coincided with pathologically elevated mutant Dyn A levels in the cerebellum and altered expression of ionotropic and metabotropic glutamate receptors and glutamate transporters. These alterations indicate an attempt to reduce glutamate-induced toxicity by the Purkinje cells, elimination of climbing fiber- Purkinje cell synapses and a decrease in long term depression. These changes in glutamate signaling are most likely due to glutamate mimicking, mutant Dyn A.
In conclusion, the PDYN-R212W mouse is a novel model for spinocerebellar ataxia and our work suggests that the elevated mutant Dyn A levels are responsible for the initiation and progression of the disease. Furthermore, this model can aid in elucidating the molecular mechanism of, and developing a treatment for SCA23.

S3-A3
Opioid neuropeptides make pores in plasma membrane: possible mechanism of signal transduction

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Dynorphin opioid peptides exert their actions primarily by interacting with kappa-opioid receptors, but may also produce non-opioid-receptor-mediated effects causing neurodegeneration, chronic pain and neurological dysfunctions. We have found that dynorphins (Dyn A, Dyn B and Big Dyn) interact with cellular membranes. Using quantitative methods with single-molecule sensitivity, fluorescence correlation spectroscopy and patch-clamp electrophysiology, we demonstrate that Big Dyn and Dyn A accumulate in the plasma membrane and induce a continuum of transient increases in its ionic conductance, which is consistent with formation of pores that are too large to possess ion selectivity (~27 Å in mode diameter). The pore forming potency of dynorphins correlates with their arginine content. The observed pore formation is not cell-type-specific and cannot be blocked by the general opioid receptor antagonist naloxone. However, it is greatly attenuated by increased external Ca2+ or by a high positive potential inside the cell. The potency of dynorphins to induce membrane pores correlates with their toxic effects on microbial cultures, mammalian cells, and with neuropathological effects in whole animals. Like other peptides disrupting the plasma membrane, dynorphins have powerful antimicrobial effects. Direct interaction of dynorphins with the plasma membrane may provide exchange of metabolites, RNAs and peptides between neurons and the extracellular milieu through large dynorphin-induced pores, thus potentially representing an ancestral form of intercellular or protosynaptic signaling. This novel mechanism may explain the unique actions of dynorphins at synaptic and extrasynaptic sites, as well as their neuropathological properties and ability to act as anti-microbial agents.

S3-A4
The left-right neurohormonal regulation in the brain: Lateralized endogenous opioid system

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In the 1920-1940s Anna Di Giorgio, an Italian scientist discovered that a unilateral injury to the brain induces the formation of postural asymmetry of the hind limbs. The asymmetry retained long after spinal cord transection suggesting the side-specific neuroplastic / molecular changes in the spinal cord. Our analysis of the neurochemical mechanisms underlying the postural asymmetry fixation identified the endogenous opioid peptides as factors that selectively modulate the left- and right-hind limb motor reflexes. Thus, Met-enkephalin induced flexion of the left leg, while Leu-enkephalin facilitated flexor reflex of the right hind limb. These effects were apparently mediated through the lateralized opioid receptors. Further studies demonstrated that the lesion to the left or right hemisphere differentially affects the opioid peptide levels; thus the opioid peptide-expressing neuronal networks may selectively mediate effects of left and right brain injury on the lateralized brain / spinal cord functions. The mechanism of the differential neurohormonal regulation of the left and right brain hemispheres / spinal cord segments is proposed and will be discussed. Recent findings on lateralization of the opioid peptides in the human anterior cingulate cortex that may underlie lateralization of positive and negative emotions will be also presented.

S3-A5

Investigation of possible interactions of pain modification actions of endogenous enkephalinergic and noradrenergic systems in Zymosan-induced chronic inflammatory pain model

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Chronic inflammatory pain, a nociceptive process associated with tissue damage due to inflammation mediated by increased sensitivity of nociceptive-specific neurons, is a feature of a large number of painful conditions for which no satisfactory treatment has been available yet. The aim of this study was to assess the possible analgesic affects of opiorphin, an endogenous enkephalinase inhibitior, and noradrenalin, known to be involved in the intrinsic modulation of nociceptive transmission, on inflammatory pain in rats. Timing the delay for the first hind paw lift to a focused thermal radiant heat, in vivo nociceptive behavioral "plantar test" was used for assessing pain sensitivity of adult male Spraque Dawley rats. After obtaining control nociceptive latency values, chronic inflammatory pain was induced by intraplantar injection of zymosan (6 mg in 200 uL) and heat stimulated nociceptive tests were repeated. Pain threshold values were determined and analyzed by a pairwise comparison between vehicle and each opiorphin treated group using a Dunnett's t-test on the ranked data. Zymosan caused a persistent significant increase in pain sensitivity as measured 15-180 minutes after its intraplantar injection (P <0.05). Systemic administration of opiorphin (0.1-0.3 mg/kg) provided a dose dependent (P >0.05 for 0.1 mg/kg opiorphin while P <0.05 for 0.3 mg/kg i.p opiorfin at 30 and 40th min) inhibition of inflammatory pain. Combination of opiorphin with noradrenalin (1 mg/kg) provided a limited synergic action, and this effect was reversible by alpha-1 adrenergic blockage (prazosin 1 mg/kg). Results from this study provides some degree of overlap between endogenous enkephalinergic and noradrenergic analgesic modification which implicates potential for development of therapeutical strategies involving combined use of enkephalinase inhibitors and noradrenergic agonists for the management of persistent pain associated with inflammatory painful conditions. " tabindex="" maxlength="2200" onkeyup="charcheck()" style="height: 326px;width: 820px;" >0.05 for 0.1 mg/kg opiorphin while P <0.05 for 0.3 mg/kg i.p opiorfin at 30 and 40th min) inhibition of inflammatory pain. Combination of opiorphin with noradrenalin (1 mg/kg) provided a limited synergic action, and this effect was reversible by alpha-1 adrenergic blockage (prazosin 1 mg/kg). Results from this study provides some degree of overlap between endogenous enkephalinergic and noradrenergic analgesic modification which implicates potential for development of therapeutical strategies involving combined use of enkephalinase inhibitors and noradrenergic agonists for the management of persistent pain associated with inflammatory painful conditions. " tabindex="" maxlength="2200" onkeyup="charcheck()" style="height: 326px;width: 820px;" >Chronic inflammatory pain, a nociceptive process associated with tissue damage due to inflammation mediated by increased sensitivity of nociceptive-specific neurons, is a feature of a large number of painful conditions for which no satisfactory treatment has been available yet. The aim of this study was to assess the possible analgesic affects of opiorphin, an endogenous enkephalinase inhibitior, and noradrenalin, known to be involved in the intrinsic modulation of nociceptive transmission, on inflammatory pain in rats. Timing the delay for the first hind paw lift to a focused thermal radiant heat, in vivo nociceptive behavioral "plantar test" was used for assessing pain sensitivity of adult male Spraque Dawley rats. After obtaining control nociceptive latency values, chronic inflammatory pain was induced by intraplantar injection of zymosan (6 mg in 200 uL) and heat stimulated nociceptive tests were repeated. Pain threshold values were determined and analyzed by a pairwise comparison between vehicle and each opiorphin treated group using a Dunnett's t-test on the ranked data. Zymosan caused a persistent significant increase in pain sensitivity as measured 15-180 minutes after its intraplantar injection (P <0.05). Systemic administration of opiorphin (0.1-0.3 mg/kg) provided a dose dependent (P >0.05 for 0.1 mg/kg opiorphin while P <0.05 for 0.3 mg/kg i.p opiorfin at 30 and 40th min) inhibition of inflammatory pain. Combination of opiorphin with noradrenalin (1 mg/kg) provided a limited synergic action, and this effect was reversible by alpha-1 adrenergic blockage (prazosin 1 mg/kg). Results from this study provides some degree of overlap between endogenous enkephalinergic and noradrenergic analgesic modification which implicates potential for development of therapeutical strategies involving combined use of enkephalinase inhibitors and noradrenergic agonists for the management of persistent pain associated with inflammatory painful conditions.

S3-A6

Hemokinin-1 is a potent inflammatory and pro-nociceptive peptide in acute and chronic mouse arthritis models

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The Tac4 gene-derived hemokinin-1 (HK-1) is expressed in the nervous system and inflammatory cells. Despite several similarities to substance P (SP), it is suggested to have different binding site at the NK1 tachykinin receptor, distinct activation mechanism and signal transduction pathways, but a specific own target was also proposed. We described its non-NK1-dependent inflammatory actions in the joints, therefore, in this study, the role of HK-1 was examined in arthritis models of distinct mechanisms using C57Bl/6 wildtype (WT) and Tac4 gene-deleted (Tac4-/-) mice.
In acute mechanism models Complete Freund's adjuvant (CFA) or the protease-activated receptor 2 agonist mast cell tryptase (MCT) was injected into the knee joint. Edema and hyperalgesia were measured with digital micrometer and aesthesiometry after 2-24 or 2-6 hours, respectively. MCT-induced vasodilation was measured for 30 minutes by laser Speckle after topical administration. In the K/BxN serum-transfer chronic polyarthritis model, arthritogenic or control serum was injected i.p. on days 0 and 3. Paw volume was measured by plethysmometry, touch sensitivity by aesthesiometry, noxious heat threshold on hot plate, cold tolerance by paw withdrawal latency from 0°C water, joint function in the grid test and arthritis severity by weight loss during 2 weeks.
CFA and MCT evoked 10-15% knee joint edema and 30-35% mechanical hyperalgesia, which were significantly reduced in Tac4-/- mice compared to the WTs. MCT-induced acute synovial vasodilation was also remarkably decreased after 15 minutes in the Tac4-/- group. In the chronic immune-arthritis model 90% joint swelling and 40% mechanical hyperalgesia detected in the WTs were significantly smaller, and the thermonociceptive thresholds were higher in Tac4-/- mice.

Cold allodynia, joint function and weight loss were not different.
Hemokinin-1 increases inflammatory swelling and hyperalgesia in both acute and chronic arthritis models. Identification of its receptors and molecular mechanisms of action might open new perspectives for arthritis therapy.

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S2-B

Nutrition and Cardiovascular Health: New Perspectives in Prevention and Therapy

S2-B1
Homocysteine and homocysteine-thiolactone induce cardiac and vascular damage: interplay with oxygen consumption, oxidative stress, and gasotransmitters

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This study deals with the effects of homocysteine (Hcy) isoforms on cardiodynamics, cardiac oxygen consumption, oxidative stress, and vascular reactivity. Firstly, cardiodynamics and oxidative stress were estimated following administration of any homocysteine isoform, i.e. DL-Hcy, DL-Hcy TLHC or L-Hcy TLHC (all 10 microM) in isolated hearts of rats (Wistar, male, groups n =6, age 8 weeks, b.m. 180–200 g, coronary perfusion pressure 70 cmH2O). Oxidative stress markers were measured in coronary effluent spectrophotometrically through TBARS, nitrites (NO2-), superoxide anion (O2-), and hydrogen peroxide (H2O2) concentration. In the effects of DL-Hcy TLHC the role of gasotransmitters (NO, H2S or CO) were estimated, through administration of L-NAME (30 microM), DL-propargylglycine (10 microM) or ZnPPR-protoporphyrin IX (10 microM). Any Hcy isoform induced decrease of cardiac contractility as well as decrease of coronary flow. NO and H2S were involved in the effects of DL-Hcy TLHC but participation of CO was not registered. Regarding the effects of Hcy isoforms on oxidative stress markers, only L-Hcy TLHC significantly affected O2- release. Secondly, transduction mechanisms were estimated following the application of 100 microM Hcy on isolated rat femoral artery ring (RFA). Hcy induced contractile response of intact RFA, which was significantly increased after endothelial denudation, while notably decreased after incubation of 10 microM urapidil, 0.1 microM nifedipine or 10 microM indomethacin. The initial RFA contraction evoked by 1 microM phenylephrine was further increased by single addition of Hcy, which was not the case when 100 microM ouabain was preincubated. Thirdly, oxygen consumption of rat cardiac homogenate was measured before and after administration of any homocysteine isoform. DL-Hcy, DL-Hcy TLHC or L-Hcy TLHC (all 10 microM) caused decrease of O2 consumption rate. Also in the effects of DL-Hcy TLHC the influences of L-NAME, DL-PAG or ZnPPR IX (30, 10 or 10 microM, respectively) were tested. L-NAME or ZnPPR IX caused a higher decrease of O2 consumption rate; however DL-PAG attenuated the effect of DL-Hcy TLHC.

S2-B2
Epigenetic modulation of cardioprotection with plant compounds

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Early reperfusion of occluded coronary arteries leads to additional myocardial injury for which effective therapy is a desirable achievement. Novel noninvasive strategies to reduce myocardial infarction size, to preserve cardiac function and to improve clinical outcomes in infarcted patients are still required. Over the past two decades, researchers have studied the ability of plant compounds to prevent or reverse the cardiac remodeling in translational models of myocardial infarction and heart failure. We have demonstrated that it is possible to increase the expression of cardioprotective factors (i.e.: VEGF, HGF, PIM-1) by inducing the acetylation of histone H4 with intramyocardial delivery of natural molecules (i.e: hyaluronan mixed ester of butyric and retinoic acid). Recently, we have also observed that lower dose of plant compounds with antioxidant property (i.e.: (1-3)beta-D-glucan) have the ability to promote angiogenesis in the presence of increased histone H4 acetylation, yet the cardiomyocyte survival is induced in an epigenetically independent manner. The same compounds have inhibitory effects on the cardiac cell viability and function at higher dose. The results from animal studies have been promising and suggest the pleiotropic effect of plant molecules to mediate cardioprotection in a dose-dependent manner. Although some mechanisms have been identified for the cardioprotective effect of selected plant compounds, there is a need for further research to identify the specific molecular mechanism of epigenetic cardioprotection of ischemic heart by bioactive compounds contained in foods of plant origin.

S2-B3
Dietary factors for favorable modulation of platelet function

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Thrombocyte activation and aggregation are crucial steps in the pathogenesis of atherothrombosis leading to severe complications, such as coronary artery disease, stroke and peripheral artery disease. The treatment of these clinical conditions is evidence-based, well established, antithrombotic agents are one of the basic drugs in therapy. A wide variety of pharmacological agents are available to inhibit thrombocyte aggregation, i.e. aspirin, ticlopidine, clopidogrel, prasugrel, etc., while others are under development. Secondary prevention of these diseases is also well described as published in the various guidelines. However, primary prevention of atherothrombosis is ambiguous. During drug prevention, the bleeding (and additional) risk should not exceed the benefit of drug use, and the decision between "to treat" and "not to treat" is not always easy. Thus when dealing with patients with a risk of atherothrombosis, it is important to consider non-pharmacological approaches that are effective and have a low side effect profile. Dietary plant polyphenols may have antithrombotic and therefore cardioprotective and vascular protective effects by interfering with platelet activation. They have been shown to inhibit platelet function in vitro and in vivo, in animal and in human studies. They also have been shown to improve endothel function.
The mechanism of actions seems to be complex. The major sources of polyphenols are cocoa, chocolate, wine, grape and berries. A significant antithrombotic effect is attributable to n-3 polyunsaturated fatty acid consumption as well. They exert their beneficial effects most probably via decreasing thromboxane A2 formation. Despite the demonstrated beneficial effects, at the moment the mentioned (and other) nutrients are not considered an accepted antiplatelet tool for clinical purpose in healthy or diseased individuals, however, it seems that they efficiently can be used in primary prevention in low- and moderate-risk patients or as an adjunct in secondary prevention in cardiovascular disease.

S2-B4
The use of dietary flaxseed to promote cardiovascular health

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Hypertension is an important silent killer strongly associated with the incidence of myocardial infarctions and stroke. Because of this, it is the leading global risk for burden of death in the world. The incidence of hypertension is rapidly increasing as well. Current medications used to control hypertension are costly, can induce unwanted side-effects and they are not always effective in controlling blood pressure (BP) in all hypertensive patients. Having a food that will control BP represents an alternative strategy that is more popular amongst patients than drugs. Flaxseed is enriched in the cardioprotective omega-3 fatty acid (alpha linolenic acid (ALA)), lignans and fiber. Peripheral arterial disease (PAD) is associated with hyperlipidemia and hypertension. The FlaxPAD Trial was initiated to determine if dietary supplementation with milled flaxseed in PAD patients could provide beneficial actions on a variety of cardiovascular disease parameters. The results of the trial will be reported here on the effects of dietary supplementation with milled flaxseed on BP regulation and circulating cholesterol levels in patients with PAD. The FlaxPAD Trial involved a clinical population with PAD who were randomized into a ground flaxseed or whole wheat placebo group. Individuals were required to consume 30g of the appropriate intervention which was incorporated into different foods daily. This FlaxPAD Trial was double-blinded and involved 110 patients. SBP was reduced by 10 mm Hg and DBP by 7 mm Hg in the flaxseed group relative to control following 6 months of intervention. Both total and LDL cholesterol levels were reduced by flaxseed by about 10-15%. The mechanisms for these effects will be discussed. We conclude that consuming ground flaxseed daily may offer a significant dietary strategy to lower both circulating cholesterol and BP.

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S2-B5
Interaction of clopidogrel and statins in secondary prevention after ischemic stroke

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AIMS. Variability in clopidogrel responsiveness is an emerging clinical problem in secondary prevention after ischemic stroke which has been postulated to be linked to competitive metabolization of cytochrome P450 (CYP) 3A4-oxidated statins such as simvastatin and clopidogrel. This study aimed to assess the hypothesis that simvastatin, in contrast to CYP 2C9-metabolized fluvastatin, reduced clopidogrel-mediated inhibition of platelet aggregation.
METHODS. A randomized, double-blind, double-dummy, crossover-study was performed in 13 patients with ischemic stroke (8 females, 5 males), ages 64.1±8.0 years (mean±SD). Following a 14-day run-in period during which all patients

received 75mg clopidogrel/die, patients additionally received either 20 mg simvastatin/die or 80 mg fluvastatin/die for 14 days. After a 14 day wash-out period, regimens were crossed over and switched regimens were continued for another 14 days. ADP-induced platelet aggregation, plasma concentrations of clopidogrel active metabolite (CAM) as well as routine laboratory parameters including prothrombin time (PT) Quick percent value were measured at baseline and after each treatment phase.

RESULTS. Clopidogrel reduced ADP-induced platelet aggregation in all patients as anticipated. Both, ADP-induced platelet aggregation and CAM plasma levels were unchanged when simvastatin or fluvastatin was added to treatment with clopidogrel. Simvastatin attenuated PT Quick percent value (reduction from 109±10.5% to 103±11%, $p < 0,05$) when co-administered with clopidogrel but was unchanged following administration of fluvastatin and clopidogrel.

CONCLUSIONS. This study indicates that administration of CYP 3A4-metabolized simvastatin does not jeopardize clopidogrel-mediated platelet inhibiton. In co-treatment of simvastatin and clopidogrel we observed attenuation of PT Quick percent value which could be explained by simvastatin-mediated reduction of prothrombin fragment 1+2 activity.

S2-B6

Effects of L-arginine, vitamin C and folic acid on coronary hemodynamics, oxidative stress markers and NO system in isolated rat heart

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The pathogenesis of all mechanisms endothelium-dependent coronary vasodilation in coronary blood vessels is still clearly not known. We examined whether supplementation with L-arginine, folic acid and vitamin C, with or without non-specific inhibition of nitirc oxide synthase by L-NAME, improve endothelium-dependent coronary vasodilation and its effects on oxidative stress markers in isolated rat hearts. The hearts of male Wistar albino rats (n=12, age 8 weeks, body mass 180-200g) were retrogradely perfused according to the Langendorff technique at gradually increased coronary perfusion pressure (CPP=40-120 cmH2O). Coronary flow and markers of oxidative stress: nitrite outflow, index of lipid peroxidation (TBARS) and superoxide anion production in coronary effluent were calculated. Study results suggest that L-arginine did not significantly improve coronary flow, and also did not significantly increase nitrite outflow, index of lipid peroxidation and superoxide anion production in isolated rat hearts. These effects were partially blocked by L-NAME. Intracoronary administration of vitamin C shows non-significantly improvement of coronary flow, nitrite outflow and decreasement of lipid peroxidation and superoxide anion production. These effects were partially blocked by L-NAME. The results also show that folic acid increased coronary flow, increased nitrite outflow, and index of lipid peroxidation and decreased superoxide anion production. These effects were blocked by L-NAME. Our study results demonstrated complicated mediation of many factors in coronary vasodilation. This confirms particapation of L-arginine, vitamin C and folic acid in NO mechanisms. This study is also suggesting less known direct vasodilatatory and antioxidative properties of folic acid.

S3-B

Signalling at Membrane Contact Sites

S3-B1

Coupling acidic organelles and the endoplasmic reticulum through Ca2+. A role for membrane contact sites?

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Cellular Ca2+ signals often derive from intracellular Ca2+ stores. By far the best characterized Ca2+ store is the endoplasmic reticulum. But acidic organelles such as lysosomes can also serve as mobilizable stores of Ca2+. These so called acidic Ca2+ stores are often tapped by the second messenger NAADP. Here I discuss the idea that Ca2+ released from acidic Ca2+ stores can trigger further Ca2+ release from the endoplasmic reticulum and that this process may involve a novel class of membrane contact sites between the two compartments.

S3-B2

Calcium and ROS signaling at the ER-mitochondrial interface

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Endoplasmic reticulum (ER) and mitochondria are functionally distinct with regard to membrane protein biogenesis and oxidative energy production, respectively, but cooperate in several essential cell functions, including lipid biosynthesis, cell signaling and organelle dynamics.

The interorganellar cooperation requires local communication that can occur at the strategically positioned and dynamic associations between ER and mitochondria. Recently, these

junctions have received attention because of their pivotal role in mediating calcium signal propagation to the mitochondria, which is important for both ATP production and mitochondrial cell death. Many of the ER-mitochondrial calcium transporters and signaling proteins are sensitive to redox changes and might be directly exposed to the reactive oxygen species (ROS) produced in the mitochondria and ER. Although ROS has been emerging as a novel signaling entity, the ER-mitochondrial redox signaling is yet to be elucidated.
We describe here a new fluorescence-based strategy to measure local calcium-ROS interactions at the ER-mitochondrial interface and provide some evidence for its relevance for the functions of mitochondria in both cell survival and death.

S3-B3
Broadband connections within the cell: How the mitochondria talk to the endomembrane?

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Mitochondria, like other organelles, need to communicate with neighboring subcellular compartments to exchange metabolites and to coordinate cellular function. This communication takes place, at least in part, at contact sites where the membranes of two organelles come to close apposition. These contact sites are organized and maintained by proteinaceous tethers. Contact sites between the mitochondria and the Endoplasmic Reticulum (ER) play major roles in lipid biosynthesis as they allow exchange of lipids between both organelles. This exchange provides lipids for the biosynthesis of both mitochondrial membranes and allows the distribution of phosphatidylethanolamine synthesized in mitochondria to the rest of the cell membranes. In yeast, one protein complex that establishes and maintain ER-mitochondria contact sites is the ER-Mitochondria Encounter Structure (ERMES), a multisubunit complex made of both ER and outer-mitochondrial membrane integral proteins. Although ERMES is involved in lipid exchange between the ER and mitochondria, its function appears to extend beyond that to mitochondrial DNA replication and protein import. How ERMES-mediated ER-mitochondria contact sites favor lipid exchange and how ERMES manages to coordinate all of its proposed functions is unknown. ERMES is regulated by the Miro GTPase Gem1, but the role of this regulation is unclear. Besides Gem1, all components of ERMES have been lost in metazoan, suggesting that the tethering role has been taken over by (an)other protein complex(es).

S3-B4
Investigating membrane contact sites between the endoplasmic reticulum and phagosomes

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Phagocytosis, the process through which immune cells engulf pathogens or foreign particles, is essential for an effective immune response. Endoplasmic reticulum (ER) membranes are recruited to phagosomes, but the role the ER plays in phagocytosis is not entirely understood. Recently, we showed that ER membranes are recruited to the vicinity of phagosomes for signaling purposes by STIM1, a key regulator of the ubiquitous store-operated Ca2+ entry pathway. Using a combination of fluorescence and electron microscopy in neutrophils from STIM1-deficient mice and in phagocytic fibroblasts lacking STIM1, we showed that STIM1 is recruited to phagosomes and helps generate membrane contact sites (MCS) between the ER and phagosomes. Our findings indicate that these MCS are required for high-efficiency phagocytosis by generating localized intracellular Ca2+ hotspots through the opening of phagosomal Ca2+ channels. Our current research efforts are focused on further dissecting the mechanisms that drive ER-phagosome MCS formation as well as their function, through both candidate as well as unbiased proteomic approaches. Our preliminary data identify novel molecules that comprise ER-phagosome contact sites and/or regulate their function as platforms that allow precise spatial localization of signaling events within the cell.

S3-B5
Differential impact of 5-phosphatase-mediated and phospholipase C-induced plasma membrane PtdIns(4,5)P2 depletion on G protein-coupled receptor endocytosis

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Phosphatidylinositol 4,5-bisphosphate (PtdInsP2), a minor lipid constituent of the plasma membrane, plays an important role in several cell functions including signal transduction and membrane trafficking. We have previously shown that depletion of PtdInsP2 by the acute plasma membrane recruitment of a 5-phosphatase (5-ptase) domain inhibits the endocytosis of various G protein-coupled receptors (GPCRs). In this study we compared the impact of this method on GPCR endocytosis with that of phospholipase C (PLC)-mediated PtdInsP2 depletion.
In our 5-ptase-based system we used the inducible heterodimerization of FKBP and FRB to translocate the PtdInsP2-degrading enzyme domain (FKBP-5-ptase) to the plasma membrane (PM-FRB); whereas wild type (wt) and internalization-incompetent mutant forms of the Gq protein-coupled type 1 angiotensin receptor (AT1R) were used for PLCβ-mediated PtdInsP2 depletion. We also created and tested AT1R-FRB fusion proteins that were capable of both 5-ptase recruitment and PLCβ activation. The rate of PtdInsP2 depletion was measured with the help of the PLCδ1 PH domain which binds PtdInsP2 specifically. We applied bioluminescence resonance energy transfer (BRET) between the luciferase-labeled β2 adrenergic receptor (β2AR) and the fluorescently tagged early endosome marker Rab5 as an indicator for receptor endocytosis in HEK-293T cells.
Confirming our previous results, β2AR internalization was inhibited after PtdInsP2 depletion by our 5-ptase-based system. A similar inhibition occurred after the activation of wt AT1R.

However, PtdInsP2 depletion by internalization-incompetent AT1R forms caused very little inhibition of β2AR internalization, despite the higher rate of lipid depletion compared to the wt receptor.
Our data suggest that stimulation of wt AT1R inhibits β2AR endocytosis, possibly through competition for the endocytic machinery. Using internalization-incompetent AT1R mutants we found that the effect of plasma membrane PtdInsP2-depletion on β2AR endocytosis depends on the method of lipid degradation suggesting the importance of local phosphoinositide pools in the regulation of receptor endocytosis.

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S3-B6
Glycine modulates membrane potential, cell volume, and phagocytosis in murine microglia

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Phagocytes form engulfment pseudopodia at the contact area with their target particle by a process resembling cell volume (CV) regulatory mechanisms. We evaluated whether the osmoregulatory active neutral amino acid glycine, which contributes to CV regulation via activation of sodium-dependent neutral amino acid transporters (SNATs) improves phagocytosis in isotonic and hypertonic conditions in the murine microglial cell line BV-2 and primary microglial cells (pMG). Phagocytosis of polystyrene microspheres was visualized by scanning electron microscopy (SEM). Gene expression was analyzed by reverse-transcriptase (RT)-PCR. Cell membrane potentials (Vmem) and Na+ currents were measured using the perforated patch clamp technique. CV was measured by flow cytometry. In BV-2 cells and pMG, RT-PCR analysis revealed expression of SNATs (Slc38a1, Slc38a2), but not of GlyRs (Glra1–4). In BV-2 cells, glycine (1 and 5 mM) led to a rapid Na+-dependent depolarization of Vmem. Furthermore, glycine (0.3, 1 and 5 mM) increased CV up to about 10%. Visualizing of phagocytosis of polystyrene microspheres revealed that glycine (1 mM) increased the number of BV-2 cells containing at least one microsphere by about 13%. Glycine-dependent increase in phagocytosis was suppressed by the SNAT inhibitor α-(methylamino)isobutyric acid, by replacing extracellular Na+ with choline, and under hypertonic conditions, but not by the GlyR antagonist strychnine or the GlyR agonist taurine. Interestingly, hypertonicity-induced suppression of phagocytosis was rescued by 1 mM glycine. From our findings we conclude that the neutral amino acid glycine increases phagocytosis in iso- and hypertonic conditions by activation of Na+-coupled neutral amino acid transporters (SNATs).

S2-C

New Therapeutical Targets in Acute Pancreatitis

S2-C1
Characterization of pancreatic acinar Ca2+ influx pathway leads to potential new therapy for pancreatitis

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In the exocrine pancreatic acinar cells, the physiological secretagogues evoke release of Ca2+ from internal stores, which in turn triggers opening of store-operated Ca2+ entry channels in the plasma membrane. The resulting repetitive short-lasting elevations (spikes) of the cytosolic Ca2+ concentration then activate fluid and enzyme secretion (1). Pathological stimulation of pancreatic acinar cells with bile acids or combinations of alcohol and fat induce massive intracellular Ca2+ release, followed by excessive entry of Ca2+ through the store-operated channels, resulting in a sustained elevation of the cytosolic Ca2+ concentration. This causes intracellular trypsin activation and necrotic cell death (1). There are probably several components of store-operated Ca2+ entry in these cells (1), but recent work indicates that the most important pathway is through classical Ca2+ release-activated Ca2+ (CRAC) channels, which are extremely Ca2+-selective (2). This conclusion, based on the detailed characterization of the Ca2+ influx pathway, gave rise to the hypothesis that specific blockade of CRAC channels could be used as a therapy against severe acute pancreatitis. The relatively selective CRAC channel blocker GSK7975A dramatically inhibited store-operated Ca2+ entry and therefore prevented the sustained elevation of the cytosolic Ca2+ concentration that normally follows emptying of intracellular stores. Most importantly, the CRAC channel blocker markedly inhibited the intracellular protease activation evoked by palmitoleic acid ethyl ester – a non-oxidative product of palmitoleic acid and ethanol, which is an important mediator of alcohol-related pancreatitis – as well as the resulting necrosis (2). These data (2) provide proof in principle that specific CRAC channel blockade can be the basis of a drug-based therapy of acute pancreatitis (3).

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S2-C2
How to target the inflammatory response in pancreatitis

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Premature intracellular protease activation is known to be the primary event in acute pancreatitis. However, severe acute pancreatitis is characterised by an early inflammatory immune response syndrome (SIRS) and a subsequent compensatory anti-inflammatory response syndrome (CARS) contributing to severity as much as protease activation does. CARS suppresses the immune system and facilitates nosocomial infections including infected pancreatic necrosis, one of the most feared complications of the disease. A number of attempts have been made to suppress the early systemic inflammatory response but even if these mechanisms have been found to be beneficial in animal models they failed in daily clinical practice. Since T-cells are known to balance immune response we have addressed the role of the Teff and Tregs in severe acute pancreatitis. In mice depleted for the co-inhibitory surface molecule CTLA-4 T-cell deregulation resulted in complete destruction of the pancreas within weeks displaying morphological features of chronic pancreatitis. When studying severe acute pancreatitis in either CTLA-4 depleted animals or animals depleted for Tregs we found that T-reg cells play a dominant role in controlling the early immune response (SIRS) in severe acute pancreatitis whereas a subsequent Th-1 activation is crucial for preventing nosocomial infections and to overcome CARS. Blockade of CTLA-4 after the onset of severe acute pancreatitis, at the peak of CTLA-4 expression, resulted in the activation of T-effector cells and helped to overcome CARS. It was beneficial with regard to survival and bacterial translocation. Thus, inhibition of the co-inhibitory surface molecule CTLA-4 represents a promising therapeutic strategy aimed at reversing the immune deficit during the most critical phase of pancreatitis.

S2-C3
Insulin protects pancreatic acinar cells from pancreatitis-inducing agents

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Acute pancreatitis is a serious and sometimes fatal inflammatory disease where the pancreas digests itself. The non-oxidative ethanol metabolites palmitoleic acid (POA) and POA-ethylester (POAEE), are reported to induce pancreatitis due to impaired mitochondrial metabolism, cytosolic Ca2+ ([Ca2+]i) overload and necrosis of pancreatic acinar cells. Metabolism and [Ca2+]i are linked critically by the ATP-driven plasma membrane Ca2+-ATPase (PMCA) important for maintaining low resting [Ca2+]i. Our previous study showed that insulin protected pancreatic acinar cells against hydrogen peroxide-induced cellular injury which mimics many of the features of pancreatitis. The aim of the current study was to test the protective effects of insulin on cellular injury induced by the pancreatitis-inducing agents, ethanol, POA and POAEE.
Rat pancreatic acinar cells were isolated by collagenase digestion and [Ca2+]i was measured by fura-2 imaging. An in situ [Ca2+]i clearance assay was used to assess PMCA activity. Magnesium green (MgGreen) and a luciferase-based ATP kit was used to assess celular ATP depletion. Propidium iodide fluorescence was used to assess acinar cell injury (necrosis).
Ethanol (100 mM) and POAEE (100 μM) induced a small but irreversible Ca2+ overload response but had no significant effect on PMCA activity. POA induced a concentration dependent ATP depletion leading to inhibition of PMCA activity and the consequent cytotoxic Ca2+ overload and necrotic cell death. Insulin pretreatment (100 nM for 30 minutes) prevented the POA-induced ATP depletion, inhibition of the PMCA, Ca2+ overload and necrotic cell death. Moreover, the insulin-mediated protection of the cytotoxic overload was partially prevented by the PI3K inhibitor, LY294002, suggesting that protection was due at least in part to the activation of the PI3K/Akt pathway.
These data provide the first evidence that insulin directly protects pancreatic acinar cell injury induced by bona fide pancreatitis-inducing agents, such as POA. This may have important therapeutic implications for the treatment of pancreatitis.

S2-C4
Inhibition of CFTR function is critical in the development of pancreatitis

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Acute pancreatitis is one the most common cause of hospitalization for non-malignant gastrointestinal diseases. The mortality of the disease is unacceptably high, moreover no specific pharmaceutical therapy is currently available. A major reason stated to account for the inability to develop effective treatments is that there are multiple pathobiologic pathways activated during pancreatitis making it difficult to choose molecular targets for therapeutic strategies. However recent advances highlighted the crucial role of pancreatic ductal epithelial cells in the pathogenesis of acute pancreatitis. This cell type secretes the HCO3- rich, alkaline pancreatic juice, which maintains the intraluminal pH and washes the digestive enzymes out from the ductal system playing an important role in the pancreatic physiology. The damage of the pancreatic HCO3- secretion can lead to pancreatic damage and to the development of acute pancreatitis, as described in the genetic disease cystic fibrosis. Recently we showed that the most common pancreatitis inducing factors (bile acids, ethanol and ethanol metabolites) and active trypsin impair the pacreatic ductal HCO3- secretion via the functional inhibition of CFTR Cl- channel. Moreover exposure of pancreatic ductal cells to ethanol decrease the cell surface expression of CFTR as well via decreased protein maturation and increased plasma

membrane turn over. We also showed that the autoactivation of trypsinogen is a pH dependent process, with increased activity in acidic environment, which means that HCO3- secretion prevents the untimely trypsinogen autoactivation. During the development of acute pancreatitis the intraductal pH drops due to the impaired ductal HCO3- secretion resulting in accelerated trypsinogen autoactivation, which will further inhibit HCO3- secretion. These observations highlight the central role of CFTR Cl- channel in the pancreatitis pathogenesis; therefore, correcting CFTR function should offer therapeutic benefit.

S2-C5
ER-PM junctions in pancreatic acinar and pancreatic cancer cells: from structure to function

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Junctions between the endoplasmic reticulum (ER) and the plasma membrane (PM) are important platforms for Ca2+ and cAMP signalling. In pancreatic acinar cells ER-PM junctions preferentially concentrate near the basolateral regions of the plasma membrane. Upon the reduction of Ca2+ concentration ([Ca2+]) in the ER lumen stromal interaction molecule 1 (STIM1), which serves as a [Ca2+] sensor in the ER, translocates into ER-PM junctions, interacts with Orai 1 (channel-forming protein) and activates Ca2+ entry. In pancreatic acinar cells ER-PM junctions are stationary. The number of junctions is not modified by the store depletion. In pancreatic ductal adenocarcinoma cells (PANC-1 cells in our experiments) ER-PM junctions are dynamic; they preferentially decorate the leading edge of migrating cells and undergo continuous cycles of formation and dissolution. The functional implications of the positioning and dynamics of ER-PM junctions will be discussed.

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S2-C6
The effect of taurocholic acid on ryanodine receptor and SR calcium pump activity

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The earliest critical event of pancreatitis is a long lasting high amplitude rise of intracellular Ca2+ concentration in the acinar cell, which can be triggered by secretagogue overstimulation or high concentration of bile acids. Although, Ca2+-release through ryanodine receptors (RyR) is involved in the process, the significance and the exact mechanism of bile acid`s action on RyR has not been fully elucidated yet. Therefore, we aimed to test whether taurocholic acid (TCA) exerts a direct effect on RyR and SERCA pump. We show that TCA enhanced RyR`s 3H-ryanodine binding in the pathologically relevant 25-500 μM range. We also found that 500 μM TCA triggered robust Ca2+-release from RyRenriched vesicles, which was prevented by the application of 12 μM dantrolene. Single channel current analysis of RyRs reconstituted into lipid bilayers demonstrated that 200 μM TCA induced a 5- fold increase in channel open probability (Kd=180 μM). Furthermore, we show that TCA suppressed Ca2+-uptake and ATP-ase activity of SERCA- enriched vesicles.
Overall, our data strongly suggest that TCA activates RyR and inhibits SERCA with an allosteric mechanism, which might contribute significantly to TCA-induced pathologic Ca2+-leak from the endoplasmic reticulum in pancreatic acinar cells.

S3-C

Epithelial Function and Repair

S3-C1
Epithelial fluid and HCO3- secretion

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HCO3- secretion is a key function of secretory epithelia and involves HCO3- entry at the basolateral membrane and exit across the luminal membrane. In most epithelia the bulk of HCO3- entry is mediated by the Na+-HCO3- co-transporter NBCe1-B and HCO3- exit is mediated by the combined and regulated action of CFTR and members of the SLC26 transporters. The function, regulation and interdependence of CFTR/SLA26 complexes are critical for the secretory process. In this presentation, the properties of and function of the HCO3- transporters by IRBIT and by intracellular Cl- will be discussed in the context of synergism in epithelial fluid and HCO3- secretion and intracellular Cl- as a key regulator of the transport process.

S3-C2
HCO3- secretorydf function of pulmonary epithelial cells

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Bicarbonate serves many functions in our body. It is the predominant biological buffer maintaining acid-base balance in the blood as well as inside all of our cells. Bicarbonate is also secreted from the blood to the mucosal surfaces of most epithelial tissues where it plays a crucial role in a myriad of processes ranging from sperm capacitation to mucosal protection and even innate defence. In the airways, HCO3- secretion likely contributes to important lung defence mechanisms, such as secretion and solubilisation of mucins, bacterial killing and regulation of ciliary beat frequency. Aberrant HCO3- secretion would therefore be predicted to predispose the lungs to mucus blockage, bacterial infection, and disease. An important challenge in epithelial biology has been to understand the molecular coordination of transepithelial bicarbonate secretion and how this simple anion impacts on such varied physiological processes. Through studies on the inherited disease cystic fibrosis (CF), it has become clear that the plasma membrane protein encoded by the CF gene, the cystic fibrosis transmembrane conductance regulator(CFTR), is essential for bicarbonate secretion, but it is only recently that we have begun to fully appreciate the complex role this anion channel plays. This talk will describe some of our recent work in airway epithelial cells that helps define the role of CFTR and other putative bicarbonate transporters in this fundamental process, as well as how pathological situations, such as hypercapnia, exposure to cigarette smoke and bacterial infection, impacts on bicarbonate secretion.

S3-C3
Physiological and pathophysiological roles of pancreatic ducts

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The pancreas is a glandular organ that has both endocrine and exocrine functions. The exocrine pancreas, including acinar and ductal cells, in humans secretes about 2.5 l of bicarbonate-rich fluid daily. Interestingly, a large proportion of this fluid is actually produced by the ductal cells which make up less than 5% of the pancreatic tissue. Therefore, it is no wonder that besides providing a structural framework, duct cells play an essential role in maintaining the homeostasis of the pancreas. The exact mechanism of ductal HCO3- secretion is unknown, but cystic fibrosis transmembrane conductance regulator (CFTR) is a key protein in orchestrating this process. In fact, CFTR not only acts as an anion channel, but also interacts with other (e.g. solute carrier 26) transporters involved in bicarbonate secretion. The importance of CFTR is highlighted by the fact that its functional defect can result in the development of cystic fibrosis (CF), which is the most common lethal autosomal recessive disorder in Caucasians. Therefore, it is not surprising that bicarbonate secretion is impaired in CF. It has been shown that the alteration of ductal bicarbonate secretion is also present in other diseases such as acute and chronic pancreatitis which may influence their course.
This presentation will focus on some interesting developments in the fields of physiology and pathophysiology of pancreatic ductal secretion. Experimental evidence suggests that the stimulation of ductal secretion may provide new prophylactic and therapeutic strategies in the treatment of pancreatic disease and inflammation.

S3-C4
Epithelial transport processes of ameloblasts leading to dental enamel formation

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Dental enamel, the hardest tissue in mammals, is produced by ameloblasts, which are electrolyte-transporting epithelial cells. Although the end product is very different, they show many similarities to transporting epithelia of the pancreas, salivary glands and kidney. Ameloblasts transport calcium and phosphate ions, the principal building blocks of hydroxyapatite crystals, into the enamel space. Enamel is produced in a multi-step epithelial secretory process. First, “secretory” ameloblasts form the entire thickness of the enamel layer, but with low mineral content. Then they differentiate into “maturation” ameloblasts, which remove organic matrix from the enamel and in turn further build up hydroxyapatite crystals. To form dental enamel large amounts of mineral ions should be transported across the ameloblast layer into the enamel space. The protons generated by hydroxyapatite formation need to be buffered, otherwise enamel will not attain full mineralization. Buffering requires a tight pH regulation and secretion of bicarbonate by ameloblasts. The whole process has been the focus of many studies but, perhaps surprisingly, the data available are almost exclusively based on ultrastructural, immunohistochemical and gene expression techniques, and the use of knock-out mice. Up till now, no functional data existed for mineral ion transport by ameloblasts. However, most recent studies including ours provided evidence for the nature of molecular mechanism of mineral transport, as well as for the bicarbonate secretory processes in enamel formation. The secretory regulation is not completely known as yet, but its significance is crucial. Impairing regulation retards or prevents completion of enamel mineralization and results in the development of hypomineralized enamel that easily erodes after dental eruption. Factors that impair this function are fluoride and disruption of pH regulators. Revealing these factors may eventually lead to the treatment of enamel hypomineralization related to genetic or environmentally induced malformation.

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S3-C5

Aquaporin-3 (AQP3) in cell proliferation, a potential target for therapeutic drugs

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Objective: Numerous studies demonstrated an abnormal AQP3 expression in tumor cells of different origins, suggesting a role for this enhanced protein expression in the tumor process. Recently we verified that the gold (III) complex Auphen, described as a very selective and potent inhibitor of AQP3´s glycerol permeability, significantly inhibits the cell proliferation of AQP3 expressing cells. Then to further understand the role AQP3 may play in the cell proliferation process we explore the effect that stable over expression of AQP3 produce over the proliferation and cell cycle of PC12 cells as cellular model.
Methods: Cell cycle by flow cytometry with propidium iodide (PI) and cell proliferation rate through cell counting and BrdU staining were used. Using Nocodazole, a typical drug used to induce cell death for apoptotic mechanisms, we evaluated the cell response to arrest its cell cycle and the resistance to apoptosis by Annexin V staining. Auphen effect over cell cycle progression and cyclins was also analyzed, and proteomic and transcriptomic techniques were perfomed with the goal of highlight key molecules implicated in cell proliferation process which expression may be altered by overexpression of AQP3.
Results: Cells with overexpression of AQP3 showed higher cell proliferation rate and larger percentage of cells in phases S and G2/M. After 24h in the presence of Nocodazole, overexpressing cells exhibited less modification of the cell cycle pattern and lower Annexin V specific staining consistent with a higher resistance to apoptosis in these cells. Additionally, in AQP3-expressing cells treated with Auphen, strong arrest of the cell cycle in the S-G2/M phases, supported by analysis of cyclins (A, B1, D1, E) levels, was observed. The RT-qPCR analysis comparing AQP3 overexpressing cells to wild type revealed interesting changes in the expression of molecules related with cell proliferation, tumor and cell cycle progression, such as, Zeb2, Jun, JunB, NF-kβ, Cxcl9, Cxcl10, TNF, and TNF receptors.
Conclusions: The significant role of AQP3 in the cell proliferation process seems to be connected to increments in the cell cycle turnover. Our results support the view that larger expression of AQP3 confers to the cell a more tumoral like phenotype and contributes to explain the presence of this protein in many different tumors. A potential therapeutic effect of Auphen in tumors where cell proliferation can be associated with AQP3 seems promising, but more studies are necessary to clarify this issue.

S3-C6

Basal ciliary activity depends on ATP release in respiratory epithelium of mouse trachea

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In mucociliary epithelia from respiratory tract, ciliary beat frequency (CBF) is the main factor that determines the effectiveness of mucociliary clearance (MCC). ATP is known to increases CBF in ciliated cells, effect that is mediated by purinergic receptor activation. The increase of CBF induced by ATP depends on intracellular Ca2+. In respiratory epithelial cells, ATP can be released constitutively or following mechanical stimulation (MS) through a mechanism possibly mediated by pannexin 1 (Panx1) or connexin 43 (Cx43) hemichannels (HCs). The aim of this study was to determine if extracellular ATP (eATP) release contributes to regulate basal ciliary activity. We used ciliated cells from primary cultures of mouse trachea. CBF was recorded using videomicroscopy and Sisson Ammons Video Analysis software, eATP was measured by luminometric assay using luciferin/luciferase and [Ca2+]i were measured using FURA 2AM. Apyrase (50 U/mL), an ectonucleotidase that hydrolyzes ATP, significantly lowered eATP levels compared to vehicle (3.8 ± 1.4 versus 8.1 ± 0.8 pmol/cm2 after 1 min. treatment, *p <0.05). Apyrase also reduced CBF in a 45.5 ± 2.3 % (n=4), effect that correlates with a [Ca2+]i reduction. Simultaneous treatment with carbenoxolone (CBX) (100 µM), a HCs inhibitor, and oxidized ATP (oATP) (100 µM), a P2X7-R antagonist, produced a reduction of CBF compare to basal activity of 57.5 ± 3.0% after 5 min of incubation, returning to baseline after 20 min. This reduction was prominent compared with oATP alone (6.8 ± 1.7 %, *p <0.05) and CBX alone (24.9 ± 6.8%, n=12). Furthermore, concomitant treatment with CBX, oATP and apyrase reduced the basal CBF in 85.2 ± 4.8% (n=10), in concordance with a reduction on eATP levels (19.9 ± 9.5 pmol/cm2 for vehicle, n=11, versus 2.6 ± 0.1 pmol/cm2 for treatment, n=4, * p <0.05).
These results suggest that ATP release from epithelial ciliated cells is necessary to maintain basal ciliary activity associated to [Ca2+]i homeostasis. The underlying molecular mechanism might involve HCs and P2X7-R, through an autocrine mechanism that regulates basal ciliary activity in respiratory epithelium. Millenium Scientific Iniciative P10-035-F.

S2-D

Lipid Homeostasis: What We Learnt from Sex Hormone Estrogens

S2-D1
Estrogen more than a sex hormone

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17β-estradiol (E2), the most effective female estrogen, controls many aspects of human physiology, including development, reproduction and homeostasis in general. The spectacular diversity of this hormone effects relies on different estrogen receptor subtypes (e.g., ERα and ERβ), which are differentially expressed in many if not all human tissues as well as on a repertoire of molecular mechanisms that are activated in E2 target cells both in female and in male individuals. Indeed, the ERs are ligand-activated transcription factors that differentially modulate gene expression in different tissues by recruiting a vast array of transcriptional co-factors (i.e., co-activators and co-repressors). However, research over the last two decades have clearly revealed that ERs are extrinsic plasmalemmal proteins with the ability to signal through many different intracellular transduction languages (i.e., kinases, ubiquitin-ligases, calcium) to the regulation of many physiological functions. Structure-function analysis have linked this so-called extra-nuclear signalling with specific ERs biochemical determinants and revealed an intimate cross-talk with the ERs-based ability to regulate gene transcription. More recently, the availability of mouse model systems strongly demonstrated the importance of the ERs extra-nuclear signalling for the body homeostasis in vivo. Therefore, the pleiotropic nature of this sex steroid hormone resides on the different and somewhat opposite mechanisms of the E2-based signalling, which also cross-talks with many other hormonal networks. In this perspective, it is not surprising that deregulation of ERs-based signalling can be a cause of many hormone-based diseases and, on the other hand, E2 can exert also protective effects in many different body districts (e.g., central nervous system). Here an updated overview of the above-mentioned E2-based mechanisms will be given with the perspective of regulation of physiological and protective functions of estrogen in male and female organs.

S2-D2
Estrogen suppresses lipid synthesis: Role of membrane estrogen receptors

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Lipid synthesis is an important part of overall lipid homeostasis. Estrogen has been reported to repress low density cholesterol synthesis and augment clearance of this lipid, while enhancing high density cholesterol synthesis. This is attributed in part to the regulation of key genes, presumably from nuclear estrogen receptor (ER) alpha action. To investigate a possible role of extra-nuclear ER action in these regards, we compared ovariectomized wild type (WT), membrane ERalpha-only (no nuclear ERalpha) (MOER), and total ERalpha deleted (ERKO) mice responses to injection for 3 days of an ERalpha agonist, PPT. Comparisons included DNA microarray in the liver that showed suppression of many lipid synthesis genes, comparably in WT and MOER mice, but absent in ERKO mice. This correlated to suppression of liver cholesterol, triglyceride, and the fatty acid palmitate content in both mice. Cultured hepatocytes from WT and MOER mice responded to insulin with increased lipid synthesis, suppressed by estradiol or PPT, only and comparably in WT and MOER mice. Further investigation implicated membrane ERalpha signaling through AMP kinase, phosphorylating SREBP1 to sequester this key transcription factor in the cytoplasm, thereby preventing lipid synthesis gene expression stimulated from insulin action (Science Signaling, 2013). More recently, we also asked whether estrogen suppressed triglyceride synthesis and prevented differentiation of pre-adipocytes (3T3-Li cells), and found an important role for membrane ERalpha action in these regards. Estrogen fails to suppress fat cell development in-vivo in both MOER mice and mice that lack the membrane receptor but retain nuclear ERalpha (NOER mice, Developmental Cell, 2014).
In summary, extra-nuclear (membrane-localized) ERalpha plays a prominent role in suppressing lipid synthesis in several organs, at times exclusive of nuclear ERalpha.

S2-D3
Complex interplay between estrogens and polyunsaturated fatty acids in hippocampal lipid homeostasis: Relevance for Alzheimer's disease

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After the adipose tissue, the brain has the highest lipid content in the body. Moreover, the brain is unique in that it contains the largest amount of long chain polyunsaturated fatty acids (LCPUFA), in particular docosahexanoic acid (DHA), which is extremely well preserved in the nervous tissue. These fatty acids play a crucial role in maintaining a number of nerve cell functions from neurogenesis and synaptic transmission to memory consolidation, and their depletion has been linked to the development of Alzheimer's disease (AD). Along with cholesterol, LCPUFA modulate the physicochemical

properties of plasma membranes and provide the lipid environment for signaling platforms known as lipid rafts. Data from different laboratories, including ours, have demonstrated that n-3 LCPUFA and cholesterol levels are altered in AD brains, a phenomenon that may greatly contribute to membrane destabilization and perturbation of neuronal homeostasis during the development of AD.
Recently, we have demonstrated that estradiol is an important modulator of brain lipid homeostasis. Thus, physiological doses of this hormone increase cholesterol and DHA levels in nerve tissues through different mechanisms, but especially by activating the DHA mobilization from the liver to the brain across the blood-brain barrier. Moreover, we also demonstrate that the brain is also capable to produce LCPUFA and cholesterol through the local synthesis from dietary precursors, and that this is probably a crucial mechanism contributing to the preservation of brain lipid matrix. Moreover, the brain capacity to synthesize LCPUFA and cholesterol is under hormonal and dietary influences. Interestingly, estrogens and DHA might act synergistically to stabilize brain lipid structure by modulating both hepatic and neuronal synthesis, even in conditions of low LCPUFA intake. Our lipidomic results in the hippocampus of wild-type and APP/PS1 transgenic mice (a familial model of AD), suggest that part of the neuroprotective effects elicited by estrogens occur through the homeostatic preservation of LCPUFA and cholesterol levels in the brain.

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S2-D4
Bisphenol-A as a potential environmental factor that alters the development

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The endocrine disrupting compound Bisphenol A (BPA) has been reported to act as an obesogen in rodents exposed perinatally. Indeed, a number of studies have determined that rodents exposed to environmentally relevant doses of BPA during prenatal and postnatal development are more susceptible to becoming obese. Our work has confirmed these effects and we have determined that early life BPA exposure alters the development of the hypothalamic pro-opiomelanocortin (POMC) system leading to altered adult expression of hypothalamic peptides associated with food intake and energy balance. For instance, Males exposed to the high dose of BPA showed impaired glucose tolerance on both the normal and high-fat diets. This was correlated with increased Neuropeptide-Y (NPY) and Agouti Related Peptide (AgRP) expression in the arcuate nucleus (ARC) and reduced pro-opiomelanocortin (POMC) fiber innervation into the paraventricular nucleus (PVN) of the hypothalamus. Females exposed to the high BPA dose were heavier, ate more and had increased adiposity and leptin concentrations with reduced POMC mRNA expression in the ARC when consuming a high fat diet. The alterations in the developmental course of the melanocortin system appear to be related to a shift in the timing of the early postnatal leptin surge that is required for the full development of the melanocortin system projections stemming from the hypothalamic arcuate nucleus (ARC) to other hypothalamic nuclei. While some of these effects were also observed in mice exposed to estrogen during development, some were only observed in mice exposed to BPA. These data support the idea that BPA at environmentally relevant doses can alter the development of the hypothalamic circuits regulating food intake and energy balance. Furthermore, they suggest that BPA may pre-program susceptibility to develop obesity in both a steroid dependant and independent manner.

S2-D5
Cholesterol homeostasis in the brain: A sex and age viewpoint

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Although a great knowledge about the patho-physiological roles of cholesterol metabolism perturbation in several organs has been reached, scarce information is available on the regulation of cholesterol homeostasis in the brain where this lipid is involved in the maintenance of a variety of neuronal processes. Currently, no study is available in literature dealing how and if sex and age may modulate the major proteins involved in the regulatory network of cholesterol levels in different brain regions. In this study we investigated the behavior of 3-hydroxy 3-methylglutaryl Coenzyme A reductase (HMGR) and Low Density Lipoprotein receptor (LDLr) in adult (3-month-old) and aged (12-month-old) male and female rats. The analyses were performed in four different brain regions: cortex, brain stem, hippocampus and cerebellum which represent brain areas characterized by different neuronal cell types, metabolism, cytoarchitecture and white matter composition.
The results show that in hippocampus HMGR is lower (30%) in adult female rats than in age-matched males. Differences in LDLr expression are also observable in old females with respect to age-matched males: the protein levels increase (40%) in hippocampus and decrease (20%) in cortex, displaying different mechanisms of regulation.
The obtained data demonstrate that age- and sex-related differences in cholesterol homeostasis maintenance exist among brain regions, such as the hippocampus and the prefrontal cortex, important for learning, memory and affection. Some of these differences could be at the root of marked gender disparities observed in clinical disease incidence, manifestation, and prognosis.

S2-D6
The determination of sodium salicylate effect to body weights and fatty acid values in the frontal lobe of brain on the immobilized rats

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The aim of this study is effects of sodium salicylate to body weights and fatty acid values in frontal lobe of brain in rats which were exposed immobilization. According to literature, sodium salicylate has protective effect against oxidative stress in rat tissues. Ethics committee approval was taken from Faculty of Medical Ethics Committee in Firat University (Turkey) and experimental applications were fulfilled in Experimental Research Unit of Firat University. 12 numbers Wistar-Albino rats that were 100-120 days and weigh 200-220g were used for this applications. Study was separated to three groups: control group (C, physiological solution, n=4), immobilization group (I, physiological solution+immobilization-15min./day, n=4) and immobilization+sodium salicylate group (I+SS, 200mg/kg+immobilization-15min./day, n=4). Fatty acids that were obtained from brain tissues were analyzed by gas chromatograph device (GC). According to experimental results, 15:1 (pentadecanoic acid), 16:0 (palmitic acid), 16:1 n-7 (palmitoleic acid), 18:0 (stearic acid), 18:1 n-9 (oleic acid), 18:2 n-6 (linoleic acid), 20:1 n-9 (eicosenoic acid), 20:4 n-6 (arachidonic acid), 22:2 (docosadienoic acid), 22:6 n-3 (docosahexaenoic acid) and 24:0 (lignoceric acid) fatty acids were calculated. 18:0 and 24:0 fatty acid values of immobilization group was statistically lower than control group ($p < 0.05$), but ∑MUFA value of immobilization groups was higher than control groups ($p < 0.05$). ∑SFA value of I+SS group was lower statistically than control group, but ∑USFA value of I+SS group was higher than control ($p < 0.05$). The average body weight changes of animals in the I group (6.4g rising) according to control group (11.86g) showed statistically decreasing ($p < 0.05$). On the other hand, the average body weight changes of animals in the I+SS (9.12g rising) was lower compared with control group ($p < 0.05$). As a result, Both immobilization and sodium salicylate affected the weight of animals and the fatty acid composition in the brain frontal lobes of rats. We suggest that immobilization may affect the fatty acids metabolism directly or indirectly in the brain.

S3-D

Novel Mechanisms Contributing to Aging

S3-D1
Functional, morphological and molecular changes in arteries as a function of age

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Background: In the present study we hypothesized that due the continuous presence of hemodynamic forces the contractile capacity of arteries increases as a function of age. Potassium chloride (KCl) was used to assess the contractile capacity of arteries, because it elicits contraction of smooth muscle without receptor mediation (which could also be affected by aging).
Methods: Carotid arteries from 8 days (0.25m), 2 months (2m), 6 months (6m), 12 months (12m), 24 months (24m) and 30 months (30m) old WKY rats were isolated and their isometric wall tensions were measured. Arterial contractions were elicited by KCl (60mM). The arterial level of oxidative stress was assessed by HPLC. The expressions of some of the key signaling molecules of contractile activity of smooth muscle were also assessed, such as PKC-pCPI-17, MLCP and RhoA-ROCK2.
Results: There were three phases of KCl-induced arterial contractions as a function of age: contractions increased from 8d to 2 months (phase I.) than it did not change until 12m (phase II.), then it increased again to the age of 30 months (phase III.). Wall thickness increased with age, most significantly in the media and adventitia. Expressions of PKC-pCPI-17, MLCP and RhoA-ROCK2 proteins showed also an age dependency. PKC-CPI17 increased substantially from newborn to senescence age, whereas RhoA-Rock2 first increased than decreased as function of age.
Conclusions: Because KCl increases smooth muscle [Ca 2+]i via voltage operated Ca2+-channels and release of Ca2+ from the sarcoplasmic reticulum thereby stimulating the PKC-pCPI-17 and the RhoA-ROCK2 pathways, the increased magnitude of arterial contraction in older age is likely due to the upregulation of these pathways, findings which may have physiological importance regarding the age dependent regulation of peripheral vascular resistance.

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S3-D2
Mechanisms of extension of cognitive health span with IGF-1

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Insulin-like growth factor-1 (IGF-1) is an important anabolic hormone that decreases with age. In the past two decades, extensive research has determined that the reduction in IGF-1 is an important component of the age-related decline in cognitive function in multiple species including humans. Deficiency in circulating IGF-1 results in impairment in

processing speed and deficiencies in both spatial and working memory. Replacement of IGF-1 or factors that increase IGF-1 to old animals and humans reverses many of these cognitive deficits. Despite the overwhelming evidence for IGF-1 as an important neurotrophic agent, the specific mechanisms through which IGF-1 acts have remained elusive. Recent evidence indicates that IGF-1 is both produced by, and has important actions on, the cerebrovasculature as well as neurons and glia. Nevertheless, the specific regulation and actions of circulating, vascular- and brain-derived IGF-1 are poorly understood. The diverse effects of IGF-1 discovered thus far reveal a complex endocrine and paracrine system essential for integrating many of the functions necessary for brain health. In this presentation the latest studies of the complex actions of IGF-1 on the cerebrovasculature, neurons and glia will be discussed. Identification of the mechanisms of IGF-1 actions will undoubtedly provide critical insight into regulation of brain function in general and the causes of cognitive decline with age.

S3-D3
Novel model of age-related cognitive impairment and molecular mechanism of synaptic failure

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Vascular cognitive impairment, Alzheimer's and other neurodegenerative diseases affect more than 40 million elderly patients world-wide. As prevalence of cognitive impairment and dementia cases is accelerating in our aging population, more efficient therapies are urgently needed. According to recent results in dementia research, a key event in the pathomechanism of dementia is the disruption of synaptic connections among neurons. Synapses are the structural elements for information processing, neuronal communication in the brain, and are essential for learning and memory as well as other cognitive processes. The core mechanism for transmitter release from synaptic vesicles requires the SNARE (SNAP Receptor) complex. Three proteins form the synaptic SNARE complex in the brain: SNAP-25, syntaxin1 and synaptobrevin.
Using the knock-out mouse strains as a novel model of dementia and fluorescence imaging functional assays, we have found that the levels of SNARE proteins significantly correlate with synaptic release. Synaptobrevin2 (syb2) and Insulin-like Growth Factor (IGF)-1 have been implicated in a transcriptional network analysis as strongly associated with brain aging and dementia. IGF-1 is an important trophic hormone and its expression decreases with age. Deficiency of this hormone therefore may influence cognitive decline in the elderly. We hypothesized direct synaptic effects of IGF-1 and tested its role in memory improvement. We performed electrophysiological and fluorescence imaging experiments on primary neuronal cultures of the synaptobrevin2 knock-out mice and present novel data on synaptic mechanisms of learning and memory after IGF-1 treatment.
Taken together, our data indicate that the level of syb2 is a limiting factor in synaptic transmission and its expression in the brain is hindered during physiological aging. Based on these new results on the effects of IGF-1 on synaptic communication, we propose the IGF-1/PI3K/Akt pathway as a possible therapeutic target and a novel approach to improve cognitive function for the elderly that could overcome synaptic dysfunction.

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S3-D4
Determination of biological age in humans: Results from the EU FP7 MARK-AGE project

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The rate of ageing in humans is not uniform, due to genetic heterogeneity and the influence of environmental factors. Age-related changes in body function or composition that could serve as a measure of "biological" age and predict the onset of age-related diseases and/or residual lifetime are termed "biomarkers of ageing". Many candidate biomarkers have been proposed but in all cases their variability in cross-sectional studies is considerable, and therefore no single measurement has so far proven to yield a useful biomarker of ageing on its own.The MARK-AGE Consortium, comprising 26 partners from 14 European countries, has therefore conducted a population study (3,300 subjects) aiming at the identification of a set of biomarkers of ageing that could serve as a measure of biological age. Two larger groups of subjects have been recruited, i.e. (i) randomly recruited age-stratified individuals from the general population covering the age range 35-74 years and (ii) subjects born from a long-living parent belonging to a family with long living sibling(s) already recruited in the framework of the GEHA project. For genetic reasons such individuals (termed GEHA offspring) are expected to age at a slower rate. They have been recruited together with their spouses as controls, thus allowing initial validation of the biomarkers identified. (iii) A small number of patients with progeroid syndromes have also included in the study. A wide range of candidate biomarkers were tested, including (a) classical ones for which data from several smaller studies have been published; (b) new ones, based on recent preliminary data, as well as (c) novel ones, based on recent research on mechanistic aspects of ageing. Bioinformatic analyses have been performed to extract a robust set of biomarkers of human ageing from the large amounts of data generated. Data on the top 10 biomarkers will be shown. Based on our results we have developed a strategy to determine biological age of men and women, respectively, which also will be shown.

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S3-D5

Age-related changes in response to ischemia and adaptation in male rat hearts: Potential molecular mechanisms behind

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Reduced tolerance to ischemia/reperfusion (IR) injury was shown in aged human and animal hearts, however, precise cellular mechanisms underlying this unfavorable phenotype are incompletely understood. Moreover, aging may interfere with the mechanisms of innate myocardial protection (preconditioning, PC) and cause defects in protective cell signaling cascades that may shift the balance from cell survival to cell death. We studied effects of aging on myocardial function and response to ischemia, as well as selected molecular pathways in the hearts from juvenile (1,5 months), younger adult (3 months), mature adult (6 months) and senescent (24 months) male Wistar rats. In Langendorff-perfused hearts exposed to 30-min I/120-min R without or with prior PC induced by one cycle of 5-min I/5-min R, we measured the size of myocardial infarction (IS), susceptibility to ventricular arrhythmias and contractile function (LVDP). In parallel groups, LV tissue was sampled at baseline (BL) and after IR, for the detection of protein levels (WB) of protein kinase B (Akt, an effector of PI3-kinase), phosphorylated (activated) Akt (pAkt), its target endothelial NO synthase (eNOS) and protein kinase Cε (PKCε) as "pro-survival" pathways linked with attenuation of apoptosis. Maturation impaired heart response to lethal IR injury (increased IS) and promoted arrhythmogenesis, and these changes were more markedly evident in the younger groups of animals, while functional recovery was not affected by aging. PC suppressed the occurrence of malignant arrhythmias, reduced the IS and improved LVDP recovery in the younger animals, while its efficacy was attenuated in the mature ones. Loss of cardioprotective effects of PC was associated with reduced BL and post-IR levels of total Akt, pAkt, eNOS and PKCε in the elder groups. Age-dependent decline in PC-induced upregulation of these proteins in the hearts of mature animals compared with younger adults was also observed. In conclusion, aging-related alterations in myocardial response to ischemia may be caused by loss of innate cardioprotection due to dysfunction of proteins involved in protective cell signaling.

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S3-D6

Aging dependent GDNF induction by hypoxia in Carotid Body: Implications for antiparkinsonian cell therapy

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Intrastriatal carotid body (CB) grafts induce trophic protection and restoration of the dopaminergic nigrostriatal pathway in rodent and primate models of Parkinson's disease (PD) by the release of glial cell line-derived neurotrophic factor (GDNF). Moreover, CB cells are physiologically resistant to hypoxia, a normal environmental condition in the brain that is accentuated inside intracerebral grafts. In this study, we analyse whether the age of the CB tissue could modify the regulation of GDNF expression under chronic hypoxia. Chronic environmental hypoxia (PO2 ~75 mmHg) induced an up-regulation of CB GDNF expression in young mice (2-3 months old) but the same treatment resulted in decreased CB GDNF expression in aged mice (>14 months old). This differential regulation of GDNF expression with aging was also observed in intrastriatal CB grafts and affects its efficacy in antiparkinsonian cell therapy. In addition, we have demonstrated that young CB implants are able to induce trophic protection of dopaminergic nigrostriatal neurons of aged host parkinsonian mice. These findings are in accord with previous clinical trials, where the efficacy of CB autotrasplantation in PD patients was inversely related to patient age. To further demonstrate this issue, we are performing human CB xenografts in chronic 1-methyl-4-phenyl-1,2,3,6,-tetrahydropyridine (MPTP)-treated PD mice model. Finally, we also are studying age-related epigenetic modifications of the GDNF promoter to unravel the molecular mechanism involved in the differential hypoxic response.
In conclusion, these findings will provide new insight on critical factors that could affect clinical efficacy of antiparkinsonian CB cell therapy.

S2-E

Regulation of Mitochondrial Function in Heart Failure: From Health to Dysfunction

S2-E1
Alternations in mitochondrial structure and function in rat myocardium in chronic heart failure

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During the disease progression of chronic heart failure, a profound remodelling process takes place in the cardiac myocyte. Alterations occur in cell size, glycogen storage, enzyme content and mitochondrial shape and function, all affecting cardiac energetic function. Cardiac mitochondrial function is not only determined by its volume density, but also mitochondrial quality. However, little is understood about the influence of alterations in mitochondrial respiration during the transition from cardiac hypertrophy (CH) to failure (HF). Recently, we developed a photometry-based technique to simultaneously measure contractile function and autofluorescence of NADH (reduced nicotinamide adenine dinucleotide) and FAD (oxidized flavin adenine dinucleotide) in thin cardiac trabeculae from rats with and without CH or HF. The contraction-energetic coupling (i.e. increased contractile output linked to an increased mitochondrial function) was disturbed in HF. High-resolution respirometry revealed that mitochondrial complex I-stimulated respiration and maximal oxidative capacity were severely affected in HF and could not be explained by changes in mitochondrial volume density or protein content. Other factors, such as ultra-structural alterations and mitochondrial super-complex destabilisation are likely contributing to a bio-energetic failure in HF.

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S2-E2
Diffusion obstacles shape the environment surrounding mitochondria in heart

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Intracellular environment in the heart cells is highly packed which could lead to the formation of diffusion obstacles. In particular, diffusion of ATP and ADP is rather slow when estimated on the basis of respiration kinetics measurements on permeabilized cardiomyocytes. Intriguingly, using kinetic experiments to estimate diffusion restrictions, it has been shown that they change significantly in disease. We have recently demonstrated that this estimation of slow diffusion is caused by intracellular structures and is not induced by possible side-effects, such as clamping of the cells or unstirred water layers. For that, we used the autofluorescence of cardiomyocytes and followed redox state of mitochondria by recording nicotinamide adenine dinucleotide (NADH) and flavorotein (FP) signal changes in a permeabilized cell. By positioning the permeabilized cells in flow chamber, following NADH and FP changes induced by variation of ADP and ATP concentration in solution, and analyzing the data with mathematical models, we were able to distinguish contribution of unstirred water layers and significant intracellular diffusion restrictions. Significant intracellular diffusion restrictions have been reveled when following diffusion of fluorescent molecules using raster image correlation spectroscopy. However, characteristics and localization of these restrictions is yet to be elucidated. While a mitochondrial outer membrane has been frequently suggested as a main diffusion restriction estimated by the kinetic measurements, recent data points to existence of diffusion restrictions in cytoplasm grouping mitochondria and ATPases.
The aim of this work is to distinguish the contribution of mitochondrial outer membrane and obstacles in cytoplasm to overall intracellular diffusion restrictions. For that, we recorded high resolution images of mouse cardiomyocyte autofluorescence changes due to ADP titration. These recordings allow us to analyze the heterogeneity of autofluorescence response. Experimental data is analyzed by mathematical modeling that enables us to estimate intracellular ADP diffusion coefficient and resistance of mitochondrial outer membrane.

S2-E3
Myocardial mitochondrial respiration in human heart failure

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Heart failure (HF) is a clinical disease entity defined by the presence of symptoms related to structural or functional abnormality of the heart, which impairs its pumping capability. The cellular mechanism associated with HF is not fully elucidated. Decreased phosphocreatine (PCr) to ATP ratios in human dilated cardiomyopathy contributed to the notion that the failing heart is “an engine without fuel” in recognition of the inability of the mitochondria to meet the energetic requirements of the contractile elements in the myocytes. Bioenergetic studies of human cardiomyocytes is limited by the availability of tissue samples (in particular left ventricular), but some data are available. In patients with and without left ventricular systolic dysfunction (LVSD) biopsies from the left ventricle was studied using high resolution respirometry. A pronounced reduction in oxidative phosphorylation capacity (OXPHOS) was seen in the patients with LVSD EF, when the respiration was sustained by both non-fatty acid as well as fatty-acid substrates. Creatine addition displayed marked effect on respiration, and revealed differences between groups in apparent Km from ADP titrations. The limited fatty acid sustained respiration was supported by decreases in proteins involved in uptake, transport and combustion of fatty acids, as well as a decrease in proteins in the respiratory chain. Promising data on a feline model of hypertrophic cardiomyopathy are now emerging, and this model may actually serve as a substitute for human disease.

S2-E4

Ca2+ microdomains and interaction between mitochondria and ER/SR: Open questions

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Mitochondria are highly pleiotropic organelles, involved in the control of many intracellular processes, ranging from ATP synthesis to programmed cell death. They play a key role in the maintenance of Ca2+ homeostasis, since they can accumulate Ca2+ within their matrix through the so-called mitochondrial Ca2+ uniporter (MCU). Being the affinity of MCU well above the Ca2+ concentration reached in the bulk of the cytoplasm following its release from the Endoplasmic Reticulum (ER), the ability of mitochondria to take up Ca2+ was highly debated. This apparent paradox was explained by hypothesizing the presence of microdomains of high Ca2+ concentration at the interface between the two organelles. Experimental evidence to support this theory lacked until the recent discovery of the first mitochondria-ER molecular tether (Mitofusin 2, whose ablation impairs mitochondrial Ca2+ uptake) and our demonstration that [Ca2+] hot-spots exists at the ER-mitochondria contact sites, as independently confirmed by Hajnoczky and colleagues.

Since then the field of mitochondria-ER/SR interaction gained more attention, and nowadays its importance for the control not only of Ca2+ homeostasis but also of the overall cell physiology is widely recognized.

Despite this, and despite the evidence that impaired mitochondria-ER interactions are present in several disease models, a lot of open questions still remain: which are the molecules that mediate the physical interaction between these organelles? Which are the signalling pathways that control, or in turn are controlled by, mitochondria-ER/SR communication? How does impairment of inter-organellar communication contribute to the onset and progression of pathological conditions, i.e. does it play a causative role? Answering these questions will improve our understanding of physiological and pathological processes, and likely open the way to the discovery of druggable targets and novel therapeutical strategies.

S2-E5

The effects of Simvastatin on skeletal muscle treated with LPS in rats

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Aim: Sepsis model in animal is comprised experimentally to intraperitoneally injection of Lypopolysaccharide (LPS). LPS causes release of proinflammatory cytokines and alters mitochondrial activities together with the changes in muscle structure. In our study, we aimed to investigate the effects of Simvastatin on muscle tissue treated with LPS in rats.
Material-Method: Male Wistar albino rats were divided into four groups: Control, LPS, Simvastatin, Simvastatin+LPS. Mitochondrial electron transport chain enzymes; citrate synthase, Complex I, Complex I+III, Cytochrome c oxidase (COX) were measured in spectrophotometer. Muscle tissue was evaluated for myopathic changes, Succinate dehydrogenase (SDH) and Cytochrome Oxidase (COX) under light microscopy.
Results: We found statistically significant increased levels of serum TNF-α and IL-10 in LPS and Simvastatin+LPS groups compared to Controls ($p < 0.01$). In all experimental groups, Complex I and Citrate synthase activities were increased compared to controls. In control and LPS groups, COX activities were significantly higher than in the Simvastatin groups. In Simvastatin and Simvastatin+LPS groups, Complex I-III activity was higher than control and LPS ($p < 0.05$). More than half of the animals treated with LPS showed myopathic changes.
Conclusions: Our findings demonstrated that simvastatin improves the alterations on enzyme activities and muscle structure after treating with LPS.

S2-E6

Exercise performed before and during sub-chronic Doxorubicin treatment mitigates cardiac mitochondrial alterations (morphology, OXPHOS, biogenesis, oxidative stress, permeability transition and apoptotic signaling)

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The role of exercise performed before and during treatment against heart mitochondrial dysfunction and cell death associated with sub-chronic Doxorubicin (DOX) is unknown. Two chronic exercise models (endurance treadmill training – TM and voluntary free-wheel activity - FW) were used. Mitochondrial ultra-structure, OXPHOS protein subunits, biogenesis, permeability transition pore (mPTP), apoptotic signaling and oxidative stress were analyzed. Thirty-six male Sprague-Dawley rats were divided into six groups (n=6 per group): saline sedentary (SAL+SED), SAL+TM (12-wks treadmill), SAL+FW (12-wks voluntary free-wheel), DOX+SED [7-wks sub-chronic DOX treatment (2 mg·kg-1·wk-1)], DOX+TM and DOX+FW. Heart mitochondrial ultra-structural alterations, OXPHOS proteins content and in gel activity, as well as proteins involved in mitochondrial biogenesis (PGC1α and TFAM) and oxidative stress (carbonyl groups, SIRT3and p66shc) were evaluated. Apoptotic signaling and mPTP opening regulation were followed by measuring, Bax, Bcl2, and cophilin expression and calcium-induced variations of osmotic swelling. DOX

treatment resulted in ultramitochondrial alterations, decreased OXPHOS, decreased complex I activity and content, mitochondrial biogenesis (TFAM) and increased oxidative stress (carbonyls, SIRT3 and p66shc(Ser36)/p66shc ratio). Moreover, DOX treatment also augmented of calcium-induced mPTP susceptibility and apoptotic signaling (Bax/Bcl2 ratio). TM and FW mitigated DOX-induced alterations in mitochondrial ultra-structure, OXPHOS, increased oxidative stress and the decrease in complex I activity and content. DOX-induced decreased TFAM and SIRT3 content were only prevented by TM and both exercise modalities increased the content of cofilin in comparison with DOX+SED group. Both TM and FW mitigated DOX-increased mPTP susceptibility and apoptotic signaling. In summary, both chronic models of physical exercise performed before and during the course of sub-chronic DOX treatment translated into improved mitochondrial fitness and limited cardiac mitochondrial-driven apoptotic signaling, which may result in part from the prevention of oxidative stress and damage.

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S3-E

Current Trends in Respiratory Physiology: From Lung Function Towards System Biology Approaches

S3-E1
The links between lung function and cardiovascular diseases

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Lung function measurements provide an insight into the mechanical function of the respiratory system. Forced vital capacity (FVC) and forced expiratory volume in one second (FEV1) are spirometric measures that reflect lung volume and airway calibre and not only used to diagnose and monitor pulmonary diseases, but can also be applied for the prediction of cardiovascular disease mortality. Loss of tissue elasticity with aging is associated with loss in FVC and lung age is suggested as a potential marker of vascular remodelling. Both lung function and vascular stiffness have heritable determinants and are also influenced by environmental factors. Furthermore, there is a potential genetic influence on parenchymal and airway changes in chronic obstructive pulmonary disease (COPD) (Tarnoki Dl et al, Acta Physiol Hung, 2014) together with a genetic predisposition for accelerated arterial stiffening (Horvath T et al Hypertens Res 2014). Recent findings show that FVC and FEV however are phenotypically, but not genetically, associated with augmentation index, a measure of wave reflection (Tarnoki DL et al, J Breath Res, 2013) emphasizing the importance of environmental and life-style factors. Furthermore, in hypertension increased cardiovascular risk and reduced lung function coexists, but the pathophysiological mechanisms behind this feature are not clear. Further investigation of this relationship may lead to a better understanding of the close interplay in the cardiopulmonary system.

S3-E2
Oxidative stress pathways as new therapeutic opportunities: from infection to lung ageing

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Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen, such as peroxide and oxygen ions. It has been widely accepted that the moderate concentrations of ROS have important roles in cell signalling, immune cell function and homeostasis, particularly host defence. However, an excess or chronic exposure of ROS or imbalance between ROS and anti-oxidants results in significant damage of molecules, cells and tissue. Thus oxidative stress is often blamed for the development of cancer and chronic inflammatory diseases such as chronic obstructive pulmonary disease (COPD) and severe asthma. Oxidative stress cascade is not simple, and recent system biological approach revealed that oxidative stress is amplified by mitochondrial dysfunction and secondary reaction of pollutants, smoke and infection rather than direct response. Elevated oxidative stress then affects inflammation, virus/fungus infection as well as lung ageing. Chronic exposure of ROS also reduced expression or function of anti-ageing molecules, such as histone deacetylase 2 (HDAC2), sirtuin 1, DNA repair protein Ku86 in lung, and this causes reduced endogenous antioxidants via defect of the large forkhead family of proteins (FOXO) and nuclear factor erythroid 2-related factor 2 (Nrf2). Defect of Nrf2 is also reported to causes an increase in influenza or respiratory syncytial virus infection, or virus infection-induced systemic effects, such as exacerbation. Even more importantly, Nrf2 activators, sulforaphane and metformin restored function of anti-ageing molecules and inhibited respiratory infection. Thus oxidative stress causes respiratory inflammation as well as accelerating lung ageing and respiratory infection in an even more complex manner. This vicious downward spiral of oxidative stress is a key factor of pathogenesis of COPD, and novel approach to oxidative stress will provide great opportunities to develop new treatment for chronic inflammatory disease, ageing or infection rather than simple synthetic anti-oxidants.

S3-E3
Mast cell biology in the human lung

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Allergic asthma is associated by involuntary constriction of the bronchial smooth muscle. This is often caused by mast cell activation. Allergen activation of mast cells causes most symptoms of allergic asthma through release of histamine, and synthesis of PGD2 and Cysteine leukotrienes, and later secretion of cytokines and chemokines. Expression of FceRI on lung mast cells varies greatly by microlocation. It is known to depend on plasma IgE concentration.

We culture mast cells from human peripheral CD133+ blood stem cells with a 7 week protocol, in which we add IL4 and human specific IgE (total IgE 80 kU/L) for the last two weeks. Mast cells cultured this way can be activated with allergen under near-physiological conditions. Mast cells cultured from both non-allergic controls and allergic asthmatics respond similarly in this model, which suggests that allergen activation is largely a function of IgE rather than an intrinsic mast cell property. The sensitivity of PBMC depends to some extent on IgE concentration, and the fraction of cells responding depends on the complexity of IgE.
The relative contribution of mast cells and IgE to allergic responses is not yet elucidated, and may vary from person to person (and patient to patient). With this model, we can keep the IgE component constant and explore properties of the mast cell per sé.

S3-E4
Systems medicine and big data to phenotype and treat chronic airway diseases

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The most prevalent respiratory diseases exhibit far from simple pathophysiology. The complex biology of asthma and COPD is currently fueling the discovery of various disease phenotypes. Nevertheless, current guidelines for diagnosis and treatment are almost exclusively based on clinical and functional characteristics. Even though single biomarkers (e.g. sputum eosinophils) are gradually introduced in the management of these diseases (based on evidence provided by 'algorithm studies'), it can be postulated that composite biomarker fingerpints will provide more comprehensive patho-biological information of these diseases and their underlying phenotypes1.
High-throughput "omics" technologies based on unbiased systems biology approaches, including transcriptomics, proteomics, lipidomics, and metabolomics are increasingly used for biomarker discovery in asthma2. This has led to unravelling and validation of e.g. transcriptomic phenotypes of asthma based on epithelial, sputum or bronchial biopsy analysis. The leading principle here is to strictly obey the recent guidelines on omics analysis in clinical medicine, thereby purposely limiting false discovery with proper (external validation)3.
There are two challenges when bringing this to clinical practice. First, the measurements should be feasible at point of care with real-time availability of results. Metabolomics of exhaled air (breathomics) qualifies for this purpose and is currently applied in longitudinal, monitoring studies. Second, the 'omics' fingerprints have to be integrated with clinical data, requiring a quantitative multi-scale systems approach. The latter is key of the U-BIOPRED project that, after recently completing its longitudinal follow-up of (severe) asthma patients, is delivering its first unbiased, bio-clinical phenotypic fingerprints (www.ubiopred.eu). It is envisaged that this will lead to effective integration of molecular fingerprints in patient phenotyping and clinical management.

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S3-E5
Bronchoconstriction and alveolar derecruitment following extracorporeal circulation: Good by(e)pass?

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Background: While cardiopulmonary bypass (CPB) exerts its adverse pulmonary effects via inducing partial ischemia-reperfusion in the lung and systemic inflammatory response leading to surfactant damage, the changes in the lung mechanics and ventilation have not been characterized. Therefore, we aimed at elucidating the effects of CPB on the airway and lung tissue mechanics, ventilation heterogeneities, and dead space parameters.
Methods: Anaesthetized, mechanically ventilated patients (n=46) undergoing elective heart surgery were studied in open-chest condition before, and 5 min after weaning from CPB. Forced oscillations were applied to measure airway resistance (Raw), inertance (Iaw), lung tissue damping (G) and elastance (H). Mainstream capnography was performed to assess third phase slope of the expired CO2 (SIII) and respiratory dead space indices. Fowler's dead space (VDF) reflecting the volume of the

conductive airways, Bohr's dead space (VDB) including also the unperfused alveolar volume, and Enghoff's dead space (VDE) comprising additionally the volume of the perfused but not ventilated alveoli were determined. The VDE VDB difference reflecting the intrapulmonary shunt was also calculated.

Results: CPB induced significant increases in Raw (143±15[SE]%), G (130±6%), H (7±0.5%) and SIII (38±17%, $p < 0.02$ for all). Conversely, Iaw (-178±25%) and the ventilation dead space variables decreased after CPB (-12±0.1% and -10±0.1% for VDB and VDF, respectively; $p < 0.01$). These changes caused significant elevation in the intrapulmonary shunt (38±0.6%, $p < 0.001$).

Conclusions: After CPB, the narrowing of the airways and their volume loss is revealed by the rises in Raw and the drops of Iaw and VDF. The increases in SIII, G and H suggest atelectasis development with concomitant augmentation of ventilation heterogeneities. Elevated intrapulmonary shunt is substantiated from the increased VDE-VDB. These findings demonstrate the deleterious pulmonary effects of CPB, with providing evidence for the rapid development of disperse mechanical and ventilation defects affecting the central and peripheral lung compartments.

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S3-E6

Metabolic risk factors in insulin resistant vs. insulin sensitive asthma patients

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The current study assesses the metabolic risk status of asthma patients visiting the outpatient unit of Dept. of Pulmonology between August 15 2012 and October 15 2013. The metabolic state was characterized by the HOMA index (insulin resistance: HOMA index ≥4.4). Lung function was quantified by whole body plethysmography. Quality of life (QoL) was characterized using the St. George Respiratory Questionnaire. Overall 164 asthmatic patients were included in our sample (90 male, 74 female, average age 46,38 ± 14,87 years), of whom 36 proved to be insulin resistant. There was no significant difference between the insulin sensitive and insulin resistant group regarding the age, gender, smoking habits, total cholesterol, LDL-C, Lp(a) and ApoB levels. In contrast, several laboratory parameters were higher in insulin resistance: HgA1c ($p=0.0002$), triglyceride ($p < 0.001$), CRP ($p=0.048$) and uric acid ($p=0.027$). In turn, HDL-C ($p=0.003$) and ApoA1 ($p=0.022$) levels were lower for the insulin resistant group. Body mass index ($p < 0.001$) and waist circumference ($p < 0.001$) were higher among insulin resistant patients. Lung function differed as well: the residual volume compared to the reference value was lower in the insulin sensitive group ($p=0.02$). Of the four domains assessing the QoL, the activity ($p=0.043$) and total score ($p=0.039$) showed significant differences. It is concluded that asthmatic patients with insulin resistance have several untoward metabolic derangement and poorer quality of life compared to those asthmatic patients who are insulin sensitive. This advocates the need for multidisciplinary action in the disease management of asthma.

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S4-A

Sodium Signalling in Astroglia

S4-A1

The mitochondrial 3Na+/Ca2+ exchanger NCLX is a hub for cellular and mitochondrial Ca2+ signaling in astrocytes. or Na+

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Powered by the mitochondrial membrane potential Ca2+ permeates into the mitochondria via the mitochondrial Ca2+ uniporter and pumped out by the activity of a 3Na+/Ca2+ exchanger. This mitochondrial Ca2+ shuttling is playing a key role in coupling Ca2+ signal to ATP production or regulation of Ca2+ signals in the ER and plasma membrane micro domains while its breakdown leads to mitochondrial Ca2+ overload, the hallmark of brain and cardiac diseases. Despite the importance of mitochondrial Ca2+ shuttling the molecular identity of the uniporter and exchanger remain elusive. In the first part of my talk I will describe the identification of the mitochondrial Na+Ca2+ exchanger termed NCLX and the tools we use to control and monitor its activity. In the second part of my talk I will focus on the physiological role of NCLX in regulating Ca2+ and Na+ signal thereby mediating executive functions ranging from neurotransmission release to proliferation of glial cells. Finally I will describe a novel communication pathway between NCLX with the plasma membrane store operated Ca2+ channel.

S4-A2

Exocytotic glutamate release from astrocytes: Intracellular Ca2+ and Na+ dynamics

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Astrocytes can exocytotically release the gliotransmitter glutamate. Increased cytosolic Ca2+ concentration is

necessary and sufficient in this process. The source of Ca2+ for the Ca2+-dependent mechanically-induced exocytotic release of glutamate from astrocytes predominately comes from endoplasmic reticulum (ER) stores; both inositol trisphosphate (IP3)- and ryanodine-sensitive receptors are involved. An additional source of Ca2+ in this process comes from the extracellular space through the plasmalemmal canonical transient receptor potential 1 protein (TRPC1), which forms channels that are activated by depletion of internal Ca2+ stores, and Na+/Ca2+ exchanger (NCX) operating in reverse mode. Mitochondria can modulate cytosolic Ca2+ levels by affecting two aspects of the cytosolic Ca2+ kinetics in astrocytes. They play a role in immediate sequestration of Ca2+ during the cytosolic Ca2+ increase in stimulated astrocytes using calcium uniporter. As cytosolic Ca2+declines due to activity of pumps, such as the smooth ER Ca2+-ATPase, free Ca2+ is slowly released by mitochondria into cytosol via the mitochondrial Na+/Ca2+ exchanger (NCLX) and transient openings of the mitochondrial permeability transition pore. Taken together, ER, extracellular space and mitochondria, can vary concentration of cytosolic Ca2+ which in turn can regulate Ca2+- dependent vesicular glutamate release from astrocytes. In parallel to this Ca2+ excitability, there are fluctuations in astrocytic cytosolic Na+ levels. While the plasmalemmal Na+/K+-ATPase and Ca2+-ATPase are the major Na+ and Ca2+ extruders in resting astrocytes, they appear less important during times of Ca2+ and Na+ cytosolic loads caused by mechanical stimulation. Unexpectedly, NCX in reverse mode appears as a major contributor to overall Ca2+ and Na+ homeostasis in astrocytes both at rest and when these glial cells were mechanically stimulated. This stimulation of astrocytes results in increases in cytosolic Ca2+ and Na+ levels that are in part due to entry of extracellular cations through TRPC1 containing channels. TRPC channels seem amenable to changes in selective filtering as antibody binding to the TRPC1 pore different

S4-A3
Astrocytic Na+ influences extracellular GABA/glutamate balance in the neocortex

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In the CNS, glutamatergic neurotransmission proceeds with high temporal and spatial resolutions. Synaptically released glutamate diffuses through the synaptic cleft and activates postsynaptic receptors, eliciting a postsynaptic current. Glutamate effects on receptors are terminated by diffusion and by the actions of glutamate transporters (EAATs). Glial EAATs strongly contribute to glutamate removal, and synaptically-activated, glutamate transporter-mediated currents (STCs) can be recorded. In this study, STCs elicited by local electrical stimulation in cortical layer 4 were recorded from layer 2/3 astrocytes. When low [Na+]i (5 mM) intra-pipette solution was used, STCs demonstrated paired-pulse facilitation (PPF) at short (<250 ms) inter-stimulus intervals (ISIs) and paired-pulse depression (PPD) at longer ISIs. Elevation of intra-pipette [Na+]i to 20 mM, i.e. close to its physiological value, reduced PPF of STCs at short ISIs, while PPD at longer ISIs was not affected. STC decay kinetics were slowed in the presence of high [Na+]i. Exogenous GABA increased astrocytic [Na+]i, reduced the mean STC amplitude, decreased PPF at short ISIs, and slowed STC kinetics. All GABA-induced changes were blocked by NO-711 and SNAP-5114, GABA transporter (GATs) antagonists. GAT blockade decreased PPF at short ISIs under control conditions, provided low [Na+]i intra-pipette solution was used. Dialysis of single astrocyte with low [Na+]i solution increased the amplitude and reduced paired-pulse ratio of evoked field potentials recorded in the vicinity of the astrocyte. Thus, endogenous GABA can influence EAAT functioning via GATs and astrocytic [Na+]i modulates the short-term plasticity of STCs and in turn the efficacy of glutamate removal.

S4-A4
Sodium signalling in astroglia

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Astrocytes exhibit their excitability based on variations in cytosolic Ca2+ levels, which leads to variety of signalling events. Only recently, however, intracellular fluctuations of more abundant cation Na+ are brought in the limelight of glial signalling. Indeed, astrocytes possess several plasmalemmal molecular entities that allow rapid transport of Na+ across the plasma membrane: (i) ionotropic receptors, (ii) canonical transient receptor potential cation channels, (ii) neurotransmitter transporters, and (iv) sodium-calcium exchanger. Concerted action of these molecules in controlling cytosolic Na+ may complement Ca2+ signalling to provide basis for complex bidirectional astrocyte-neurone communication at the tripartite synapse.

S4-A5
A putative counter-apoptotic role of the non-gastric H+/K+-ATPASE ATP1AL1 (ATP12A)

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The non-gastric H+/K+-ATPase ATP12A (ATP1AL1) is expressed in a broad variety of tissues, but the knowledge about its function is sparse. We found by RTPCR and/or western blotting, intracellular pH measurements, electron microprobe analysis, cell volume (CV) measurements and flow cytometry that ATP12A is expressed in human myelomonocytic HL60 cells, rat insulinoma Ins-1E cells, human pancreatic islets, the prostate cancer cell lines LNCaP, PC3 and DU-145 as well as in normal and cancerous human prostate tissue. Treatment of HL60 cells with low (1mM) concentrations of butyrate leads to monocyte-directed differentiation whereas higher (5-10mM) concentrations

induce apoptosis as assessed by flow cytometric determination of CD86 expression, CV, cell granularity, caspase activity, phosphatidylserine exposure on the outer plasma membrane leaflet, cell cycle analysis and cell proliferation. Transcriptional up-regulation of ATP12A is evident during apoptosis in HL60 and Ins-1E cells and both cell types exhibit apparent apoptotic volume decrease (AVD). An effect of the H+/K+-ATPase inhibitor SCH28080 is not evident in untreated HL60 cells, but becomes visible in cells are stimulated with 1mM butyrate. Moreover, AVD from 24-72h is not affected by SCH28080 in the untreated non-apoptotic cells. Inducing the cells with 1 mM butyrate reveals the same behavior in PS- cells, however PS+ cells resist to shrink and have finally a significantly higher CV than PS- cells after 72h and the CV of PS+ cells is now sensitive to SCH28080. i.e. PS+ cells treated with SCH28080 have the same cell volume as butyrate treated non-apoptotic cells. This indicates that AVD may depend on differentiation and/or exposure of phosphatidylcholine to the outer leaflet of the cell membrane. Moreover ATP12A expression is altered in tissue from benign hyperplasia of human prostate and in prostate cancer. In summary it is shown that ATP12A is functionally active, plays a role during early apoptosis in HL60 cells and Ins-1E cells, is differently expressed in normal and pathological prostate tissue.

On the other hand, increased levels of 20-HETE mediates cerebral microvessels' vasoconstriction and increases tissue damage after stroke in rats and in human, while increasing levels of EETs or decreasing levels of 20-HETE have beneficial effects. EETs are anti-inflammatory, sustain endothelium-dependent vasodilation in human essential hypertension, and decreased EETs are associated with salt-sensitive hypertension.

The increased production of reactive oxygen species (ROS) underlies disbalance of vasodilator vs. vasoconstrictor eicosanoids. ROS are generated by a variety of enzymes in vascular tissues, but also in circulating and adhering leukocytes.There is a circuitous relationship between ROS and cyclooxygenases (COX1,2) upregulating each other. 20-HETE significantly increases vascular production of ROS. ROS mediate non-enzymatic peroxidation of AA, which gives rise to isoprostanes, particularly to potent vasoconstrictors, 8-epi-PGF2α and 8-epi-PGE2. In oxidative stress, blood levels of isoprostanes are much higher than those of COXs' products.

In conclusion, there is a communication network among various eicosanoids. In physiological conditions eicosanoids are important in maintenance of vascular tone and reactivity, but in condition with increased ROS/isoprotanes formation, the imbalance of the PGI2/TXA2 system and/or in 20HETE/EETs system can occur, which may become deleterious to vascular function, such as in stroke and hypertension

S5-A

From Macro- to Microvessels: Function, Structure and Molecular Mechanisms

S5-A1
Role of metabolites of arachidonic acid in regulation of vascular function

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Eicosanoids, metabolites of arachidonic acid (AA), are crucial in maintenance of vascular tone and reactivity to various physiological stimuli, contributing to blood pressure regulation, appropriate tissue perfusion, and protection of, or susceptibility to atherosclerosis and thrombosis.While the importance of prostaglandin (PGs)/TX balance is well documented, there is an increasing body of evidence that hydroxylation or epoxygenation of AA via CYP450 enzymes significantly contribute to physiological maintenance of vascular tone and vascular reactivity of resistance vessels.20-HETE mediates arterial myogenic constriction and arteriolar response to increased pO2 in vitro and the autoregulation of cerebral blood flow in vivo.

S5-A2
Angiotensin II and leukocyte trafficking: New insights for an old vascular mediator

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Angiotensin-II (Ang-II) is implicated in atherogenesis. We demonstrated that 4h exposure to Ang-II in vivo caused arteriolar leukocyte adhesion in the mesenteric microcirculation of the rat through the interaction with its AT1 receptor (1). While mononuclear cells were the main leukocytes attached to the arteriolar endothelium, neutrophils were predominantly cells interacting with the venular endothelium. Since, the same cell adhesion molecules (CAMs) were expressed on both the arteriolar and venular endothelia in response to Ang-II (1), other mechanisms seemed to be responsible for the differential cellular distribution. Indeed, arteriolar mononuclear leukocyte recruitment by Ang-II was found to be largely mediated by tumor necrosis factor-alpha (TNFalpha) (2) and, fractalkine (CX3CL1) expression was detected in both cremasteric arterioles and post-capillary venules 24h after Ang-II intrascrotal injection (3). Arteriolar leukocyte adhesion was the unique parameter significantly reduced (83%) in animals lacking CX3CL1 receptor (CX3CR1). When human arterial and venous umbilical endothelial cells (HUAEC and HUVEC) were stimulated with 1 μM Ang-II increased CX3CL1 expression was detected, yet neutralization of CX3CL1 activity only significantly inhibited Ang-II-induced mononuclear cell-HUAEC interactions. The use of siRNA revealed the involvement of TNFalpha in Ang-

II-induced CX3CL1 up-regulation and mononuclear cell arrest. Nox5 knock down with siRNA or pharmacological inhibition of ERK1/2, p38 MAPK and NFκB also abolished these responses. These results suggest that targeting CX3CL1-CX3CR1 axis may constitute a new therapeutic strategy in the treatment of Ang-II-associated cardiovascular disorders.

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S5-A3
Microvascular mechanisms of age-related cognitive decline

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Hypertension in the elderly substantially contributes to cerebromicrovascular damage and promotes the development of vascular cognitive impairment. Despite the importance of the myogenic mechanism in cerebromicrovascular protection, it is not well understood how aging affects the functional adaptation of cerebral arteries to high blood pressure. Hypertension was induced in young (3 months) and aged (24 months) C57/BL6 mice by chronic infusion of angiotensin II (AngII). In young hypertensive mice, the range of cerebral blood flow autoregulation was extended to higher pressure values, and the pressure-induced tone of middle cerebral artery (MCA) was increased. In aged hypertensive mice, autoregulation was markedly disrupted, and MCAs did not show adaptive increases in myogenic tone. In young mice, the mechanism of adaptation to hypertension involved upregulation of the 20-HETE (20-hydroxy-5,8,11,14-eicosatetraenoic acid)/transient receptor potential cation channel, subfamily C (TRPC6) pathway and this mechanism was impaired in aged hypertensive mice. Downstream consequences of cerebrovascular autoregulatory dysfunction in aged AngII-induced hypertensive mice included exacerbated disruption of the blood-brain barrier and neuroinflammation (microglia activation and upregulation of proinflammatory cytokines and chemokines), which were associated with impaired hippocampal dependent cognitive function. Collectively, aging impairs autoregulatory protection in the brain of mice with AngII-induced hypertension, potentially exacerbating cerebromicrovascular injury and neuroinflammation.

S5-A4
Role of antioxidant genes and microRNAs in revascularisation after hind limb ischemia

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Nuclear factor, erythroid 2-like 2 transcription factor (Nrf2, encoded by NFE2L2) and heme oxygenase-1 (HO-1, encoded by HMOX-1 gene), one of its major targets, are crucial mediators of cellular defence response against oxidative stress. Accordingly, their involvement in numerous diseases has been widely investigated. However, it appears that the functions of both Nrf2 and HO-1 extend much beyond their canonical protective activity. We and others have elucidated that the HO-1 is playing a role in blood vessels formation and such an effect of HO-1 in regeneration after skin injury, hind limb ischemia and myocardial infarction has been demonstrated. Our recent studies showed also that angiogenic response of endothelial cells and bone-marrow derived proangiogenic cells is impaired in the absence Nfe2l2 and Hmox-1. Accordingly, lack of HO-1, as evidenced by the studies of Hmox-1 deficient animals attenuates regeneration after hind limb ischemia and even haplodeficiency of Hmox-1 decreased revascularisation in diabetic animals. Genetic overexpression of Hmox-1 by hypoxia-regulated vectors provides faster restoration of vascularisatiuon and, additionally, combined overexpression of HO-1 and VEGF-A is able to fully restore hind limb circulation in Hmox-1 knockout mice. Surprisingly, in contrast to HO-1, the lack of Nrf2 promotes blood vessel regeneration after hind limb ischemia, indicating for the context-dependent role of Nrf2 in inflammatory and non-inflammatory angiogenesis.

Recent studies indicate for the significant role of non-coding microRNAs in blood vessel formation. Among angiomirs the miR-378 appears to be crucial for revascularisation. The cross-talk interactions of this miRNA with HO-1 have been recently described by us. Interestingly, the lack of miR-378 appears to significantly affect the vasculature regeneration after hind limb ischemia.

Understanding of the role of antioxiodant genes and their interactions with non-coding RNAs can provide the rationale for novel approaches in revascularisation therapies.

S5-A5
A new functional role of Ca2+ sensitization mechanisms in the regulation of vascular smooth muscle contraction

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Introduction: Cardiovascular diseases related to sustained arterial contraction are an important cause of mortality and morbidity in humans. Although the increase in [Ca2+]i can cause contraction of smooth muscle, arterial contraction can be regulated even if the concentration of this ion remains constant. This mechanism, called "Ca2+ sensitization", participates in the maintenance of arterial contraction through the inhibition of the activity of myosin light chain phosphatase (MLCP). Although RhoA/Rho kinase (ROCK) and PKC can both inhibit MLCP, these two pathways not only work in parallel, but they can also interact. We have studied the role of both, PKC and RhoA/ROCK, sensitization pathways in PDBu-treated arteries.

Materials and Methods: Isometric force was measured in rat femoral arterial rings. Immunofluorescence experiments were performed on isolated myocytes. PKC and RhoA were analyzed by Western blot and G-LISA in arterial rings precontracted with PDBu, a PKC activator.
Results: PDBu induced the activation and translocation of PKCα to the plasma membrane. PDBu also evoked an increase in cytosolic RhoA, suggesting its inhibition. Simultaneous application of ROCK and PKCα inhibitors induced a synergistic, non-additive, vasorelaxant effect on sustained contraction in PDBu-treated arteries.
Discussion: Here we describe a new form of crosstalk between PKCα and RhoA pathways. As PKCα negatively regulates RhoA activity, PKCα inhibitors could facilitate the vasorelaxant effect of ROCK inhibitors. Simultaneous treatment with ROCK and PKCα inhibitors could provide a new strategy to optimize the therapeutic treatment of pathological processes that mediates arterial vasospasm.

S5-A6
Myeloperoxidase promotes the vasoconstrictive effects of hydrogen-peroxide

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Myeloperoxidase (MPO) is a central participant in inflammatory processes; however recent studies emphasize the involvement of MPO in the development of cardiovascular diseases. MPO catalyzes the reaction of hydrogen-peroxide (H2O2) with chloride (Cl-) to form hypochlorous acid (HOCl).
We detected the changes of internal diameter (id.) of cannulated and pressurized (80 mmHg) skeletal muscle arterioles (SKA, id.: 160±6 μm) and coronary arterioles (CA, id.: 178±14 μm), moreover we examined the changes of the contractile force of the basilar artery (BA). The changes in [Ca2+]i by Fura-2 fluorescence ratio (F340/380) were also determined in SKA.
H2O2 has a biphasic effect in the SKA and the BA (constriction at lower, and dilation at higher concentrations) however; it evokes only vasodilation in the CA. In the presence of MPO (5 nM) the H2O2-induced constriction was increased in SKA (47±11% constriction vs. 50±21% dilation at 1 mM H2O2, P=0.004), and in the BA (114±14% vs.88±6% of KCl evoked maximal constriction measured at 100 μM H2O2). The H2O2-evoked dilation turned into constriction in CA (6±3% constriction vs. 13±4% dilation at 100 μM H2O2, P=0.006) after incubation with MPO. The mechanism of the MPO evoked increased constriction was investigated in SKA. The HOCl scavenger L-methionine inhibited the MPO and H2O2 induced constriction (91±2% dilation vs. 47±11% constriction at 1 mM H2O2, P <0.0001). In the absence of endothelium the constriction was still observed in lower concentrations of H2O2 (31±8% vs. 26±4% constriction at 100 μM H2O2) but it was abolished in higher concentrations of H2O2 (28±21% dilation vs. 47±11% constriction at 1 mM H2O2). Furthermore, the constriction was converted to dilation (70±21% dilation vs. 47±11% constriction at 1 mM H2O2, P=0.001) by thromboxane A2 (TXA2) receptor inhibition. MPO and H2O2 induced constriction was not accompanied by significant changes in F340/380.
Our results suggest that MPO increases the H2O2-evoked constriction by the generation of HOCl which causes the synthesis of TXA2 in the endothelial and smooth muscle cells leading to an increase in the Ca2+ sensitivity of the vascular smooth muscle.

S4-B

Lipid GPCRs in Physiology and Disease

S4-B1
Novel GPCRs for lysophosphatidylserine; their structure and function

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It is now accepted that lysophospholipids (LPLs) have a wide variety of functions as lipid mediators that are exerted through G protein-coupled receptors specific to each lysophospholipid. While the roles of some LPLs, such as lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P), have been thoroughly examined, little is known about the roles of several other LPLs, such as lysophosphatidylserine (LysoPS), lysophosphatidylthreonine (LPT), lysophosphatidylethanolamine (LPE), lysophosphatidylinositol (LPI) and lysophosphatidylglycerol (LPG). Recently, three GPCRs (GPR34/LPS1, P2Y10/LPS2 and GPR174/LPS3) were found for LysoPS. In this talk, I will focus on these newly identified GPCRs and summarize the actions of LysoPS as lipid mediators.

S4-B2
Cannabinoid type 1 receptor in noradrenergic/adrenergic cells and its role in metabolism and stress

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Neuropeptide Y (NPY) and stress has been associated with obesity. However, it is still unknown why some people lose weight under stress. Our study suggests that specific deletion of cannabinoid type 1 receptor (CB1) from dopamine

β-hydroxylase (dbh) expressing neurons in mice disturbs the homeostatic equilibrium between β-adrenergic and NPY transmission, making mutant mice resistant to diet induced obesity through increased β-adrenergic transmission in white and brown adipose tissue (BAT). However, these mutant mice when exposed to chronic stress gain weight through increased NPY transmission in visceral fat independent of ongoing higher thermogenesis in BAT.
Our study suggests CB1 signaling in dbh neurons may be crucial for sensitivity to stress-induced weight gain or loss. Furthermore, stress- and NPY-induced obesity cannot be corrected by increasing thermogenesis alone and requires inhibition of NPY transmission in visceral fat.

S4-B3
New functions for short chain fatty acid and prostanoid receptors

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G-protein-coupled receptors (GPCRs) have traditionally been regarded as receptors for hormones, neurotransmitters, and other mediators which are produced solely for the purpose of carrying a signal and to serve cell-cell communication. This view has changed during the last decade, as a growing number of GPCRs are being identified, for which the ligands are energy substrates or metabolic intermediates. Among these ligands are saturated and unsaturated free fatty acids (FFAs), which exert cellular effects through GPCRs named FFA1-FFA4. These receptors are widely expressed in the human body and regulate the metabolic, endocrine or immune system to maintain homeostasis under changing dietary conditions. Data will be presented on the role of FFA2 and FFA3 in the regulation of metabolic functions. In addition, recent research on the identification of novel lipid ligands of prostanoid receptors will be discussed.

S4-B4
Control of gastrointestinal epithelial integrity by lysophosphatidic acid GPCR

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Lysophosphatidic acid (LPA) is a pleiotropic growth factor-like lipid mediator that acts via at least six GPCR. Although LPA is produced in blood and other biological fluids from lysophosphatidylcholine and lysophosphatidylserine by the lysophospholipase D autotaxin, it is also present in foods. The LPA2 GPCR is abundantly expressed in gastrointestinal epithelial cells and for this reason we have studied its role in the maintenance of gastrointestinal epithelial function. In this talk we will review the following aspects of LPA2 signaling in the GI epithelium:

1) Inhibition of CFTR-mediated secretory diarrhea by the LPA2-NHERF2 macromolecular complex.
2) Protection of the small intestinal stem cells from radiation- and chemotherapeutic-induced apoptosis by the LPA2-Siva-1 and LPA2-NHERF2-TRIP6 macromolecular signalosome.
3) Enhancement of DNA repair mediated by LPA2.
4) Protection against NSAID-induced gastric erosions by LPA2-selective agonists.

Our results point to a comprehensive role of the LPA2 GPCR in the maintenance of the functional integrity of the gastrointestinal epithelium.

S4-B5
Signaling pathways of thromboxane receptor-mediated vasoconstriction: Major role of phospholipase C epsilon

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We aimed to elucidate the intracellular signaling pathways of the sustained vasoconstriction and hypertension induced by thromboxane A2 (TXA2) via stimulation of TP prostanoid receptors. Isometric tension recording was performed in thoracic aortic segments (TAs) isolated from wild-type mice (WT) as well as from mice deficient in TP receptors (TP-KO), the alpha subunits of q/11 or 12/13 G-proteins (Gq/11-KO and G12/13-KO) or PLCε (PLCε-KO). Phosphoinositol-hydrolysis was monitored by measurement of 3H-inositol-phosphate (3H-InsP) formation in TAs. Changes of intracellular calcium levels [Ca2+]i in TAs and primary cultures of vascular smooth muscle cells (VSMC) were assessed by ratiometric measurement of Fura-2-AM fluorescence. Arterial blood pressure was determined in ketamine/xylazine anesthetized animals. 12/13-KO mice showed decreased contraction and [Ca2+]i elevation in response to the TP receptor agonist U-46619, which failed to induce any effect in TP-KO vessels. Interestingly, the U-46619 induced Ca2+ signal in Gq/11-KO VSMC persisted in Ca2+-free medium and was unaffected by the Rho-kinase (ROCK) inhibitor Y-27632, but almost completely abolished by the RhoA inhibitor TAT-C3. Since RhoA can activate reportedly PLCε we further investigated this pathway. TAs from PLCε-KO mice showed significantly decreased vasoconstriction, 3H-InsP accumulation and [Ca2+]i, elevation upon administration of U-46619 whereas the effects of the alpha1-adrenoreceptor agonist phenylephrine remained unchanged. In accordance, the hypertensive effect of U-46619 but not that of norepinephrine decreased in PLCε-KO as compared to WT mice.αTAs from both Gq/11-KO and G Our results indicate that q/11 and 12/13 G-proteins are

simultaneously involved in the mediation of TXA2-induced vasoconstriction. While the Gq/11-dependent effect is likely to be mediated by PLCbeta, the Gq/11-independent pathway also involves phosphoinositol-hydrolysis and subsequent intracellular Ca2+ release in a RhoA-dependent, but ROCK-independent manner.
Our results suggest that G12/13- and RhoA-mediated activation of PLCε plays a major role in TXA2-induced vasoconstriction and hypertension.

S4-B6
Mutations in the conserved 'DRY' motif of the CB1 cannabinoid receptor result in functionally selective receptor conformations

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The role of the highly-conserved 'DRY' motif in the signaling of the CB1 cannabinoid receptor (CB1R) was investigated by introducing single, double and triple alanine mutations into this site of the receptor. We found that the CB1R-R3.50A (CB1R-DAY) mutant displays a partial decrease in its ability to activate heterotrimeric Go proteins (~85% of wild-type CB1R (CB1R-WT)). Moreover, this mutant showed impaired β-arrestin binding in response to agonist stimulus, although its basal β-arrestin binding was enhanced. More strikingly, the double mutant CB1R-D3.49A/R3.50A (CB1R-AAY) was biased toward β-arrestins, as it gained a robustly increased β-arrestin2 binding ability compared to the wild-type receptor, while its G protein activation was substantially decreased. In contrast, the double mutant CB1R-R3.50A/Y3.51A (CB1R-DAA) proved to be G protein-biased, as it was practically unable to recruit β-arrestin2 in response to agonist stimulus, while still activating G proteins, although at a lowered level (~75% of CB1R-WT). Agonist-induced internalization as well as ERK1/2 activation of the CB1R mutants showed good correlation with their β-arrestin binding ability, but not with G protein activation. Our results suggest that G protein activation and β-arrestin binding of the CB1R are mediated by distinct receptor conformations and that the conserved 'DRY' motif plays different roles in the stabilization of these conformations, thus mediating both G protein- and β-arrestin2-mediated functions of CB1R.

S5-B

Physiology and Regulation of K2P Channels

S5-B1
Excitability tuning by two-P-domain channels: From inhibitory potassium-selective channels to excitatory cationic channels

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Potassium channels set the resting membrane potential, favouring hyperpolarization and inhibition of cell excitability. Gene mutations leading to a loss of selectivity for potassium in different potassium channels are related to severe disorders in mouse and human.
We have shown that such a change in ion selectivity is not only observed in pathological conditions but also in normal physiology. The background K2P channel TWIK1 is the very first example of a channel that reversibly shifts from a potassium-selective state to a sodium-permeable state upon physiological stimuli. This result suggests that a single channel can fulfill opposite roles traditionally attributed to different classes of ion channels: either hyperpolarizing and inhibitory such as potassium and chloride channels, or depolarizing and excitatory such as sodium, calcium and cationic channels. Dynamic ion selectivity also occurs in other mammalian K2P channels, as well as likely in those of Caenorhabditis elegans. This broad distribution and evolutionary conservation among K2P channels indicates that dynamic selectivity constitutes a new regulatory mechanism of cellular excitability, whose significance is only now becoming appreciated.

S5-B2
The intracellular traffic of the two-pore-domain potassium channel TASK-1

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Background. It is still unclear to what extent membrane proteins determine the itinerary and the destination of the transport vesicles in which they reside. In many cases the intracellular traffic of membrane proteins is controlled by specific interacting proteins. We have shown previously that the K2P-channel TASK-1 interacts with 14-3-3 proteins and with p11 (S100a10). Recently, we found that TASK-1 also interacts with the endosomal SNARE protein syntaxin-8 and studied the functional relevance of this interaction.
Principal findings. TASK-1 and syntaxin-8 were expressed in Xenopus oocytes and mammalian cell lines. Co expression of syntaxin-8 caused a four-fold reduction in TASK-1 current, a marked reduction in the expression of TASK-1 at the cell surface and an increase in the rate of endocytosis of the channel. Co-expression of the SNARE protein syntaxin-7 had no effect on TASK-1 currents. Systematic mutagenesis experiments showed that the C-terminus of TASK-1 interacts with a 40 amino-acid region proximal to the SNARE domain of syntaxin-8. The stimulatory effect of the SNARE protein on the endocytosis of the channel was abolished when both an endocytosis signal in TASK-1 and an endocytosis signal in syntaxin-8 were mutated. A syntaxin-8 mutant which cannot

assemble with other SNARE proteins had virtually the same effects as wild-type syntaxin-8. TIRF micro¬scopy showed formation and endocytosis of vesicles containing fluorescence-tagged clathrin, TASK-1 and/or syntaxin-8.
Conclusions. Our results suggest that the unassembled form of syntaxin-8 and TASK-1 are internalised via clathrin-mediated endocytosis in a co-operative manner. The adaptor protein AP-2 possesses separate binding sites for tyrosine and di-leucine-based endocytosis signals. In vitro binding assays suggested that AP 2 exhibits tighter binding to a membrane harboring both di-leucine- and tyrosine-based sorting signals [6]. Now we have shown for the first time that this is indeed the case in intact cells. This implies that syntaxin-8 regulates the endocytosis of TASK-1, and vice versa. In addition, our study shows that SNARE proteins can have functions unrelated to membrane fusion.

S5-B3
Pharmacological and genetic recovery of current through truncated and mutated K2P channels

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Two pore domain potassium (K2P) channels contribute to background potassium conductances in many cell types, being active over the entire voltage-range. However their activity can be up or down regulated by a variety of physiological and pharmacological mediators (Enyedi & Czirják, 2010). Using molecular modelling, site directed mutagenesis and electrophysiological approaches, my laboratory is investigating how both gain of function mutations and physiological and pharmacological mediators can give insight into the functional properties of K2P channels and what regions of the channel are important for both gating and the transduction of a regulatory signal to the gate. In this presentation, I will focus on our recent experiments on two K2P channels, TREK1 (TWIK-related K channel 1, K2P2.1, KCNK2) and TASK3 (TWIK-related acid-sensitive K channel 3, KCNK9, K2P9.1).

TREK1 can exist in two forms following alternative translation initiation (Thomas et al 2008). Each of these forms is expressed as protein in both neurons and expression systems The shorter, N-terminus truncated form of TREK1 gives rise to a current with a much reduced open probability, a reduced potassium selectivity and a measurable permeability to sodium (Thomas et al., 2008). We have found that the current through this short form of TREK1 is greatly enhanced by non-steroidal anti-inflammatory drugs such as flufenamic acid (FFA). In addition, FFA alters the ion selectivity of this channel so that it becomes a K selective channel, like the long form of TREK1. The effects of these drugs are also seen following gain of function mutations of the short form of the channel (Veale et al., 2014b).

TASK3 channels are the only K2P channels known to be genetically imprinted, in that they are expressed only from the maternal allele. Indeed a mutation in TASK3 (G236R) is responsible for a maternally transmitted developmental disorder, Birk Barel mental retardation dysmorphism syndrome (Barel et al., 2008). We have considered the functional properties of G236R mutated TASK3 channels in some detail. Expression of TASK3_G236R channels gives rise to small but detectable functional current. In contrast to WT TASK3 channels, the current is inwardly rectifying and this current is differentially sensitive to a number of regulators such as extracellular acidification and activation of G protein coupled receptors. Importantly, the reduced current through mutated TASK3_G236R channels can be overcome, at least in part, by both application of FFA and by a gain of function mutation of TASK3 channels (Veale et al., 2014b).

Our work on truncated TREK1 channels and mutated TASK3 channels shows that it is possible to restore current through these channels through manipulations that alter channel gating. Pharmacological manipulation of these channels, in particular, may have potential therapeutic applications.

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S5-B4
TRESK background K+ channel is regulated by calcineurin and other interacting proteins

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Members of the K2P channel family are controlled by a great diversity of regulatory mechanisms. Excitable cells typically express different combinations of K2P channels in order to adjust their resting membrane potential and counter excitatory stimuli. TRESK (K2P18) is predominantly expressed in the sensory neurons of dorsal root and trigeminal ganglia, together with TREK-2 (K2P10) and minor amounts of other K2P channels. At present, the specific physiological roles of these K2P components in pseudounipolar neurons are incompletely understood, although the inactivating mutation of TRESK was reported to be linked to a rare form of familial migraine, suggesting the functional relevance of the channel.
In the past decade we have investigated the regulatory mechanisms of TRESK in vitro and in heterologous expression systems. We have established that in contrast to the other K2P channels, TRESK is regulated by the elevation of the cytoplasmic calcium concentration. This activation depends on the calcium/calmodulin-dependent protein phosphatase calcineurin. Alanine-scanning mutagenesis indicated three serines in TRESK as the putative targets of dephosphorylation. Calcineurin directly associates to TRESK via a Nuclear Factor of Activated T cells (NFAT)-like binding site located in the cytoplasmic loop of the channel. Such a mechanism of calcineurin targeting is unique within the ion channel superfamily. In the present lecture, we are going to reveal a previously unknown level of the intimate interaction of the channel with the phosphatase.
Basal channel activity is maintained, and calcineurin-dependent activation is reversed after the cessation of the

calcium signal, by inhibitory kinases. One regulatory serine is phosphorylated by protein kinase A (PKA) and this phosphorylation allows the anchoring of 14-3-3 adaptor protein to the channel. 14-3-3 may have inhibitory effect by preserving the phosphorylation of the channel under resting conditions. We have also detected the interaction of a third protein with TRESK by a recent affinity chromatography approach, however, it remains to be established whether this interaction is relevant in vivo.

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S5-B5
Amusing functions of TWIK-1 in the brain

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TWIK-1 is the first identified K2P channel which is highly expressed in the brain. However, its physiological role has been elusive because it has been demonstrated as a non-functional channel. Recently, we found that TWIK-1 form a heterodimer with TREK-1 to be functional in astrocytes. These heterodimeric channels via disulfide bridge formation mediate the potassium passive conductance and Gβγ-induced glutamate release in astrocytes. In my talk, I will discuss the role of TWIK-1 in astrocytes and present some of our recent data on its function in CNS neurons.

S5-B6
Stable gene silencing of TASK-3 channels in melanoma cells induce intrinsic apoptosis

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We have previously demonstrated a primarily mitochondrial localisation of the TASK-3 potassium channels in cultured melanoma cells. We hypothesised that mitochondrial TASK-3 channels may exert antiapoptotic effects via contributing to mitochondrial function, most likely by maintaining mitochondrial membrane potential. To confirm this hypothesis and to study possible other functions of TASK-3 channels, we employed RNA interference. Our present experiments were conducted on WM35 cells in which TASK-3 biosynthesis was stably knocked-down. WM35 cells that were stably transfected with a scrambled RNA sequence served as control. To monitor mitochondrial function, Jc-1 fluorescent dye was applied at a concentration of 5 µg/ml. Mitochondrial depolarisation was evoked by carbonyl cyanide m-chlorophenylhydrazone (50µmol/l CCCP). TASK-3 knock-down cells had depolarised mitochondrial membrane potential. In addition, their mitochondrial membrane could be more easily depolarised, suggesting that melanoma cells having reduced TASK-3 expression are less capable of increasing their mitochondrial activity in response to metabolic challenges. An MTT assay, that measures mitochondrial reducing capacity, also indicated reduced mitochondrial function in the knock-down cell cultures. In addition, TASK-3 gene-silenced cells showed slower proliferation rate (confirmed by Cyquant assay) and increased Annexin V binding. The latter observation indicates that knock-down melanoma cells are more prone to apoptotic cell death. Knock-down cells also had decreased cell volume which may be the result of apoptotic volume decrease. During the apoptotic events the translocation of AIF from mitochondria to cytosol and cell nuclei occurs.
Our data indicate that reduced TASK-3 expression of the melanoma cells results in mitochondrial depolarisation, reduced mitochondrial function, decreased rate of proliferation, and a markedly increased rate of intrinsic apoptosis.
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S4-C

Physiology and Pathophysiology of Bicarbonatesecretion in the Airways – Key to Therapy of Cystic Fibrosis

S4-C1
Anion secretion by calcium-activated anoctamin chloride channels: A direct or indirect mechanism?

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Cystic fibrosis (CF) is characterized by reduced Cl- secretion due to impaired function of the cystic fibrosis transmembrane conductance regulator (CFTR) and probably enhanced Na+ absorption through amiloride-sensitive epithelial sodium channels (ENaC). Electrolyte secretion by the airways is necessary to produce the airway surface liquid that allows for mucociliary clearance of the lungs. More recently the requirement of CFTR for airway bicarbonate secretion has been increasingly recognized, leading a decrease of the pH in the airway surface liquid of CF patients and CF-animal models. Anion secretion is driven by opening of Cl- selective ion channels in the apical membrane of airway epithelial cells,

through either receptor mediated increase in intracellular cAMP or cytosolic Ca2+. Traditionally cAMP-dependent and Ca2+-dependent secretory pathways are regarded as independent. However, this concept has been challenged recently. With identification of the Ca2+ activated Cl- channel TMEM16A (anoctamin 1) and with increased knowledge of the cAMP-regulated cystic fibrosis transmembrane conductance regulator (CFTR) Cl- channel, it became possible to look more closely into the relationship of both ion channels. Mice lacking expression of anoctamin 1 in the airways demonstrate a CF-like lung phenotype, suggesting that anoctamin 1 and possiblly additional anoctamin paralogues also expressed in airway epithelial cells are essential in mouse airways for Cl- and possibly bicarbonate secretion.
Thus anion secretion by anoctamins may occur by forming separate apical exit pathways, or indirectly by supporting the secretory function of CFTR.

S4-C2
HCO3-, fluid, mucus and the structure of small airways

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The surfaces of the lumens of small airways are continually exposed to exogenous debris and pathogens as well as accumulated endogenous products. Since these surfaces, excepting the alveoli, represent the largest surface of the lung, extremely efficient mechanisms of clearance must operate to defend the tissue from infection, inflammation, and obstruction while maintaining lumens patent for airflow. Toward that end, the surfaces maintain a liquid layer that normally flows easily along the luminal surface without obstructing the lumen.
Since secretory glands are few or absent from the smaller airways, the structures must perform both fluid absorption and fluid secretion to maintain a critical balance between dehydration and inundation of the airway. Microanatomy shows that these airways are anastomosing tubes whose luminal surface is corrugated into longitudinal folds and pleats that run in parallel from the respiratory bronchioles to the larynx. Accepting that the anatomy allows expansion and contraction of the tubes without a change in the epithelial surface, our data indicate that the folds and pleats, respectively, are also the sites of concurrent fluid absorption and fluid secretion. Moreover, in general, mucin-secreting goblet cells tend to be aggregated in the basal zones of the pleats.
Results also show that: a.) small airways continuously secrete HCO3-, b.) both cAMP and Ca++ mediated agonists can modulate HCO3- secretion, and c.) like fluid secretion, such HCO3- secretion likely occurs in the basal regions of pleats as well. Given the recent reports that HCO3- is critical for bactericidal properties of innate anti-microbial peptides and that HCO3- is essential for transforming mucins compacted in granules into hugely expanded networks of transportable mucus gels, the structures of pleats and folds appears to be crucial for local integration of: a.) epithelial HCO3- secretion, b.) water and solute coupling for isotonic fluid secretion, and c.) strategic maintenance of surface fluid volumes, into a local mechanism for clearing pathogenic material from small airway surfaces.

S4-C3
The importance of bicarbonate and proteases for mucin secretion and mucus formation

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In contrast to previous ideas, mucin is packed in the mucin granulae of goblet cells in a highly organized way by calcium and low pH mediated interactions of the N-terminal part of the mucin (1). Further structural studies have revealed molecular details that explain how the mucin packed on concatenated rings are organized in relation to each other. This will allow an organized release that is triggered by removal of calcium ions. The natural way of accomplishing this is by sufficiently high concentrations of bicarbonate. Cystic Fibrosis (CF) is caused by a non-functional chloride and bicarbonate ion channel (CFTR) and mice lacking functional CFTR have to humans similar ileal problems with mucus stagnation. Interestingly, the ileal CF mucus adhered to the epithelium, was denser, and less penetrable than that of wild-type mice (2). The adherent ileal CF mucus was normalized by secretion into a high concentration sodium bicarbonate. However, deeper studies of this process revealed that the unfolding of the main mucus component, the MUC2 mucin, was not sufficient to detach the mucin as a specific enzyme was also necessary to cleave in the MUC2 mucin. The lower levels of secreted bicarbonate in CF hindered this cleavage as the mucin was not sufficiently unfolded to allow the cleavage to take place. This knowledge allows novel studies of novel ways of testing mucus release and therapeutic options for a mucus stagnation.

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S4-C4
Effects of bicarbonate and pH on bacterial growth and MIC of Erythromycin

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In cystic fibrosis (CF), defective transepithelial anion transport is associated with retention of thick mucus, lower pH and reduced bacterial killing in the airways. Aerosolizing bicarbonate into CF pig airways increased bacterial killing in vivo. Thus, we investigated the effects of external pH and bicarbonate on the growth of different bacteria, as well as on the efficacy of erythromycin against S. aureus. We compared the effects of adding NaHCO3 or NaCl to media on the growth of E. coli, S. aureus, S. agalactiae, E. faecalis and H. influenzae. The growth rate of bacteria was followed by measuring the optical density. Erythromycin MIC was determined by agar dilution: a) in the presence and absence of NaHCO3, b) in ambient air versus 5%/20% CO2, and c) in a pH range of 7.1-9.0. We found that NaHCO3 significantly inhibited bacterial growth in all strains, whereas NaCl did not influence growth. Bicarbonate was bacteriostatic rather than bactericidal. Alkalinizing external pH reduced the MIC values of erythromycin significantly. However, bicarbonate did not alter erythromycin MICs when both external pH and CO2 concentrations were held constant. The data indicate that bacterial growth is inhibited by HCO3- per se rather than by HCO3--induced alkaline pH or higher osmolality. Increased efficacy of erythromycin appeared to be due more to alkaline pH and not solely to bicarbonate alone. Administration of aerosolized bicarbonate may transiently raise airway pH, inhibit bacterial growth and improve the efficacy of antibiotic therapy in CF and other inflammatory lung diseases.

S4-C5
HAT-7 cells, a new model to study the intracellular pH regulation and bicarbonate transport of ameloblasts

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Formation and growth of hydroxyapatite crystals during amelogenesis by ameloblasts generates a large number of protons that must be neutralized. Ameloblasts express a number of transporters and channels involved in bicarbonate transport in other epithelia. However, to date there is no functional evidence for bicarbonate transport in these cells. Therefore, we aimed to develop a novel two dimensional monolayer to model the bicarbonate transport by ameloblasts using HAT-7, a rat cell line of ameloblast origin. HAT-7 cells were seeded onto Transwell permeable filters. We used three different media to test tight junction formation. Transepithelial resistance (TEER) was measured by an epithelial volt-ohm meter. The expression of transporters and tight junction proteins was investigated by RT-PCR. Intracellular pH regulation and bicarbonate transport was measured by microfluorometry. HAT-7 cells formed polarized epithelia on permeable supports. TEER was significantly higher in Hepatostim medium and, to a lesser extent, in Differentiation medium (DMEM-F12 containing 2.1 mM calcium and 10 nM dexamethasone) compared to control DMEM-F12, indicating the formation of tight epithelia. HAT-7 cells in all three media expressed tight junction proteins and key transporters previously identified in ameloblasts. The intracellular pH increased rapidly in response to basolateral CO2/HCO3- containing solution, as bicarbonate entered the cells. The bicarbonate uptake was sensitive to 0.1 mM acetazolamide (carbonic anhydrase inhibitor) and 0.5 mM H2DIDS (AE/NBC inhibitor) indicating intracellular HCO3- accumulation in HAT-7 cells by anion transporters and/or carbonic anhydrases. Furthermore, cells were polarized for membrane CO2 permeability as the apical membrane has much higher permeability than the basolatereal one. In conclusion, HAT-7 cells i) can form tight junctions, ii) express typical tight junction proteins and electrolyte transporters, iii) are functionally polarized and iv) can accumulate bicarbonate from the basolateral side. Therefore, the HAT -7 cell line is suitable to study bicarbonate transport by ameloblasts.

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S4-C6
The role of aquaporins in pancreatic ductal cells

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Background. Acute pancreatitis (AP) is a multicellular disease in which pancreatic ductal cells play an important role. Toxic agents inducing AP inhibit pancreatic ductal bicarbonate secretion, however, no information is available concerning their effects on the regulation of aquaporins (AQPs). Therefore, the aim of this study was to investigate the effects of bile acids, ethanol and its metabolites on the expression of AQPs.
Methods. CAPAN-1 cells were treated with ethanol (EtOH; 1-100 mM), chenodeoxycholate (CDC; 0.1-0.5 mM), glycochenodeoxycholate (GCDC; 0.1-0.5 mM) palmitoleic acid (POA; 10, 100 and 200 uM) and palmitoleic acid ethyl ester (POAEE; 10, 100 and 200 uM) for 6, 12, 24 and 48 hours and the expression of AQP isoforms (AQP1-12) was examined by real-time RT-PCR and immunocytochemistry.
Results. All 12 AQPs were expressed in the CAPAN-1 cell line to a certain degree. AQP1, 3, 5, 6 and 11 were expressed at the highest levels while AQP2 and 4 were hardly detectable. In almost all treated group, the expression of AQPs decreased both at mRNA and protein levels dose- and time-dependently. Notably, a 72-hour incubation in culture media restored the expression of AQPs in the 6- and 12-hour CDC- and GCDC-treated groups and in the 24-hour EtOH-treated group.
Conclusion. The role of AQP in the pathogenesis of AP needs further investigations.

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S5-C

From Cell Signalling to Bioenergetics and Cell Damage

S5-C1
Pathways to calcium mediated neuronal injury: Starvation in the midst of plenty

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Neuronal cell death is attributed to abnormal calcium signalling in a number of different pathological states in the CNS. We have shown that amyloid beta and glutamate toxicity both cause abnormal calcium signals that culminate in neuronal death. In both cases, calcium mediated toxicity is associated with oxidative stress. Our data suggest that the enzyme PARP plays a key role in these pathophysiological models as an intermediary between oxidative stress and mitochondrial dysfunction. I will show data to suggest that activation of PARP mediated by oxidative stress causes consumption of cytosolic NAD+, inhibition of mitochondrial substrate supply and impaired mitochondrial function suggesting a number of potential therapeutic targets that are neuroprotective in these model systems and that may be considered in the associated pathologies.

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S5-C2
The crucial role of mitochondrial damage and consequent breakdown of bioenergetics in acute pancreatitis

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Acute pancreatitis is an inflammatory disease with no specific treatment. One of the main reasons behind the lack of specific therapy is that the pathogenesis of acute pancreatitis is poorly understood. During the development of acute pancreatitis, the disease-inducing factors can damage both cell types of the exocrine pancreas, namely the acinar and ductal cells. Because damage of either of the cell types can contribute to the inflammation, it is crucial to find common intracellular mechanisms that can be targeted by pharmacological therapies. Despite the many differences, recent studies revealed that the most common factors that induce pancreatitis cause (1) uncontrolled Ca2+release leading to intracellular Ca2+ overload and toxicity and (2) mitochondrial damage with the consequent breakdown of bioenergetics, that is, ATP depletion in both cell types.
This presentation summarizes the variety of Ca2+ signals and the mitochondrial function and damage within both pancreatic acinar and ductal cells. We also suggest that colloidal ATP delivery systems for pancreatic energy supply may be able to protect acinar and ductal cells from cellular damage in the early phase of the disease. An effective energy delivery system combined with the prevention of further mitochondrial damage may, for the first time, open up the possibility of pharmacological therapy for acute pancreatitis, leading to reduced disease severity and mortality.

S5-C3
The mitochondrial calcium uniporter: Molecular identity and physiological role

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Mitochondria rapidly accumulate Ca2+ through a low-affinity uptake system (the mitochondrial Ca2+ uniporter, MCU) because they are exposed to high [Ca2+] microdomains generated by the opening of ER Ca2+ channels. These rapid [Ca2+] changes stimulate Ca2+-sensitive dehydrogenases of the mitochondrial matrix, and hence rapidly upregulate ATP production in stimulated cells. On the other hand, Ca2+ also sensitizes to cell death mediators, e.g. ceramide, and anti-apoptotic oncogenes reduce the Ca2+ transfer from the ER to mitochondria, Molecular identification of the mitochondrial Ca2+ transporter(s) was thus much awaited. In my presentation, I will present the most recent molecular information on MCU, identified by our group in 2011, and the newly identified regulators (MCUb, MICU1, MICU2). I will also show how the availability of molecular tools for MCU now allows to carry out experiments in intact cells and whole organisms that highlight and clarify the importance of mitochondrial calcium homeostasis in physiology and pathophysiology.

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S5-C4
Imaging incretin-regulated bioenergetics in intact pancreatic islets

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Type 2 diabetes currently affects ~8% of the population worldwide and involves impaired insulin secretion and action. Incretins such as glucagon-like peptide 1 (GLP-1) are released from the gut and potentiate insulin release in a glucose-dependent manner, in part by increasing the connectivity between individual betas cells in the islets 1. While this action is generally believed to hinge on cAMP and protein kinase A (PKA) signalling, up-regulated beta cell intermediary metabolism may also play a role in incretin-stimulated calcium dynamics and insulin secretion. By employing recombinant probes to image ATP dynamically in situ within intact mouse and human islets, we have shown that GLP-1 engages a metabolically-coupled subnetwork of beta cells to increase cytosolic ATP levels, an action independent of prevailing energy status2. We also demonstrate that inactivation of the type 2 diabetes genes ADCY5, encoding adenylatecyclase 5, interferes with normal glucose, but not incretin-stimulated ATP changes and calcium increases, providing a mechanism through which polymorphisms in this gene affect disease risk 3.Optogeneticapproaches are currently being used to identify and analyse individual beta cells responsible for coordinating beta cell behaviour islet-wide.

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S5-C5
Cardiac calsequestrin and heart failure

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Cardiac calsequestrin (CSQ2) is located in the junctional sarcoplasmic reticulum (SR) of mammalian cardiac myocytes. To further characterize the role of CSQ2 for regulation of cardiac contractility we generated CSQ2-null (CSQ2-/-) mice by targeted deletion of the first exon (heterozygous: CSQ2+/-; wild type: CSQ2+/+). In the targeted CSQ2 allele the coding region of exon 1 is replaced by a loxP site leading to a nonsense mutation. Chimeric mice were crossed into a C57BL/6 background to get CSQ2+/- mice for further breeding. Successful targeting of the CSQ2 allele was confirmed by Western blotting. CSQ2-/- mice are viable. At an early time point (6 months of age) normal ejection fraction was measured using echocardiography by means of a Vevo 2100 system and isofluran anesthesia. However, even at this time point alterations in ventricular function as assessed by Langendorff perfusion were noticed: basal contractility in CSQ2-/- mice was higher than in CSQ2+/- and CSQ2+/+ mice. Furthermore, the contractility in CSQ2-/- mice could not be stimulated by perfusion of the isolated heart with isoprenaline, a ß-adrenoceptor agonist, at concentrations that greatly elevated force of contraction in CSQ2+/- and CSQ2+/+ mice. Additionally, at six months gross morphology of the heart as well as absolute and relative heart weight were not different in CSQ2-/- mice compared to CSQ2+/+ or CSQ2+/-. However 20-22 months old mice we measured profound impairment of cardiac function as assessed by decrease ejection fraction in CSQ2-/- mice (29 ± 6.1%, n= 4) compared to six month old CSQ2-/- mice and to 20-22 months old CSQ2+/- mice 68 ± 6.4%, n=5 $p < 0.05$), whereas heart rates were not significantly different. In summary, we present the first evidence that not only overexpression but also but also deletion of CSQ can lead to cardiac failure.

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S4-D

Revealing the Prominent Role of Neuroglia in Neurodegeneration

S4-D1
Neuroglial morphological and metabolical alterations during the progression of Alzheimer´s disease and ageing

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Neuroglial cells are fundamental for brain homeostasis and therefore represent the intrinsic brain defence system. Thus, all forms of neuropathological processes inevitably involve glial cells. Neurodegenerative diseases, including Alzheimer´s disease (AD) disrupt brain connectivity affecting neuronal-neuronal, neuronal-glial and glial-glial interaction. Furthermore, neurodegenerative processes trigger universal and conserved glial reactions classically represented by astrogliosis and microglial activation. The recently acquired knowledge allows us to regard the neurodegenerative diseases as primarily gliodegenerative processes, in which glial cells determine the progression and outcome of neuropathological processes such as AD and ageing. We have recently probed this active pathological role, by showing: (i) an astroglial generalised atrophy with a concomitant astrogliosis just restricted to Aβ plaques presence, ii) alterations in glutamate glial metabolism, iii) changes in S-100β trophic factor and (iv) an early resting microglial recruitment in the affected areas,

even before the presence of activated/macrophagic microglial cells. These glial alterations, which are complex and region dependent are fundamental for the disruption of neural networks connectivity as well as with the neurotransmitters imbalance that underlie the mnesic deficits associated with AD. However, we have recently demonstrated that psychostimulative processes, such as exposure to enriched environment and voluntary running, can not only revert this generalized astrocytic hippocampal and cortical atrophy but also potentiate their hypertrophy which could trigger new insights into the search for a potential therapeutic treatment of AD and pathological ageing.

S4-D2
Dysfunction of AMPA-type glutamate receptors in microglia may cause neurodegeneration

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Microglia express AMPA (alpha-amino-hydroxy-5-methyl-isoxazole-4-propionate)-type of glutamate (Glu) receptors (AMPAR), which are highly Ca2+ impermeable due to the expression of GluA2. Glu-induced currents in the presence of cyclothiazide (CTZ), an inhibitor of AMPAR desensitization, showed time-dependent decrease after activation of microglia with lipopolysaccharide (LPS) in GluA2+/+ microglia, but not in GluA2-/- microglia. Upon activation of microglia, expression level of surface GluA2 subunits significantly increased, while expression of GluA1, A3 and A4 subunits on membrane surface significantly decreased. These results suggest that nearly homomeric GluA2 subunits were the main reason for low conductance of AMPAR in activated microglia. Increased expression of GluA2 in microglia was also detected partially in brain slices from LPS-injected mice. Cultured microglia from GluA2-/- mice showed higher Ca2+-permeability, consequently inducing significant increase in the release of proinflammatory cytokine, such as TNF-α. The conditioning medium from KA-treated GluA2-/- microglia had more neurotoxic effect on wild type cultured neurons than that from KA-treated GluA2+/+ microglia. These results suggest that membrane translocation of GluA2-containing AMPARs in activated microglia has functional importance and thus, dysfunction or decreased expression of GluA2 may accelerate Glu neurotoxicity via excess release of proinflammatory cytokines from microglia. Since low expression of GluA2 was reported in some neurodegenerative diseases, it may be one of the reasons of the neuronal death.

S4-D3
Does innate immunity contribute to the pathogenesis of Alzheimer's disease?

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Generation of neurotoxic amyloid-ß peptides and their deposition along with neurofibrillary tangle formation represent key pathological hallmarks in Alzheimer's disease (AD). Recent evidence suggests that inflammation may be a third important component which, once initiated in response to neurodegeneration or dysfunction actively contributes to disease progression and chronicity. Microglia is being activated by binding of aggregated proteins or aberrant nucleic acids to pattern recognition receptors which elicit an innate immune response. The latter is characterized by the release of inflammatory mediators including complement activators and inhibitors, chemokines, cytokines, radical oxygen species and enzyme systems. Exogenous as well as endogenous factors may promote and facilitate neuroinflammation in the AD brain. Thus, degeneration of aminergic brain stem nuclei including the locus ceruleus and the nucleus basalis of Meynert may drive inflammation in their projection areas given the antiinflammatory and neuroprotective action of their key transmitters norepinephrine and acetylcholine. Inflammation may not just occur secondary to degeneration, but actively drive amyloid beta aggregation and APP processing. Inhibition of the microglia driven innate immune response at key signalling steps may provide protection. Therefore, antiinflammatory treatment strategies should be considered. Data on microglial activation in AD along with suggestions to modify and alter the pro- into an antiinflammatory phenotype will be reviewed and discussed.

S4-D4
The response of NG2-glia (oligodendrocyte precursors) to aging in an animal model of Alzheimer's Disease

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Oligodendrocytes are the myelinating cells of the CNS. Myelin enables the rapid transmission of signals that underlies the massive computting power of the human cortex. However, there is a loss of myelin and white matter shrinkage in the ageing brain that is associated with cognitive decline. Notably, oligodendrocytes are continuously generated trhoughout life by endogenous oligodendrocyte precursors (OPs), or NG2-glia. NG2-glia (OPs) are unique amongst glia in that they form direct synapses with neurons, which are proposed to regulate the differentiation of NG2-glia (OPs) into oligodendrocytes. A diminished capacity for NG2-glia (OPs) to regenerate oligodendrocytes is proposed to be a critical factor underlying myelin loss in the ageing brain. Moreover, myelin loss is accelerated in Alzheimer's Disease (AD), suggesting the function of NG2-glia (OPs) is disrupted in this disease. We have examined this in the 3xTg mouse model of

AD and provide evidence that NG2-glia (OPs) display atrophic changes at an early stage of the disease. In contrast, at later stages of the disease NG2-glia (OPs) appear hypertrophic closely associated with amyloid beta (Abeta) plaques, and their proliferation may be decreased. Notably, there is evidence that neuronal/synaptic loss and myelin disruption occur at the earliest signs of Abeta plaques, which correlates with the observed changes in NG2-glia (OPs). The mechanisms regulating NG2-glia (OPs) and oligodendrogenesis in the adult and aging brain are unresolved, but Wnt signalling is essential for synaptic function and is deregulated in AD. Significantly, we have identified a profound role for Wnt signalling in driving oligodendrogenesis in vivo in the mouse brain and demonstrate that this is deregulated in ageing white matter. These studies suggest a sequence of interrelated events in which deregulation of Wnt signalling is associated with disruption of synaptic function and reduced differentiation of NG2-glia (OP) into oligodendrocytes with resulting myelin loss, which in turn leads to axon/neuron degeneration and a vicious cycle of events that accelerates cognitive decline in AD.

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S4-D5
Release of 4-hydroxynonenal and 4-hydroxyhexenal-modified proteins in exosomes

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Exosomes are vesicles released from mammalian cells. They contain a diversity of proteins and siRNAs. Presumably, they play an important role in intercellular communication. 4-hydroxynonenal (4-HNE) and 4-hydroxyhexenal (4-HHE) are major lipid peroxidation products of polyunsaturated fatty acids. 4-HNE and 4-HHE react with nucleophilic sites in DNA and proteins, and, accordingly, affects activity of enzymes, transporters, and ion channels. In microglial cells, one target of 4-HNE are swelling-activated Cl- channels (see Abstract by Schmölzer and colleagues). In the present study, we documented the intra- and extracellular distribution of HHE-modified proteins in the primary murine microglial cells. 4-HHE-modified proteins were detected using an antibody to 4-HHE-modified proteins. Membranes were stained with rhodamin-labeled concanavalin A. Immune-labeled as well as concanavalin A-labeled areas were visualized using confocal laser microscopy. HNE-modified proteins were visualized in discrete areas of the cell. Most immune-labeled patches were localized close to the plasmamembrane or close to the nucleus. In addition, we localized HHE-modified proteins and apolipoprotein E (ApoE) in exosomes. ApoE is involved in oxidative stress and neurodegenerative processes. Whether release of exosomes containing HNE and HHE-modified proteins is cellular strategy to expel waste proteins or may have additional biological functions remains to be analyzed.

S4-D6
Galanin is a modulator for phagocytosis in microglial cells

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The biological significance of inflammation is clearance of affected areas. The bioactive peptide galanin, is up-regulated during inflammatory processes in the brain. Notably, microglial cells, monocyte-derived immune cells in the central nervous system, reveal galanin-dependent release of cytokines. Galanin suppresses LPS-induced release of the pro-inflammatory cytokine TNF alpha, and induces up-regulation of class II major histocompatibility complex. In the present study, we characterized galanin-receptors using RT-PCR as well as the consequences of galanin on phagocytosis in murine microglia. In BV-2 cells, a murine microglial cell line, RT-PCR revealed mRNA transcripts for galanin receptor (GalR)2, GalR3, and galanin, but not for GalR1, alarin- or galanin-like peptide. For phagocytosis experiments, microglial cells were exposed to uncoated and IgG-coated polysterene beads (microspheres, MB) for 15 minutes. In primary microglial cells, galanin in the pM and nM range impaired uptake of MBs compared to basal phagocytic activity. In contrast, in BV-2 cells, galanin facilitated engulfment of MBs. Since the widely used GalR3 specific antagonist SNAP 37889, induced cell blebbing, an indicator for cell death processes, the contribution of GalR2 and GalR3 to phagocytosis could not be distinguished. The comparison between BV-2 cells and primary microglial cells indicates that depending on the galanin receptor expression pattern, galanin either facilitates or suppresses phagocytosis. Furthermore, our observations support the assumption that galanin modulates neuroinflammatory responses by affecting phagocytosis.

S5-D

Pulmonary Surfactant: From Molecule to Function

S5-D1
Exocytosis of the lamellar body, a calcium mobilizing secretory lysosome

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Lamellar bodies (LBs) are large vesicles (∅ ≈ 1 μm) within the type II pneumocyte, an epithelial cell type of the pulmonary alveolus, the terminal structure of the airways where gas exchange takes place. LBs contain surfactant, a lipid-rich, lipoprotein-like substance reducing the surface tension of alveoli and preventing their collapse. LBs are lysosome-related organelles, and live cell fluorescence imaging experiments revealed that they are secreted into the alveolar lumen by regulated exocytosis, i.e. by low threshold elevations of the cytoplasmic Ca2+ concentration above ≈ 320 nmol/l. The exocytotic process consists of a pre-, hemi-, and post-fusion step, and during the post-fusion step, fusion pores expand slowly and discontinuously. Laser tweezer experiments revealed that LB fusion pores may represent a significant mechanical barrier for the release of surfactant into the extracellular space. This necessitates active extrusion mechanisms by actin coat formation and contraction around fused LBs. Although the molecular mechanisms involved herein are still not entirely resolved, some unique principles have been elucidated:
Ca2+ entry from fused LBs into the cytoplasm through P2X4 receptors located within the limiting LB membrane plays an important role for fusion pore dilation. We termed this mechanism fusion-activated cation entry (FACE), and demonstrated that it is activated by ATP from the extracellular (apical) compartment, when ATP is able to diffuse into the LB lumen through the exocytotic fusion pore. FACE induces an expansion of the fusion pore FACE, in part by Ca2+ binding to synaptotagmin 7. FACE also leads to osmotic cell swelling and fluid reabsorption, which may facilitate fusion pore expansion as well and support surfactant incorporation at the air-liquid interface.

S5-D2
Misfolding of surfactant protein C and how it is solved by Nature and by rational design

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Lung surfactant protein C (SP-C) is unique in many aspects; it is the only known secreted transmembrane protein, it lacks homologous proteins and it is only synthesized by one cell type - the alveolar type 2 cell. Moreover, SP-C spontaneously converts from α-helix to amyloid-like β-sheet polymers, but the helical state is kinetically stabilized by an unusually high activation barrier for unfolding. Thus, in contrast to the general behavior of protein helices, the SP-C α-helix is not able to unfold and refold. These features suggest an elegant explanation to the aggregation encountered by many groups when synthesizing SP-C for formulation of an artificial surfactant for treatment of respiratory distress syndrome (RDS). We solved this problem by replacing all Val residues in SP-C (with high β-strand propensity) with Leu (with high α-helix propensity), which yields a thermodynamically stable helix peptide, which can be synthesized in large amounts without aggregation. This SP-C analogue is active in animal models of RDS and is currently undergoing clinical trials for treatment of RDS in premature infants. By an innovative approach based on spider silk proteins we have recently developed a method to make large amounts of the SP-C analogue recombinantly. The inability of the SP-C α-helix to refold in vitro raises questions about its formation in vivo.
We have discovered that a BRICHOS domain of proSP-C works as a molecular chaperone that prevents aggregation of the transmembrane SP-C part, and promotes its folding into a helix. Intriguingly, mutations that segregate with human lung fibrosis are localized to the BRICHOS domain, and our recent determination of the first crystal structure of a BRICHOS domain can explain how mutations inactivate its chaperone function. We have found that BRICHOS mutations lead to amyloid disease; BRICHOS is thus the first described example of an endogenous factor that guards extraordinarily amyloidogenic peptides. Such factors are attractive candidates to harness for treatment of amyloid diseases, and recent findings have motivated us to explore its ability to treat and prevent Alzheimer's disease.

S5-D3
The role of surfactant in host defence

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Surfactant coats the alveolar surface which is an enormous surface and interface towards the environment. Millions of bacterial, viral and fungal pathogens are inhaled by all of us every day. Our immune system must handle the challenge to avoid the invasion of pathogens into the lung parenchyma and the blood stream on the one side without constantly overreacting on the other side. Surfactant plays a crucial role in this context.

The mechanical properties of surfactant keep the airways open and facilitate mucociliary clearance. The phospholipids that are spread on the surface exert a mechanical barrier function. Surfactant in itself and antibacterial peptides bound to surfactant have been demonstrated to interfere with bacterial growth in vitro. In vivo experiments with newborn rabbits demonstrate that surfactant limits bacterial growth and translocation to the blood stream e.g. in group streptococcal and E. coli pneumonia. Surfactant protects the airways from ventilator

induced lung injury and lack or dysfunction of surfactant seems to play a role in the pathophysiology of ventilator associated pneumonia. In addition, surfactant has a potential for use as carrier for antibiotics or immunoglobulins in experimental pneumonia.

A focus in surfactant research has been directed to the role of surfactant proteins in host defense. The hydrophobic surfactant proteins SP-B and SP-C play a major role for the biophysical function of surfactant and both the natural proteins or synthetic analogues are contained in the surfactant preparations that are in clinical use. The hydrophilic "large" surfactant proteins SP-A and SP-D have a collectin structure. They are water soluble and thus removed during the extraction/production procedure of the commercially available surfactants. SP-A and SP-D exert a variety of important functions in the pulmonary immune defense. However, results on SP-D knock mice demonstrate demonstrate that SP-D m may be of importance both for "stability and sterility". Animal derived surfactants have in use for more than 2 decades, but clinical trials with preparations containing both synthetic lipids and synthetic proteins are under way.

S5-D4
Surfactant inhibition and its reversal

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Pulmonary surfactant is a mixture of phospholipids and specific proteins that reduces surface tension of the alveolar lining layer, prevents transudation of oedema fluid into the alveolar spaces and plays a role in local immune mechanisms.
Therapy with exogenous surfactant is an established part of routine clinical management of newborns with respiratory distress syndrome (RDS). In recent years the indications have been widening to the "non-RDS" respiratory disorders with the secondary inhibition of surfactant function. Inhibition, or inactivation, refers to those processes that decrease or abolish the normal surface activity of pulmonary surfactant. Surfactant may be inactivated by wide spectrum of substances that appear in the alveolar space under pathological conditions, e.g. plasma proteins, unsaturated membrane phospholipids, free fatty acids, cholesterol, meconium, reactive oxygen species, and enzymes. Currently, there is an evidence that also inhaled nanoparticles interfere with the biophysical function of the surfactant system.
At least two distinct inhibitory mechanisms arise from in vitro studies: competitive adsorption of plasma proteins and fluidizing surfactant film by lipids.
Preventing surfactant inactivation is an important approach that could increase the therapeutic effects of exogenous surfactants in various forms of lung disease. The harmful effect of some inhibitors can be counterbalanced by increasing surfactant concentration and/or enrichment of the surfactant preparations with SP-A, or by addition of polymers such as dextran, polyethylene glycol or hyaluronan. Also polymyxin B increases the resistance of natural modified surfactant to inactivation by albumin. On the other side, the effect of plasma proteins, e.g. fibrinogen is not always inhibitory. At high phospholipid concentration used in clinical practice fibrinogen has a protective effect on properties of natural modified surfactant subjected to surface area cycling.
For the further development of surfactant therapy it is important to study and better understand behaviour of pulmonary surfactant both in vitro and in vivo.

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S5-D5
Lymphatic function is required prenatally for lung inflation at birth

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Mammals must inflate their lungs and breathe within minutes of birth to survive. A key regulator of neonatal lung inflation is pulmonary surfactant, a lipoprotein complex which increases lung compliance by reducing alveolar surface tension. Whether other developmental processes also alter lung mechanics in preparation for birth is unknown. We identify prenatal lymphatic function as an unexpected requirement for neonatal lung inflation and respiration. Mice lacking lymphatic vessels, due either to loss of the lymphangiogenic factor CCBE1 or VEGFR3 function, appear cyanotic and die shortly after birth due to failure of lung inflation. Failure of lung inflation is not due to reduced surfactant levels or altered development of the lung but is associated with an elevated wet/dry ratio consistent with edema. Embryonic studies reveal active lymphatic function in the late gestation lung, and significantly reduced total lung compliance in late gestation embryos that lack lymphatics. These findings reveal that lymphatic vascular function plays a previously unrecognized mechanical role in the developing lung that prepares it for inflation at birth. They explain respiratory failure in infants with congenital pulmonary lymphangiectasia, and suggest that inadequate late gestation lymphatic function may also contribute to respiratory failure in premature infants.

S5-D6
The N-terminal domain of spider silk proteins for synthetic surfactant production

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Lung surfactant keeps the alveoli from collapsing at end expiration and spreads rapidly over a mucosal surface.

Animal-derived surfactant preparations have successfully been employed for treating neonatal children with respiratory distress syndrome (RDS), but due to high cost and limited supply, lung surfactant is not available for other uses, e.g. surfactant could act as vehicle for drug delivery to the alveoli after topical administration. Artificial surfactant preparations based on designed, synthetic surfactant protein analogues work in animal models of RDS and are in clinical development, but peptide synthesis is costly and generates unwanted by-products.

Spider silk proteins are large and prone to aggregate, but their N-terminal domain prevents precocious aggregation during storage in the spiders' silk glands. By an innovative approach, using the spider silk proteins' N-terminal domain as a solubility tag, large amounts of surfactant protein analogues (SP-C33 and KL4) can for the first time be produced in heterologous hosts. The bacterial production of SP-C33 and KL4 fused to NT results in high yields of soluble fusion proteins and by a simple precipitation procedure, a 99% pure and functional target peptide can be obtained without the use of chromatographic steps. A surfactant made from our recombinant SP-C33 mixed with synthetic phospholipids has the appropriate spreading capabilities and is effective in animal models of respiratory distress syndrome.

S4-E

Cardiovascular Exercise Physiology

S4-E1

Use of virtual patients in teaching veterinary physiology at the Faculty of Veterinary Science, Szent István University, Budapest

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According to the teachers' experiences and feedback from the learners, the first and second year students of the veterinary and medical universities often wonder why they need to study basic sciences. They want to learn more clinical related subjects and do not want to "waste" their time on biochemistry or physiology as they applied to learn veterinary medicine.

This known approach raised the demand to include modern methods in the teaching of basic sciences based on the needs and different attitude of the new generation.

An EU project "Use of virtual problems/virtual patients in veterinary basic sciences" (vetVIP) was granted and started in 2012. Three universities are involved namely the University of Veterinary Medicine Hannover Germany (TiHo), the University of Life Sciences, Faculty of Veterinary Medicine, Lublin Poland and the Szent István University, Budapest Hungary. The TiHo has a long history of using different e-learning methods in the teaching, but the Polish and the Hungarian faculties are novices in this field.

The aim was to use case-based learning to connect the basic sciences with clinical aspects and to use a "real" clinical case to explain the processes in physiology or biochemistry.

The national teams are working together in the project to prepare 30 virtual patient cases for the students. These cases cover important fields of veterinary biochemistry or physiology such as blood coagulation, metabolism of carbohydrates or lipids, hormonal dysfunctions, inflammatory processes or even bone formation.

All the cases have listed teaching aims on the first card, and they follow similar structure and length. The cases are designed to motivate self-study as they are based on a current, often seen clinical case and have a basic story with an animal patient and its owner to simulate an everyday veterinary situation. The cases are planned to have an authentic veterinary aspect, to be relevant for the curriculum of veterinary students and to integrate theoretical with clinical knowledge. All of them are based on citable literature, illustrated with pictures and videos. To check the improvement of the students different types of multiple choice questions are integrated. The right and even the wrong answers are explained in the Comments. Further, more detailed information can be found in the Expert comments.

The cases undergo different levels of evaluation: technical, didactic and meritoric checking. The authors together with clinicians evaluate the cases of the other two national teams in terms of content. The acceptance of the new methods was measured with online surveys, the learning success of the students is analysed based on pre- and post-tests showing the improvement after using the cases.

The cases are prepared in the CASUS learning system provided by one of the partners, INSTRUCT AG which is a small enterprise specialized in e-learning, located in Munich Germany and has its roots in the academic world.

S4-E2

The effect of detraining on the characteristics of the athlete's heart

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Relatively short-term detraining often occurs during an athlete's carrier due to injuries, holidays, army service etc. It is a very important question how the well defined characteristics of the athlete's heart move back to the non-trained values. Different authors (1-3) describe that different characteristics do not change equally after cessation of regular training.

In the present study data of non-athletes (113 males, 117 females), of actually trained athletes (948 males, 486 females) and of athletes after detraining (99 males 101 females) were analysed in the function of the days of detraining (1-20, 21-40, 41-60, 61-180, more then 180).

The earliest (20-40 days) finding during detraining was a slightly higher cardiac output and stroke volume, the next modification was a decrease of the relative left ventricular

(LV) muscle mass and an impairment of the diastolic function characterized by the E/A quotient, the resting heart rate (HR) seem to elevate after half a year cessation of regular training only.

We suppose that stop of regular training first induce an increase of the reduced sympathetic tone inducing an enhanced activity of the LV myocardium, which doesn't have parasympathetic innervation. LV musculature seems to lose athletic characteristics (hypertrophy, improved diastolic function) after two months of detraining, the late modification of the HR suggest a stability of parasympathetic regulation.

Modifications are more marked in the males than in the females and athletic characteristics are more stable in the top-athletes than in the lower class or leisure-time athletes.

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S4-E3

Effects of Darbepoetin-alpha treatment and TNF-alpha blockage on cardiovascular parameters, blood cCells, and body and kidney weights in L-NAME induced hypertensive rats

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Aim: We aimed to study effects of Darbepoetin-alpha (Da- a long acting erytropoietin anolog) treatment and/or TNF-α inhibition by Infliximab (inf) on cardiovascular parameters, cell blood counts, body and kidney weights in L-NAME induced hypertensive rats.

Material and Methods: Total 64 Sprague-Dawley rats were randomly grouped within 8 groups and all injections were given for 30 days; A: daily 1ml/kg ip SF injection. B, C0.1, C0.25, C0.5, D and E groups all, given daily 20 mg/kg ip L-NAME injection. C0.1 0,1µg/kg, C.25 0,25µg/kg, C0.5 0,5µg/kg Da given once in 3 days; D; 5 mg/kg daily inf, E; 5 mg/kg daily inf and once in a 3 day 0.25µg/kg Da injections besides L-NAME. F group given once in a 3 day 0.25µg/kg Da injection. Biopac MP 100 data acqusition system and Abacus Junior Wet blood analysis sytem were used for cardiovascular and blood parameters. Data were analysed statistically (Grap Pad) and expressed as mean ± SD where $P < 0.05$ accepted as significantly different.

Results: Given L- NAME injection induced hypertension. Da alone or together with inf didn't show any significant effect on systolic, diastolic or mean arterial blood pressure and also on heart rate in L-NAME induced hypertension. In group D, inf increased the pulse pressure significantly. There were no satistical difference on WBC, PLT, RBC, %Hct, %Hb values among the groups treated with 0.25 µ/kg Da but they were significantly high in C0.5, E and F groups. B and D groups lost but Da alone treated F group gain body weight during one month injection period. There were no diffirence on kidney weight or % kidney weight between and among the groups.

Conclusion: It has been first time showed that one month treatment of 0.1 and 0.25 except 0.5 doses of Da treatment did not significantly change both cardiovascular and hematologic parameters in L-NAME induced hypertensive rats. Da treatment stops body weight lost seen in L-NAME induced hypertension. But TNF-alpha blokage has no effect on the weight while it is increasing pulse pressure. We are searching the kidney tissue histologies, blood EPO evaluations and molecular apoptotic mechanisms in our ongoing studies.

S4-E4

The effects of provinol on cardiodynamics and coronary flow in isolated rat heart

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Despite well-known contribution of wine polyphenols in the prevention of cardiovascular diseases, there are still lack of data about their potential direct effects on myocardial function and coronary circulation. This study was aimed to assess direct impact of Provinol® (an alcohol-free extract of red wine) on cardiodynamics and coronary flow in isolated rat hearts. Hearts (n=48, 12 for each experi¬mental group) excised from male Wistar albino rats (age 10 weeks, BM=250 g) were retrogradely perfused according to the Langendorff technique at gradually increased coronary perfusion pressure (CPP=40-120 cmH2O). After control sets of experiments, hearts were administered with 1) 1% DMSO, 2) 0.5 mg Provinol, 3) 1 mg Provinol, and 4) 5 mg Provinol. After the insertion of sensor in the left ventricle, the parameters of heart function: (maximum rate of left ventricular pressure development (dp/dt max), minimum rate of left ventricular pressure development (dp/dt min), systolic left ventricular pressure (SLVP), diastolic left ventricular pressure (DLVP), and heart rate (HR) were continuously registered. Coronary flow (CF) was measured flowmetrically. In comparison to the control conditions, the lowest dose of Provinol (0.5 mg) significantly reduced CF (at CPP= 40 cm H2O). Medium dose of Provinol (1 mg) did not show any significant effects, except decrease in dp/dt max (again, at CPP= 40 cm H2O). On the other hand, the highest dose of this compound (5 mg) induced significant increase of dp/dt max, dp/dt min, HR and CF. Our results suggest that direct effects of Provinol on the myocardium seems to be dose dependent. While in lower doses Provinol shows even slight impairement of cardiac function, and drop in CF, in the highest dose this wine polyphenol improved cardiac contractility and induced coronary vasodilation.

S4-E5

Evolution of spreading depolarizations in a rat cerebral microembolic stroke model

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Aging is the most important independent risk factor for the incidence of cerebral ischemic stroke. Recurrent waves of spreading depolarization (SD) spontaneously occur minutes after the onset of focal ischemia in the brain but whether SDs are elicited during microembolisation-induced ischemia has been unexplored. We set out to (i) visualize the focal area of SDs, (ii) determine the hemodynamic status of this region parallel with changes in membrane potential, and (iii) characterize hemodynamic responses associated with SDs propagating over the ischemic cortex.

Multifocal ischemia was produced in halothane-anesthetized rats (n=7) by infusion of polyethylene microspheres (d=45-53μm, 2000 particles/0.6 ml vehicle) into the left common carotid artery. Synchronous image sequences were taken at specific illuminations through a cranial window created above the ipsilateral frontoparietal cortex to visualize: Changes in membrane potential (voltage sensitive dye method); cerebral blood flow (CBF; laser speckle contrast imaging); cerebral blood volume (intrinsic optical signal, IOS at 540-550 nm); and hemoglobin (Hb) deoxygenation (red IOS at 620-640 nm). A total of 31 SD events were detected in 7 experiments. The foci of 5 SDs were identified in the cranial window, which were ignited at a site where CBF prior to SD elicitation was the lowest (56.9±9%). The CBF responses to most propagating SDs were hyperemic, but red IOS intensity changes indicated 3 different kinetics of Hb saturation: dominant Hb oxygenation; initial Hb oxygenation followed by Hb desaturation; and dominant Hb desaturation. More accentuated Hb desaturation was associated with larger initial drop in CBF shortly after ischemia induction.

We have shown that microsphere-induced embolization leads to SDs evolution in the rat cerebral cortex, which is clinically relevant for the pathophysiology of small embolic infarcts in patients. We propose that SD occurrence during the early phase of ischemia is not related to immediate infarct evolution. Hyperemic responses to SDs were shown to be coupled with various kinetics of Hb saturation, which may determine the metabolic consequences of individual SDs.

S4-E6

Reduced dietary zinc and selenium levels impairs vascular function via oxidative stress in Sprague-Dawley rats aortas

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Objective: Trace elements zinc (Zn) and selenium (Se) are important components of antioxidative enzymes. The aim of this study was to determine if reduced dietary Zn and Se levels affect vascular function by increasing oxidative stress.

Materials and Methods: Thirty three male Sprague Dawley rats were fed with 4 types of custom made rat chow (Faculty of Agriculture University of Osijek, Croatia) from weaning for 10 weeks: a) high Zn(70.81 mg/kg)-high Se (0.363 mg/kg) (N=5); b) high Zn(70.81 mg/kg)-low Se(0.043 mg/kg) group (N=10); c) low Zn(30.16 mg/kg)-high Se (0.363 mg/kg) group (N=10) and d) low Zn(28.56 mg/kg)-low Se(0.030 mg/kg) group (N=8). Prior to decapitation, rats were anesthetized with 75 mg/kg ketamine+2.5 mg/kg midazolam. Dose responses to ACh (10-9-10-5 M) and response to reduced pO2 (bath gas mixture containing N2 95%, CO2 5%) were tested in noradrenaline-precontracted aortic rings in the absence/presence of the NOS inhibitor L-NAME, COX-1,2 inhibitor indomethacin (INDO) and superoxide scavenger Tempol in tissue bath. To test differences among groups Two-way ANOVA or One-way ANOVA was used when appropriate (SigmaPlot v11.2, Systat Software, Chicago, USA).

Results: ACh induced relaxation (AchIR) was reduced in low Zn-low Se group compared to other groups ($P < 0.001$ for 10-8-10-5M ACh). L-NAME blocked AChIR in all groups of rats ($P < 0.01$ for 10-8-10-5M ACh), while INDO reduced AChIR only in low Zn-high Se group ($P < 0.5$ for 10-7-10-5M ACh). Tempol improved AChIR in low Zn-low Se group ($P < 0.01$ for 10-7-10-5M ACh). Hypoxia induced relaxation (HIR) was increased in high Zn-low Se group compared to high Zn-high Se group and low Zn-low Se group ($P < 0.05$). While INDO significantly blocked HIR in all groups of rats except high Zn-high Se group, L-NAME and TEMPOL did not have significant effect on HIR in any group of rats.

Conclusion: Zn and Se dietary deficiency affects vascular reactivity. Since AChIR (predominantly mediated by NO) is more affected than HIR (predominantly mediated by COX-1,2 metabolites), data suggest that that increased oxidative stress decreases NO bioavailability in rat aortas thus affecting NO-mediated response.

S4-E7

Different expression and localization pattern of MT1 melatonin receptor between conduit and resistant arteries can be involved in positive effects of melatonin on blood pressure control

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Background: Cardiovascular diseases are primarily cause of mortality and morbidity. Melatonin (MEL) may have hypotensive effects, but mechanisms how it can modulate blood pressure (BP) are unclear.

Aims: To evaluate expression and localization of specific MEL receptors in the thoracic aorta (TA) and mesenteric artery (MA) and effects of MEL treatment on BP in hypertensive SHR rats.

Methods: Tissues collected from normotensive Wistar rats (WKY) were analyzed by RT-PCR as well as RT-qPCR for the expression of MT1 and MT2 mRNA. Moreover, protein localization was investigated by immunofluorescence microscopy on 4µm paraffin-embedded tissue sections. Hypertensive SHR rats were exposed to physiological (D1: 2.5 mg/kg) and pharmacological (D2: 10 mg/kg) doses of MEL in drinking water during the dark phase and BP was measured by radiotelemetry. Sympathetic (LF) and parasympathetic (HF) markers of heart rate variability and spontaneous baroreflex sensitivity (BRS) were calculated from telemetry data.

Results: In WKY rats, we found mRNA expression of MT1 but not MT2 receptor with a significant difference ($p < 0.001$) between TA (20.0 ± 0.5 ΔCT) and MA (12.5 ± 0.3 ΔCT). In TA, MT1 was localized mainly in the endothelium and adventitia while in MA, the signal was prominent in the smooth muscle layer and endothelium. We observed a decrease ($p = 0.056$) of BP after MEL treatment: SHR (LD: 191 ± 2 mmHg; D1: 193 ± 2 mmHg; D2: 196 ± 2 mmHg) vs. SHR with MEL (LD: 187 ± 2 mmHg; D1: 187 ± 2 mmHg; D2: 185 ± 2 mmHg). On the other hand, MEL had no impact on relative changes of BRS ($p=0.56$) while LF and HF were improved mainly during the dark phase (LF: SHR -4.7%, SHR-MEL: -7.9%; HF: SHR: +2.1%, SHR-MEL: +4.3%) after D2 concentration. Locomotor activity was not affected by MEL.

Conclusions: Localization of MT1 receptors in different vessels supports the possibility that MEL can influence BP via specific membrane receptors mainly in resistant vessels, such as MA. Observed anti-hypertensive effects of MEL suggest a potential to develop a novel anti-hypertensive compounds with peripheral as well as central mechanisms of action on BP control.

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S4-E8

Angiogenic properties of human pluripotent stem cell-derived arterial and venous endothelial cells

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Endothelial derivatives of human pluripotent stem cells hold out for therapeutic angiogenesis in ischemic cardiovascular diseases. Our aim was to investigate the arterial and venous phenotype of stem cell derived endothelials and their angiogenic potential after in vivo engraftment.

H7 human embryonic stem cells (hESC) were differentiated via embryoid body (EB) formation in normoxygenic (20%) and hypoxic (5%) conditions as well as in monolayer. Human induced pluripotent stem cells (hIPSC) were differentiated under normoxygenic condition via EB formation. CD31-positive endothelial cells were sorted by FACS and characterized for morphology, immunocytochemistry, gene expression and proteome profiling pattern. For engineering 3D constructs human aortic wall samples were decellularised. Human ESC-EC and hIPSC-EC were seeded onto human ECM matrix. Stem cell-derived endothelials were transplanted into three months old athymic nude rats.

As shown by immunocytochemistry, hESC-EC and hIPSC-EC were stained positive for anti-CD31, von Willebrand factor and ve-cadherin; cells formed capillary-like tubules on Matrigel and took up acetylated-LDL. Quantitative PCR showed expression of arterial (EphrinB2, Notch1-2) and venous (EphB4) endothelial markers. Wide range of angiogenesis-related proteins were detected in all endothelial types. The mRNA levels of angiopoietin2 increased significantly in hESC-EC when differentiated with EB method (EB normoxia 353.17 ± 86.29; EB hypoxia 323.89 ± 86.63, $p < 0.001$ $n=3$, mRNA levels are normalized to those in hESC). Endothelial cells remained viable upon in vivo engraftment. Marked increase was found in mRNA levels of all arterial and venous marker genes in re-isolated cells. Endothelial cells seeded onto decellularised human extracellular matrix remained viable as shown by calcein AM staining.

Differentiating endothelial cells from human pluripotent stem cells via EB method increased the angiogenic potency in vitro. After in vivo conditioning endothelial markers increased, suggesting that endothelial cells linked to host microcirculation. These cells may be used as novel angiogenic therapies in the future.

S5-E

Micro RNA Networks and Potential Clinical Implications in Cancer, Cardiovascular and Renal Diseases

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S5-E1

MiRNAs in renal glomerular disease: Novel insights into pathogenic Mechanisms and clues for treatment

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Focal segmental glomerulosclerosis (FSGS) is a frequent and severe glomerular disease characterized by destabilization of podocyte foot processes. We report that transgenic expression of the microRNA miR-193a in mice rapidly induces FSGS with extensive podocyte foot process effacement. Mechanistically, miR-193a inhibits the expression of the Wilms' tumor protein (WT1), a transcription factor and master regulator of podocyte differentiation and homeostasis. Decreased expression levels of WT1 lead to downregulation of its target genes PODXL (podocalyxin) and NPHS1 (nephrin), as well as several other genes crucial for the architecture of podocytes, initiating a catastrophic collapse of the entire podocyte-stabilizing system. We found upregulation of miR-193a in isolated glomeruli from individuals with FSGS

compared to normal kidneys or individuals with other glomerular diseases. Thus, upregulation of miR-193a provides a new pathogenic mechanism for FSGS and is a potential therapeutic target.

S5-E2
MicroRNAs in ischemia/reperfusion injury and cardioprotection by ischemic conditioning: ProtectomiRs

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Cardioprotection by ischemic conditioning leads to characteristic changes in gene expression profile. Cardioprotection by conditioning is attenuated by the presence of cardiovascular risk factors and co-morbidities including metabolic diseases such as hyperlipidemia and diabetes. These risk factors also lead to changes in the gene expression profile of the heart. Fine tuning regulators of mRNA expression, miRNAs may contribute to cardioprotective gene expression response of the heart.
Therefore, he we aimed to characterize early changes in microRNA expression in acute cardioprotection by ischemic pre- and postconditioning in rat hearts. Hearts isolated from male Wistar rats were subjected to i) time-matched non-ischemic perfusion, ii) ischemia/reperfusion (30 min coronary occlusion and 120 min reperfusion), iii) preconditioning (3x5 min coronary occlusion) followed by ischemia/reperfusion, or iv) ischemia/reperfusion with postconditioning (6x10s global ischemia/reperfusion at the onset of reperfusion, respectively. Infarct size was significantly reduced by both interventions. Out of 350 different microRNAs assessed by microarray analysis, 147-160 showed detectable expression levels. As compared to microRNA alterations induced by ischemia/reperfusion vs. time-matched non-ischemic controls, 5 microRNAs were significantly affected by both pre- and postconditioning (microRNA-125b*, 139-3p, 320, 532-3p, 188), 4 microRNAs by preconditioning (microRNA-487b, 139-5p, 192, 212), and 9 by postconditioning (microRNA-1, let-7i, let-7e, let7b, 181a, 208, 328, 335, 503), respectively. Expression of randomly selected microRNAs was validated by QRT-PCR. By a systematic comparison of the direction of microRNA expression changes in all groups, we identified microRNAs, specific mimics or antagomiRs of which may have pre- and postconditioning-like cardioprotective effect (protectomiRs). Transfection of selected protectomiRs (mimics of microRNA-139-5p, -125b*, let-7b, and inhibitor of microRNA-487b) into cardiac myocytes subjected to simulated ischemia/reperfusion showed significant cytoprotective effect. This is the first demonstration that ischemia/reperfusion-induced microRNA expression profile is significantly influenced by both pre- and postconditioning, which shows the involvement of microRNAs in cardioprotective signaling. Moreover, by analysis of microRNA expression patterns in cardioprotection by pre- and postconditioning, specific protectomiRs can be revealed as potential therapeutic tools treating ischemia/reperfusion injury.

S5-E3
MicroRNA-25 regulates NOX4 in the hypercholesterolemic heart

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Diet-induced hypercholesterolemia leads to oxidative/nitrative stress and subsequent myocardial dysfunction. However, the regulatory role of microRNAs in this phenomenon is unknown. We aimed to investigate, whether hypercholesterolemia-induced myocardial microRNA alterations play a role in the development of oxidative/nitrative stress and in subsequent cardiac dysfunction. Male Wistar rats were fed with 2% cholesterol/0.25% cholate-enriched or standard diet for 12 weeks. Serum and tissue cholesterol levels were significantly elevated by cholesterol-enriched diet. Left ventricular end-diastolic pressure was significantly increased in cholesterol-fed rats both in vivo and in isolated perfused hearts, indicating diastolic dysfunction. Myocardial expression of microRNAs was affected by cholesterol-enriched diet as assessed by microarray analysis. MicroRNA-25 showed a significant down-regulation as detected by microarray analysis and QRT-PCR. In silico target prediction revealed NADPH oxidase 4 (NOX4) as a putative target of microRNA-25. NOX4 protein showed significant up-regulation in the hearts of cholesterol-fed rats, while NOX1 and NOX2 remained unaffected. Cholesterol-feeding significantly increased myocardial oxidative/nitrative stress as assessed by dihydroethidium staining, protein oxidation assay, and nitro-tyrosine ELISA, respectively. Direct binding of microRNA-25 mimic to the 3'UTR region of NOX4 was demonstrated using a luciferase reporter assay. Transfection of a microRNA-25 mimic into primary cardiomyocytes decreased superoxide production, while a microRNA-25 inhibitor resulted in an up-regulation of NOX4 protein and an increase in oxidative stress that was attenuated by the NADPH oxidase inhibitor diphenyleneiodonium. Here we demonstrated for the first time that hypercholesterolemia affects myocardial microRNA expression, and by down-regulating microRNA-25 increases NOX4 expression and consequently oxidative/nitrative stress in the heart.
We conclude that hypercholesterolemia-induced microRNA alterations play an important role in the regulation of oxidative/nitrative stress and in consequent myocardial dysfunction.

S5-E4
miR-200 in extracellular vesicles promotes metastasis of breast cancer cells

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Not all cancer cells in a tumor are capable of metastasizing. The miR-200 microRNA family, which regulates the mesenchymal-to-epithelial transition, is enriched in the serum of patients with metastatic cancers, and ectopic expression of miR-200 can confer metastatic ability to poorly metastatic tumor cells in some settings. Here we investigated whether metastatic capability could be transferred between metastatic and nonmetastatic cancer cells via extracellular vesicles. Metastatic breast cancer cell lines highly expressing miR-200 secrete miR-200 microRNAs in extracellular vesicles and transfer them to otherwise weakly metastatic cells either nearby or at distant sites and confer upon them the ability to colonize distant tissues in a miR-200-dependent manner. Thus, uptake of extracellular vesicles can transfer metastatic capability.

S5-E5
Targeting basal-like Triple Negative Breast Cancers and epithelial tumor-initiating cells with aptamer-siRNA chimeras

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RNA interference (RNAi) offers the exciting therapeutic possibility of selectively knocking down disease-causing genes. Recent Phase I and II clinical trials of small interfering RNAs (siRNAs) have shown promising gene knockdown and clinical benefit in a handful of diverse diseases caused by aberrant liver gene expression. In these studies siRNAs were targeted to the liver by encapsulation in lipoplexes or by conjugation with a sugar selectively recognized by hepatocytes. The major obstacle to harnessing RNAi for treating cancer is the difficulty delivering RNAs into disseminated cancer cells. Giangrande, McNamara and Gilboa developed a flexible and effective multifunctional RNA delivery platform using aptamer-siRNA chimeras (AsiC), to accomplish this goal. RNA aptamers, structured RNAs that bind with high affinity to a protein, covalently linked to siRNAs, cause knockdown selectively in cells bearing the receptor recognized by the aptamer. Although treatment for breast cancer has improved with the development of targeted therapeutics, there is still a need for new approaches for poor prognosis breast cancers. In particular, there is no targeted therapy for triple negative breast cancers (TNBC), which often relapse after current therapy; Her2+ breast cancers frequently develop resistance to targeted therapy with trastuzumab or lapatinib. We have developed AsiCs that target a receptor highly expressed on epithelial cancer cells and on the tumor-initiating cells (also known as cancer stem cells) within them. All basal-A TNBC and luminal breast cancer cell lines examined strongly expressed the receptor recognized by the aptamer, while a normal breast cancer epithelial line and mesenchymal TNBCs had close to background levels. RNA uptake, gene knockdown and the cytotoxic effect of these AsiCs were robust in basal-like TNBC and luminal breast cancer lines, but not in normal epithelial cells and mesenchymal cells. Treatment of mice bearing basal-like TNBCs on one flank and mesenchymal breast tumors on the other caused selective uptake and rapid (within a week) and sustained complete regression of the basal-like tumors, but had no effect on the mesenchymal tumors.

S5-E6
microRNA expression might predict prognosis of epithelial hepatoblastoma and sorafenib treated hepatocellular carcinoma

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Background and aims: The more differentiated fetal component of hepatoblastoma (HB) is suggested to associate with better survival when compared with embryonal component. Sorafenib represents the first effective targeted therapy for advanced stage hepatocellular carcinoma (HCC), however, adequate patient stratification regarding sorafenib-responsiveness is still missing. MicroRNA (miRNA) expression patterns were investigated in epithelial subtypes of HB and in pretreatment HCC under sorafenib treatment. and related to survival.
Material and methods: 20 cases of epithelial HBs subtyped as pure fetal components (n=12) and embryonal components (n=8), along with 15 non-tumorous surrounding liver (SL) samples were analysed. Further, diagnostic fine needle aspiration (FNA) cytology smears of 20 advanced stage HCC patients were investigated. Relative expressions of miR-17-5p, miR-18a, miR-21, miR-34a, miR-96, miR-122, miR-181a, miR-195, miR-210, miR-214, miR-221, miR-222, miR-223 and miR-224 were determined by TaqMan MicroRNA assay.
Results: No significant differences were revealed in overall survival between fetal and embryonal/fetal types of HBs. High miR-21, low miR-222 and low miR-224 levels proved to be independent prognostic factors for HB and were associated with significantly increased overall survival ($p < 0.03$). Among the analysed miRNAs high expression of miR-214 was associated with smaller tumor size ($p=0.019$); high miR-17-5p expression correlated with better ECOG performance status ($p=0.003$). Survival analysis revealed that high miR-224 expression was associated with increased progression-free and overall survival (PFS $p=0.029$; OS $p=0.012$).

Conclusions: miR-21, miR-222 and miR-224 levels could serve as valuable tools to predict overall survival of HB patients regardless of epithelial subtype. Pretreatment microRNA profiling, especially miR-224 expression might serve as an ancillary tool for better assessment of expected survival under sorafenib treatment.

S5-E7
Exosomal microRNA in biofluids - Robust biomarkers for disease

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MicroRNAs constitute a class of small cellular RNAs (typically 19-23 nt) that function as post-transcriptional regulators of gene expression. Current estimates indicate that more than one third of the human cellular transcriptome is regulated by this small class of RNA (~2000 miRNA).
The high relative stability of microRNAs in common clinical source materials (FFPE blocks, plasma, serum, urine, saliva, etc.) and the ability of microRNA expression profiles to accurately classify discrete tissue types and specific disease states have positioned microRNAs as promising new biomarkers for diagnostic application in cancer. The study of extracellular microRNAs and their potential as pathophysiological markers has greatly expanded in the last couple of years. MicroRNAs have been shown to be actively exported from tissues into the circulation through a variety of mechanisms including complexing with RNA binding proteins or HDL and through exosome and microvesicle transport.
Exosomes are nanovesicles secreted into the extracellular environment by a wide range of mammalian cell types under normal and pathological conditions. As the profile of exosomal microRNAs may be a fingerprint of the releasing cell type and because they are released in easily accessible body fluids such as blood and urine, their microRNA content holds potential as biomarkers for early detection of malignancy. Using exosomal microRNA as a starting point for biomarker studies will reduce the risk of false negative results when the study involve detection of low abundance microRNAs.
We recently developed a new exosome enrichment method and will present a comparison of microRNA profiles obtained with this method to profiles obtained with different commercial available exosome isolation methods and to standard profiles of whole plasma and serum. We have applied our highly sensitive LNA™-based qPCR platform for detection of microRNAs, which has enabled microRNA profiling in biofluids where levels are extremely low. The platform uses a single universal RT reaction to conduct full miRNome profiling and allows high-throughput profiling of microRNAs without the need for pre-amplification.
Summary: Exosomal microRNAs have recently gained considerable interest as potential pathophysiological markers. We have developed a new miRNA purification method using exosome enrichment and compared microRNA profiles to profiles obtained with different commercial exosome isolation methods and standard profiles of whole plasma and serum.

S6-A

The Calcifying Vessel: New Genetic Findings for Future Patho-physiological Avenues

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S6-A1
Mechanisms of arterial calcification: Lessons learned from rare monogenic disorders

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Arterial calcification significantly contributes to morbidity and mortality. Major insight into the pathophysiological mechanisms contributing to arterial calcification has been provided trough three rare monogenic disorders associated with spontaneous, premature artery media calcification. Monogenic NPP1, ABCC6, and CD73 deficiencies each drive a molecular pathophysiology of the closely related, but phenotypically different human diseases Generalized Arterial Calcification of Infancy (GACI), Pseudoxanthoma Elasticum (PXE), and Calcification of Joints and Arteries (CALJA) respectively, in which premature onset of arterial calcification is a prominent but not sole feature. Based on the similarities of GACI, PXE and CALJA, it can be speculated that the underlying disease genes, ENPP1, ABCC6 and NT5E, drive a cohesive arterial molecular pathophysiology system modulated by ATP metabolism, inorganic pyrophosphate, adenosine, inorganic phosphate generation and functional activities.

S6-A2
The role of ABCC6 in chronic and acute calcification, a tale of 3 diseases

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Abnormal mineralization occurs in the context of several common conditions, including advanced age, diabetes, hypercholesterolemia, chronic renal failure and certain genetic conditions. Metabolic, mechanical, infectious, and inflammatory injuries promote ectopic mineralization through overlapping yet distinct molecular mechanisms of initiation and progression. The ABCC6 protein is an ATP-dependent transporter primarily found in the plasma membrane of hepatocytes and appears to prevent ectopic mineralization by inducing the cellular release of nucleotides, including nucleotides triphosphate (NTPs). Surface ectonucleotidases hydrolyze NTPs into pyrophosphate, a potent inhibitor of calcification, which is then released for systemic circulation. ABCC6 deficiency is the primary cause for chronic and acute

forms of ectopic mineralization described in diseases such as pseudoxanthoma elasticum (PXE), beta-thalassemia, and generalized arterial calcification of infancy (GACI) in humans and dystrophic cardiac calcification (DCC) in mice. These pathologies are characterized by mineralization of cardiovascular, ocular, and dermal tissues. PXE and some cases of GACI are caused by inactivating ABCC6 mutations, whereas the mineralization associated with beta-thalassemia patients derives from a liver-specific change in ABCC6 expression. DCC is an acquired phenotype resulting from cardiovascular insults (ischemic injury or hyperlipidemia) and secondary to ABCC6 insufficiency. Abcc6-deficient mice develop ectopic calcifications similar to both the human PXE and mouse DCC phenotypes. Although, we have acquired a better understanding of the physiological role of hepatic ABCC6, its precise molecular and cellular functions still remain unknown. In addition, new evidences are now emerging suggesting that ABCC6 physiological function is not limited to preventing ectopic calcification.

S6-A3
Tissue-wide mineralization: What can we learn from the vasculature

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Soft tissue mineralization is a complex process, the molecular and cellular mechanisms of which are not fully understood. Mineralization is necessary for the formation of skeletal tissues but it needs to be rigorously controlled and restricted to specific regions. In many cases, ectopic mineralization results from a disturbance of the complex interplay between mineralization propagators and antagonists set out to regulate this process. Although most soft tissues can undergo calcification, certain ones - including the skin, the kidneys, the cartilage and tendons, the eyes and the vasculature - are considerably more prone. Pathologic mineralization in one of those tissues may result in disease which can be very debilitating, painful and typically destructive for the compromised tissue.

The knowledge on the molecular background of soft tissue mineralization largely comes from insights in vascular calcification, with involvement of the osteoinductive Transforming Growth Factor beta (TGFβ) family (TGFβ1-3 and Bone Morphogenetic Proteins [BMP]), together with ectonucleotides (ENPP1), Wnt signalling and a variety of local and systemic calcification inhibitors. Questions which arise are whether the cellular pathways and mechanisms documented to induce vascular calcification can simply be extrapolated to other soft tissues that are prone to mineralization and how relevant these pathways are for diseases which are characterized by a more generalized ectopic mineralization. In this presentation, concepts will be presented on the relevance of cardiovascular mineralization mechanisms for soft tissues in general and how these can contribute to our understanding of rare and more common ectopic mineralization disorders and to the identification of genetic modifiers which can be used to foresee the phenotype of an individual patient more accurately.

S6-A4
Arterial calcifications and cardiovascular diseases: Clinical and therapeutic issues

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Calcification is an actively regulated biological process that is normally restrained to bony structures in physiological conditions. Ectopic calcifications in soft tissues, such as the arterial wall, is part of normal aging, but can also occur in acquired metabolic diseases e.g. diabetes and chronic renal insufficiency and in several inherited diseases. Arterial calcification is currently recognized as an independent risk factor for cardiovascular diseases. In atherosclerosis, calcification localizes mainly within the intimal layer whereas the accumulation of hydroxyapatite within the media layer (i.e. mediacalcosis) is observed mainly in arteriosclerosis. Arterial calcification within the media lead to arterial wall stiffness thus contributing to the development of a systolic hypertension. Intimal calcification, however, leads to plaque rupture leading to thrombosis and ischemic lesions. Based on existing literature and our data, we will review the different methods to evaluate the impact of the calcifying phenotype on the arterial function. Data obtained from studies of inherited genetic calcifying diseases will be also presented since they represent excellent models for the understanding of the mechanisms linking calcification to the arterial dysfunction and possible therapeutic applications.

S6-A5
Conformation correction therapy in arterial calcification disorders, PXE and GACI

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Arterial calcification is a leading cause of death, especially in the Western countries. Little is known about the mechanisms regulating the calcification process, however, some rare monogenic disorders gave insight into the pathophysiological mechanisms contributing to arterial calcification.

Mutations in the gene of the ABCC6 transporter, a member of the ABC protein family, are responsible for the development of two genetic diseases: Pseudoxanthoma elasticum (PXE, OMIM 26480) and Generalized Arterial Calcification of Infancy (GACI, OMIM 208000). Furthermore, a missing allele of ABCC6 is a genetic risk factor in coronary arterial disease (CAD).

In order to better understand how mutations in the ABCC6 gene lead to development of abnormal calcification symptoms, and to establish potential therapeutic treatment for patients carrying ABCC6 mutations, we have set up a complex experimental

strategy to determine the structural and functional consequences of disease-causing mutations in the human ABCC6 transporter. The transport activity of the protein was determined by biochemical transport assays, and the subcellular localization of wt and mutant proteins was investigated both in vitro and in vivo. The major aim of our study was to identify mutants with preserved transport activity but failure in intracellular targeting, as these mutants are candidates for functional rescue. Sodium 4-phenylbutyrate (4PBA), an FDA-approved drug, has been shown to act as a chemical chaperon and to restore the reduced cell surface expression of certain mutated plasma membrane proteins.

To analyze the effect of 4-PBA, not only to the subcellular localization but also to the function of ABCC6 mutants, an in vivo functional assay was set up, using the cardiac freeze-thaw injury method to induce an acute Abcc6-dependent phenotype referred to as dystrophic cardiac calcification (DCC). The expression of the human WT ABCC6 in mouse liver reduced significantly the DCC phenotype. On this line we showed that while disease-associated missense ABCC6 mutants expressed in the liver of Abcc6-/- mice do not reduce calcification, administration of 4-PBA attenuates in vivo calcification by directing the mutants to the plasma membrane.

These results indicate that 4-PBA treatment restored both the localization and the physiological function of certain disease-causing ABCC6 mutants.

S6-B

Physiology of Interaction Between RAS, IRAP and Glucose Metabolism

S6-B1

Does insulin-regulated aminopeptidase play a role in regulating glucose uptake in neurones?

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IRAP (insulin-regulated aminopeptidase) is a type II transmembrane protein with two functional domains. Its N-terminal cytoplasmic domain contains trafficking motifs and this protein was originally identified in fat cells, co-localized with the insulin-regulated glucose transporter, GLUT4, in intracellular vesicles. IRAP is translocated together with GLUT4 to the cell surface following insulin stimulation. Its larger extracellular domain contains classical aminopeptidase motifs and has been shown to cleave a number of substrates including vasopressin, oxytocin, and neurokinins A & B. IRAP is also known as oxytocinase because of its role in regulating circulating oxytocin levels during pregnancy. Our group has demonstrated that peptide inhibitors of IRAP, Angiotensin IV (Ang IV) and LVV-hemorphin 7 (LVV-H7), elicit robust effects on accelerating spatial learning and facilitating memory consolidation in rodents. More importantly, these peptides restore memory in animals with experimental amnesia. We propose that IRAP is a exciting target for the development of a novel class of cognitive enhancers. We have developed a series of small molecular weight compounds targetting the catalytic domain of IRAP that have memory-enhancing properties, offering proof-of-principle that inhibition of the enzyme have beneficial effects on memory. We are currently elucidating the mechanisms by which these IRAP inhibitors facilitate learning and memory. In neurons in the hippocampus and entorhinal cortex, IRAP is found almost completely co-localized with GLUT4 as is the case in insulin-responsive tissues. We also demonstrated that IRAP inhibitor treatment results in enhanced glucose uptake into neurons via a GLUT4 mediated mechanism. Exogenous glucose administration has been shown to improve memory and increased glucose uptake has been detected in the hippocampus of rats during the performance of memory tasks. Therefore we hypothesize that one of the mechanisms by which IRAP inhibitors enhance memory is via facilitation of glucose uptake into neurons.

S6-B2

Presence, regulation and function of insulin-regulated aminopeptidase in macrophages

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Angiotensin II-(3-8) is a fragment of the cardiovascular hormone Angiotensin II that incited the interest of several research groups because of its ability to facilitate memory as well as reversal of memory deficits in animal behavior models. These effects are mediated by its interaction with the membrane-bound insulin-regulated aminopeptidase (IRAP) which was formerly denoted as AT4 receptor. Using our recently developed IRAP selective analogue [3H]-IVDE77 as well as by western blot analysis and real-time PCR we demonstrated the presence and regulation of IRAP in mouse as well as human macrophages. Macrophages are first-line defense cells that can adopt distinct activation states in response to particular cytokines. Interestingly IRAP mRNA expression in mouse thioglycolate-elicited primary pertitoneal macrophages was increased by the pro-inflammatory mediators interferon-γ (IFN-γ) and lipopolysaccharide (LPS) but not by anti-inflammatory cytokines (IL-4, IL-10, TGF-alpha). In agreement the corresponding [3H]-IVDE77 binding to intact macrophages was increased after IFN-gamma pretreatment during increasing periods of time. On the other hand only a relatively short pre-incubation (2 hours) with LPS enhanced [3H]-IVDE77 binding which then declined after longer pre-incubations. Subsequently we investigated whether Angiotensin IV and/or IRAP selective analogues have an influence on the expression of pro- and/or anti-inflammatory genes, macrophage mediated phagocytosis, antigen cross-presentation and glucose uptake. Human peripheral blood mononuclear cells were isolated and differentiated into mature macrophages with colony-stimulating-factor. The presence of IRAP in these cells was shown by the specific binding of [3H]-AL11, a former analogue of IVDE77. Similar as in mouse cells, IFN-gamma and LPS upregulated mRNA levels of IRAP in human macrophages.

Further work is needed to elucidate whether IRAP is involved in the normal and/or pathological functions of macrophages. These findings may extend our knowledge of RAS components beyond the classical cardiovascular functions.

S6-B3

Ex vivo assessment of tissue angiotensin metabolism. Focus on Ang I/Ang IV/IRAP axis in various kinds of fat tissue in rat model of obesity and insulin resistance

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Next to angiotensin II (Ang II), several "new" biologically active peptides [e.g. Ang-(1-7), Ang-(1-12), Ang IV, Ang-(2-10)] and enzymes [e.g. angiotensin converting enzyme 2 - ACE2, IRAP/oxitocinase] have been described; at least one of them - Ang-(1-7) - has been shown to oppose actions of Ang II. Several lines of evidence suggest that adipose tissue could be a rich source of various angiotensins, which may play a multiple roles in regulation of adipose tissue physiology and constitute a link between obesity and cardiovascular disorders.

Based on LC-ESI-MS method we established ex vivo system for assessment of angiotensin metabolites. Here, we present the short summary of our results regarding angiotensin I metabolism in various animal tissues, with focus on of fat tissue from different depots (epididymal, subcutaneous, periaortic and retroperitoneal) in rat model of obesity and insulin resistance - in young (12 w/o) and old (33 w/o) Zucker rats. For comprehensive identification and quantitation of production of angiotensin peptides LC-ESI-MS method was used. Additionally, qPCR measurements of mRNA expression of main enzymes involved in Ang I metabolism as well as determination of IRAP activity were performed.

In every type of fat, there was prevalence of production of Ang-(1-7) over Ang II and Ang IV; the generation of Ang IV was most efficient in subcutaneous and periaortic fat. The use of ACE inhibitor (perindoprilat) resulted in inhibition of Ang II and Ang IV and increase of Ang-(1-7) formation. In all kinds of fat the ability to generate Ang IV tended to decrease with age of animals. In both lean and obese animals plasma IRAP activity decreased with age. Interestingly, in epididymal fat of old obese Zucker rats there was a significant increase in IRAP expresssion as compared to old lean animals.

The quantitative and qualitative description of Ang I metabolism in various kinds of fat tissue in Zucker rats poses the questions about the biological roles of particular peptides [esp. Ang-(1-7)/MAS and Ang IV/IRAP axes] both in local regulation of fat tissue functions and in cardiovascular complications of obesity and diabetes.

S6-B4

Activity assays for proteolytic enzymes in complex biological samples - Technical aspects and novel methods for measuring angiotensinase and oxytocinase activities

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Peptide hormones are crucial regulators of multiple physiological functions resulting in a major scientific interest in the enzymes involved in their metabolism. Despite the fact that the chemical nature of a proteolytic cleavage is well defined, the accurate determination of a certain protease activity in a biological sample remains a significant analytical challenge. The availability of a variety of artificial chromogenic and fluorogenic substrates suggests the easy and rapid measurement of different protease activities in tissue homogenates and body fluids. However, biochemical considerations regarding substrate specificity and sample complexity put a question mark over the use of artificial substrates for protease activity measurements. The availability of mass spectrometry as a novel sensitive and highly specific tool for the quantification of peptide hormones opens up new possibilities for the analysis of peptide hormone cascades in tissue homogenates and body fluids. The utilization of natural substrates for the quantification of enzymatic activities abolishes the presence of false positive and unspecific signals, focusing on the metabolism of physiologically relevant substrates. Mass spectrometry based characterization of peptide hormone metabolism will be discussed by reference to the renin angiotensin system and IRAP aiming to create awareness about methodological limitations and frequent pitfalls coming along with the use of artificial substrates in the measurement of proteolytic enzyme activities.

S6-B5

Effect of high fat diets on angiotensinase and IRAP activities. Their role in blood pressure and glucose homeostasis control

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High fat diets have been related to the development of hypertension and other metabolic dysfunctions such as obesity or type 2 diabetes. However, these effects depend on the specific profile of the fatty acids in the diet. Extra virgin olive oil (EVOO), the main fat source of Mediterranean diet and specially rich in monounsaturated fatty acids, has demonstrated a positive role in prevention of hypertension and cardiovascular diseases, but the physiological and molecular mechanisms of these effects are not totally understood.

All the components of the renin-angiotensin system (RAS) are present locally in tissues such as kidney, brain or white adipose tissue. They play a critical role not only during development of hypertension but also in the progression of insulin resistance and type 2 diabetes. In the RAS, AngII is hydrolyzed by aminopeptidase A to generate Ang III. Subsequently this peptide is metabolized to Ang IV by aminopeptidase N, being

these enzymes called angiotensinases. The specific receptor for Ang IV (AT4) identified as the insulin-regulated aminopeptidase (IRAP), is associated with the Glut4 transporter in intracytoplasmatic vesicles, which suggests a possible link between RAS, blood pressure control and glucose uptake. These activities have demonstrated significant local changes between normotensive and hypertensive animal models, after anti-hypertensive drug treatments and compared with healthy controls, in plasma of patients with type 2 diabetes and metabolic syndrome.
Results obtained in our laboratory by measuring blood pressure, body weight and fasting insulin and leptin levels, demonstrated a possible beneficial effect when diets enriched with EVOO were compared with a high fat diet enriched with saturated fatty acids and cholesterol; and some of these effects have also been observed in spontaneously hypertensive rats. This effect was related to specific changes of angiotensinase and IRAP activities in the above-mentioned tissues.
Taking together, these results indicate a role of dietary fat in the regulation of angiotensinase and IRAP activities, and open a new approach to the study and treatment of metabolic dysfunctions.

S6-B6
Chronic treatment of rats with oxytocin upregulates renin and (pro)renin receptor expression in kidney

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Oxytocin (OT) has been shown to induce renin release by kidney during acute peptide infusion in rats1,2. The aim of our study was to evaluate whether the low dose peripheral OT chronic administration leads to renal renin-angiotensin system (RAS) activation in the kidney of lean and obese Zucker rats. We administered OT by osmotic minipumps into male lean (+/?) and obese (fa/fa) 10-week-old Zucker rats for 14 days at a dose rate of 3.6 μg/100 g/day. Rats were classified into four groups: control lean group, OT-treated lean group, control obese group and OT-treated obese group. Controls received isotonic saline. Animals were given ad libidum access to standard diet and water. The water intake had been monitored daily during OT treatment period. Gene expression of renin, (pro)renin receptor, angiotensinogen, ACE, ACE2, AT1 receptor, AT2 receptor, Mas receptor and neutral endopeptidase in kidney was determined. Data were analyzed by two-way ANOVA test with variables genotype and treatment. We found significant main effect of OT treatment on kidney weight ($p < 0.05$), relative kidney mass ($p < 0.001$) and the gene expression of the renin ($p < 0.001$) and (pro)renin receptor ($p < 0.05$) and we observed an increase in all of these parameters. The gene expression of other RAS components was not changed. OT administration had no significant effect on the water intake. No interaction of genotype x OT treatment effect on all measured parameters was revealed. Present study demonstrates stimulatory effect of OT administration on renin expression in kidney in lean and obese Zucker rats. Increased renin production in our experiment may contribute to regulation of cardiovascular homeostasis and/or, considering elevated (pro)renin receptor expression, may have impact on intrarenal angiotensin II formation and thus physiology of the kidney. Observed alterations at mRNA level prompt to determine protein expression, renin activity and renal function test.

1 Huang W. et al. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278: R226, 2000.
2 Loichot C. et al. Eur. J. Pharmacol. 454: 241, 2002 The study was supported by grants VEGA 2/0174/14, MZ 2012/51-SAV-1 and APVV-0028-10.

S6-C

Basic Research Meets Clinical Endocrinology

S6-C1
Organogenesis in a petridish - How to generate functional thyroid tissue from mouse embryonic stem cells

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Congenital hypothyroidism (CH), the most common congenital endocrine disorder in humans, affects approximately 1/3000 live births. Most CH cases are due to thyroid dysgenesis (TD). However, the molecular mechanisms underlying TD remain poorly understood; in part, due to our limited understanding on how intrinsic factors and extrinsic signaling cues orchestrate thyroid follicular cell (TFC) differentiation and thyroid morphogenesis. Embryonic stem cells (ESC) emerged as a powerful model system to define signaling pathways and gene networks controlling cell differentiation and tissue morphogenesis. Until recently, the toolkit to interrogate thyroid organogenesis was lacking a protocol for efficient generation of TFC from ESC. Directed differentiation of ESC into a given cell type relies on step-wise adjustments of culture conditions in order to reproduce in vitro the sequential events that characterize the normal ontogenesis of a given cell type. However, given the lack of knowledge on the in vivo signals regulating TFC differentiation, we employed an alternative differentiation strategy (direct programming) which relies on forced expression of key transcription factors to promote TFC differentiation. For this purpose, we generated recombinant mESC lines that permit inducible overexpression of Nkx2.1 and Pax8 upon doxycycline (Dox) treatment. Using this model system, a transient overexpression of Nkx2.1 and Pax8 for 3 days was sufficient to generate cell cultures displaying a TFC-like gene expression profile. Application of recombinant hTSH to cell cultures immediately after Dox treatment further stimulated the formation of follicular organoids displaying characteristics reminiscent of thyroid follicles in intact animals. Analyses of iodide uptake and organification in such cell cultures demonstrated the generation of functional thyroid tissue. Importantly, when follicular organoids were grafted in

vivo into athyroid mice, substantial reconstitution of functional thyroid tissue was observed at the grafting site. Moreover, athyroid mice grafted with follicular organoids displayed normalization of thyroid hormone plasma levels as well as symptomatic recovery.

S6-C2
Central regulation of hypothalamic-pituitary-thyroid axis under physiological and pathophysiological conditions

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The primary central regulator of the hypothalamic-pituitary-thyroid (HPT) axis is the thyrotropin-releasing hormone (TRH), a tripeptide amide that functions as a neurotransmitter but also serves as a neurohormone. Hypophysiotropic TRH neurons involved in this neuroendocrine process are located in the hypothalamic paraventricular nucleus and secrete TRH into the pericapillary space of the external zone of the median eminence for conveyance to anterior pituitary thyrotrophs. The released TRH controls the activity of thyroid gland through the regulation of TSH synthesis, glucosylation and release in the anterior pituitary. The activity of hypophysiotropic TRH neurons is regulated by the negative feedback effects of thyroid hormone to ensure stable concentration of circulating level of thyroid hormone. This mechanism involves complex interactions between hypophysiotropic TRH neurons and the vascular system, cerebrospinal fluid, and specialized glial cells called tanycytes. The set point of this feedback regulation, however, can be altered under certain conditions to adjust the circulating thyroid hormone concentration to the actual demand. This mechanism facilitates adaptation of the organism to changing environmental conditions, including the shortage of food and a cold environment. Circulating hormones, neuronal inputs and the tanycytes play critical role in this adaptation. Adverse conditions such as infection, also alter the feedback regulation of this axis, however, it is not completely understood whether the illness induced central hypothyroidism is an adaptive or maladaptive response.

S6-C3
Regulation of cell cycle by microRNAs and its implication in the pathogenesis of endocrine tumors

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Dysregulation of cell cycle has been implicated in the pathogenesis of various tumors including tumors originating from endocrine glands. MicroRNAs (miRNAs) are small noncoding RNAs that negatively regulate gene expression. Their role has been demonstrated in the regulation of numerous physiological and pathophysiological processes including regulation of cell cycle.
Our group studied the pathogenesis of adrenal and pituitary tumors. Multi-omics approach was used for integrating data obtained from various high-throughput technologies including whole comparative genomic hybridization, gene, miRNA and protein expression data.
Our data revealed that both in adrenal and pituitary gland tumors a dysregulation of cell cycle by aberrant miRNA expression might have an important role in the pathogenesis of these tumors. In adrenocortical cancer dysregulation of the G2/M transition of cell cycle by aberrant mRNA and miRNA expression was confirmed. In pituitary tumors underexpression of Wee1 kinase and overexpression of CDC25 phosphatases (both key regulators of cell cycle G2/M transition) together with the opposite expression of their 3' UTR targeting miRNAs were demonstrated. In addition, CDC25A targeting miRNAs showed a strong negative correlation with tumor size suggesting that these miRNAs may have an important role in tumor growth.
Our data suggest that tumors of endocrine glands have differentially expressed miRNAs compared to their normal counterparts and these miRNAs are involved in the regulation of G/2M checkpoint of cell cycle.

S6-C4
The roll of microRNAs in preimplantation embryos and pluripotent stem cells

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MicroRNAs (miRNAs) are a class of small non-coding RNAs that regulate multiple biological processes. Increasing experimental evidence implies an important regulatory role of miRNAs during early embryonic development and in embryonic stem cell biology.
We analyzed the expression profile of pluripotency-associated miRNAs in rabbit embryos and embryonic stem-like (ES-like) cells. The rabbit specific ocu-miR-302, ocu-miR-290 clusters and three homologs of human C19MC cluster (ocu-miR-512, ocu-miR-520e and ocu-miR-498) were identified in rabbit preimplantation embryos and ES-like cells. Ocu-miR-498, ocu-miR-520e and ocu-miR-512-5p exhibited an interesting expression pattern, because gene cluster members are extensively expressed in extra-embryonic tissues.
The ocu-miR-302 cluster was highly similar to its human homolog, while ocu-miR-290 revealed a low level of evolutionary conservation with its mouse homologous cluster. The expression of ocu-miR-302 cluster members began at 3.5 dpc early blastocyst stage and they stayed highly expressed in rabbit ES-like cells. In contrast, high expression level of ocu-miR-290 cluster members was detected during preimplantation embryonic development, but low level of expression was found in rabbit ES-like cells. This results show that the expression of ocu-miR-302 cluster is characteristic for the rabbit ES-like cell, while the ocu-miR-290 cluster may play a crucial role during early embryonic development.

It has been recently demonstrated that miR-99a modulates TGF-β induced epithelial to mesenchymal transition. Therefore expression of ocu-miR-99a-5p and ocu-miR-125b-5p in epiblast cells of 6 dpc blastocysts might have a direct correlation with epithelial-mesenchymal transition of epiblast cells and formation of mesoderm cells prior to implantation. In the future we would like to examine the expression of ocu-miR-99a-5p and ocu-miR-125b-5p in epiblast cells compared to hypoblast and trophoblast cells in 6 day old rabbit embryos.

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S6-C5

Novel insight in the regulation of the brainrenin-angiotensin system and itsconnectionwith hypertension

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The hyperactivity of the brainrenin–angiotensin system (RAS) has been implicated in the development and maintenance of arterial hypertension (HTA). We first demonstratedthataminopeptidase A (APA) and aminopeptidase N (APN), two membrane-bound zinc metalloproteases, are involved in the formation and metabolism of brainangiotensin III (AngIII), respectively. Then, wedesigned the specific and selective APA and APNinhibitors, EC33 and PC18. By blockingin vivobrain APA and APN withthese compounds, weshowedthatAngIII and not AngII, as established in the peripheryis one of the main effector peptides of the brain RAS, exerting a tonic stimulatory control over blood pressure (BP) in hypertensive rats. Central injection of EC33 blocks the formation of brainAngIII and normalizes BP in hypertensive rats, suggestingthatbrain APA maybepotentialtherapeutictarget for HTA treatment

For a clinical use of APA inhibitors, we, therefore, designed 4,4 -dithio{bis[(3S)-3-aminobutyl sulfonicacid]} (RB150),a systemically active prodrug of EC33 obtained by disulfide bridge mediateddimerization. RB150, givenorally in conscious DOCA-salt rats or spontaneously hypertensive rats (SHRs), crosses the intestinal, hepatic and blood-brainbarriers, enters the brain, whereitiscleaved by brainreductases, generatingtwo active molecules of EC33 whichinhibitbrain APA activity, block the formation of brainAngIII and induce a marked and sustaineddecrease in BP. The RB150-induced BP decreaseis due to a decreasedvasopressin release in the blood circulation, whichresults in increaseddiuresis, thusreducingextracellular volume and to a decrease in sympathetictone, leading to a reduction of vascularresistances. Furthermore, in SHRs, concomitant RB150 oral administration withenalapril, a systemic RAS blocker, potentiates the RB150-induced BP decrease. RB150 alsonamed QGC001 wasthenevaluated in humans and PhaseIclinical trials have shownthatthis compound isclinically and biologicallywell-tolerated in healthynormotensive male subjectsafter a single oral dose up to 2,000 mg or afterrepeated doses up to 750mg b.i.d. for 7 days. RB150 is the first drug candidate of a new class of antihypertensive agents targeting the brain RAS, the clinicalefficacy of whichwillbesoonevaluated in hypertensive patients.

S6-C6

Mutation of the palmitoylation site of Estrogen Receptor ERα in vivo reveals tissue-specific roles for membrane versus nuclear actions

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Estrogen Receptor alpha (ERα) activation functions AF-1 and AF-2 classically mediate gene transcription in response to estradiol (E2). A fraction of ERα is targeted to plasma membrane and elicits membrane-initiated steroid signalling (MISS), but the physiological roles of MISS in vivo are poorly understood. We therefore generated a mouse with a point mutation of the palmitoylation site of ERα (C451A-ERα) to obtain membrane-specific loss-of-function of ERα.

The abrogation of membrane localization of ERα in vivo was confirmed in primary hepatocytes, and it resulted in female infertility with abnormal ovaries lacking corpora lutea and increase in luteinizing hormone levels. In contrast, E2 action in the uterus was preserved in C451A-ERα mice and endometrial epithelial proliferation was similar to wild-type. However, E2 vascular actions such as rapid dilatation, the acceleration of endothelial repair and endothelial NO synthase phosphorylation were abrogated in C451A-ERα mice. A complementary mutant mouse lacking the transactivation function AF-2 of ERα (ERα-AF2°) provided selective loss-of-function of nuclear ERα actions. In ERα-AF2°, the acceleration of endothelial repair in response to estrogen-dendrimer conjugate, which is a membrane-selective ER ligand, was unaltered, demonstrating integrity of MISS actions. In genome-wide analysis of uterine gene expression, the vast majority of E2-dependent gene regulation was abrogated in ERα-AF2° whereas in C451A-ERα it was nearly fully preserved, indicating that membrane-to-nuclear receptor crosstalk in vivo is modest in the uterus.

Thus, this work is the first to genetically segregate membrane versus nuclear actions of a steroid hormone receptor and to demonstrate their in vivo tissue-specific roles.

POSTER SESSION

P1

Teaching & History of Physiology

P1.1
Replacement of animal use in medical physiology

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The replacement of animal use in medical education with non-animal methods and techniques often yields both, ethical and technical advantages. Particular emphasis is related to ensure the efficiency and validity of computer simulations regarding animal use replacement. The aim of the study was to clarify the medical student attitude of computer simulation during the laboratory practice in medical physiology. A number of 89 medical students (31 male and 58 female; 20+/-2 years old) at Skopje's Faculty were given questionnaires regarding computer program: Renal function in humans by Sheffield BioScience Programs, Great Britain. The students usually used PCs as following: regularly 57%; fairly often 17.9%; sometimes 17.9%; and 1.1% rarely with learning purpose of 51%. At the end of the laboratory classes the students had to answer with: strongly disagree (SD); disagree (D); neutral (N); agree (A); and strongly agree (SA). To study physiology using animal experiments the students answered with: A, 40.4% and SA, 20.2% while with D, 8.9% and SD, 14.6%. The most of the students preferred working with computer simulations in small groups: 47.1% A and 38.2% SA, and with its good data presentation 70.7% A and 10.1% SA. For replacement in animal use 16.8% A and 20.2% SA, but D 24.7% and SD 19.1%. From the obtained results we may conclude that the medical students accept the computer simulation in medical physiology due to good data presentation, studying together in small groups but still don't completely accept to leave the conventional experiments within animal use.

Key words: non-animal methods; medical physiology; medical students.

P1.2
Ivan Petrovich Pavlov – the Nobel laureate for Physiology or Medicine

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Russian physiologist, Ivan Petrovich Pavlov, is a well-known personality in academic and scientific world. During centuries his findings and scientific research have not lost on actuality, they gained even more significance. In 2014, Pavlov's 165. Anniversary is celebrated. The publication of Pavlov's monograph „Lecture about the activity of the main digestive glands" in 1897 brought Pavlov the world-wide popularity. The monograph was translated into European languages and honoured with the Nobel Prize in 1904. On 7th of 2014 is the 110. Anniversary of Pavlov's Nobel Prize Awarding.

Pavlov's Nobel Prize Diploma says that he has revolutionized the physiology of digestion. Indeed, the Nobel Committee has awarded Pavlov for his contribution in understanding of the mechanism of neural regulation of digestion. Pavlov's idea of the organism's holism supports the principle of unity and interaction with the surrounding.

Pavlov's and his colleagues experiments support the understanding of the central nervous system, of the brain and of the whole nervous activity. This knowledge is directly connected to physiology, biology, psychology, psychiatry, neurology, pharmacology etc.

P1.3
Physiology in context of moral and political philosophy: Support of UNO-Agenda 21

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INTRODUCTION: Nobel Price discipline *physiology* is fundamental science for biology, ecology, medicine. 200 years after *Kant* science is considered in context of epistemology & moral philosophy. Progressive increase of *global health & ecological problems* needs new conceptions for physiology.

CONCEPTION: a *Integration* of scientific-theory/ethics in physiological education & research, **b** implication of *physiological topics to intern. congresses* for philosophy-FISP, psychology-IUPsyS, biophysics-IUPAB, chemistry-IUPAC, also in clinical medicine (IUPHAR/FIGO/ISIM/SIU etc). **c** Foundation of elementary *philosophical units* to some institutes for *physiology=**IP***, **d** foundation of *international **IP** via network* of units from selected countries in context of creation of *international universities* proposed by Bertrand Russell (British Nobel Laureate) as paradigm for better education-research-technology.

Fundamentals for a-d could be 1. *Common educational/research programmes.* **2.** Common elementary *administration & laboratories.* **3.** Recognition of participants as *intern./continental professors/doctors, etc.* **4.** Possibility for *regular work (not only guests)* to institutes/branches in Africa-America-Asia-Australia-Europe. **5.** *Regular successive financial support* for participants/projects by nat. ministries, industry, Eur. Union, UNESCO, etc. **6**. *Possibility for whole life work* after 60years as senior-scientists as honorary professors, institute-directors, etc.

CONCLUSION: Implication of a-d & 1-6 resp. in physiology supports ***UNO-Agenda21*** by better therapy & ecological protection, counteraction of misuse of physiol. discoveries, better health, education & ecology in all countries. Social responsibility of science needs support of proposals by FISP/IUPS/FEPS/IUPHAR.

DEDICATION to moral support 2014-1980 of Nobel Laureates/HonICSD-members: **Africa/**W.SOYINKA Bishop TUTU **America-Asia**/H.G.KHORANA/India-USA Y.T.LEE/China L.PAULING/USA **Europe/**M.EIGEN E.NEHER/FRG B.JOSEPHSON F.SANGER Lord A.TODD/GB B.SAMUELSON/Sweden.

REFERENCES: Michailov Neu Welscher et al: **FISP** (WorldCongrPhilos) **2013-Athens** AbsBook 464-5&503-4&766; **2008-Seoul** DVD2010 (ISBN-13:978-1-889680-835) 4:101-8&20:203-14&37:195-202&45:229-37. **CongrProcIUPS** (IntUnPhysiolSci) **2009-Kyoto** 22:JPhysiolSci **59**/S1:168&214&447-448. **IUPHAR** (IntCongrPharm) **2010-Copenhagen** B&ClPharm&Tox 17/S1:454-5&488-9. **EUROTOX-2012-Stockholm** ToxLett **211**/S211-2; **2011-Paris 205S**:S203&S298. **IUPAC** (WorldCongrChem) **2013-Istanbul** AbsBook 224&611&613&1526.

P1.4
On integrative Physiology in education and research (Part II): Summary of systematic investigations

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INTRODUCTION: Model for *integrative physiology & regular participation in scientific congresses* include reports about **A-***philosophy of physiol.* (epistemology/ethics & psychophysiol.), observations about *electro-&motor activities on* **B** *cellular, organ&system levels of* **C** *cardiovascular,* **D** *urogenital systems (see Michailov/Neu et al FEPS2014 part II:*ref.1-7).

RESULTS-METHODS: *Recent investigations* related to summarized long-term are given (**2014-1970:** A-D).

A-PHILOSOPHY: Scientific & ethical limits of physiol. observations on patients, human relevancy & reduction of animal experiments by application of human surgical-tissue&animal prepar. (fish heart,etc).

B-MYOCYTES-*vascular* (guinea-pig *portal vein* n=120): Complex electrical reactions (intracellular-rec) after Ca-antagonists/nipfedipin, anaesthetics/pentobarbital, procain,etc. include change of burst-patterns into spikes-discharges,etc. *Detrusor myocytes:* After critical stretch (3-80mN) spike activity (63.29±4.96/min) is transformed into burst-plateaus (1.54±1.18/min) via *mechanosensitive ionic-channels* (Ca-activated-K).

C-CARDIO-VASCULAR-SYSTEM: *Fish heart (salmo gairdneri;n=55) as pathophysiological indicator* - generation of various motor-patterns by hormones-adrenalin/drugs-ouabain/toxicants-HgCl2-pyrethroids. *Rat blood pressure*(n=65): High complex hormonal pressor-depressor biphasic reactions (acetylcholine/vasopressin/5-HT) after nicotine&2-mercaptoethylguanidine/MEG suggest hypothesis about *3 mechanisms of hypertonia idiopathica: Sensitization of* CNS (formatio reticularis/hypothalamus), spinal sympathetic neurons (cholinergic-nicotinic)&vascular effector/myocytes, endothel.

D-UROGENITAL-SYSTEM (guinea-pig:n=125): Generation of slow tonic contractions/STC *in-vivo* (isovolumetric cystotonometry) after critical pressure (>20mmHg) and *in-vitro* (stretch >3-5mN) from *vesical trigone* (0.28±0.15/min) suggest *new hypothesis for micturition*: STC mediates transition from collecting (sympathetic) to expulsion (parasympathetic) phase by transformation of electrical spike activity into burst-plateaus after critical stretch leading to detrusor excitation (see B).

CONCLUSION: Implication of *integrative physiology* in biology & medicine leading to better education could support ecological protection, therapy, prophylaxis, esp of arrhythmia/hypertension, vesical incontinency, overactive bladder, e.g. by combined electropharmacological-therapy,etc. in context of better health&science in all countries acc. to **UNO-Agenda21.**

P1.5
On integrative Physiology (Part II): Regular congress participation and reports

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INTRODUCTION: Systematic investigations **(2014-1970)** about integrative *physiology*, esp **A** *philosophical fundamentals & psycho-physiology as well as electro-&motor-activities of* **B** *myocytes C cardio-vascular* **D** *urogenital-systems* [1-7] are summarized (no internet info) *reflecting regular participation in physiological congresses.*

RESULTS: (recent related to earlier) *see Neu et al FEPS-2014 (part I).*

CONTRIBUTIONS: [1] FISP-2013-Athens (WorldCongrPhilos) Proc464-5&503-4; **-2008/10-Seoul** Proc.DVD/ISBN-13, 195-202[A]

[2] CongrProcIUPS (IntUnPhysiolSci) **23 Birmingham-2013**:AbsBook LB44&83&137[BCD] **22 Kyoto-2009**:JPhysiolSci 59/S1:168&214&447-8[ABCD] **21 San-Diego-2005:**Faseb-J 19/4:139.4/351.21, 19/5:772.14/772.15[ABCD] **20 Christchurch-2001**:CD:ID334&291[CD] **19 StPetersburg-**

1997:P036.03& P058.38& P036.02& 036.04[BC] **18 Glasgow-1993**:140.31&140.32&211.2[BCD] **17 Helsinki-1989**:529&529[BD] **16 Vancouver-1986**:P170.12&P170.11[CD] **15 Sydney-1983**:Spl[CD] **14 Budapest-1980**:2428&2429&2430[ABC] **13 Paris-1977**:1497-9[CD] **11 New-Delhi-1974:**273&378&1133[ACD] **Jerusalem-1984** RegMeet:285[D].

[3] ISP (IntSocPathophysiol): **2006-Beijing:** ChinJPathophysiol **22/S13**:228[D] **2002-Budapest**: ActaPhysiolHung **89/1/3**:77[C] **1998-Lahti**: Pathophysiol **5/S1**:245&246[BCD].

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[5] FEPS (FedEurPhysiolSci) ActaPhysiol: **2011-Istanbul** PC28[BD] **2007-Bratislava 191/S658**:49&52&91[ABCD] **2006-München 186/S1**:OT06-36&PM09A-7[AD] **2003-Nice** Press FEPS London 121&123[BD] **1999-Prague** PhysiolRes 48/S1:S96&S138[BD] **1995-Maastricht** EurJPhysiol **430/4/S**:521&161&604[CD].

[6] DPG (GermanSocPhysiol) EurJPhysiol **2002-Tübingen** 443/S[BD] **1997-Rostock 433/6**:R65[BD] **1992-Düsseldorf 420/1**:R99[AB] **1991-Freiburg 419/1**:R98[BD] **1984-Dortmund 402**:R15&R48[BCD] **1983-Mainz 398**:R25[BC] **1982-Giessen 392/S**:R36&R37[BD].

[7] Experientia-1969-25:621-2[C].

CONCLUSION: Clarification of observations A-D leading to better therapy of cardiovascular/hypertension, etc & urogenital/incontinency,etc diseases could support better health in all countries acc to **UNO-Agenda21.** Reports about long-term research projects not only in journals *eg Physiol Rev*, but also to scientific congresses for physiology,etc initiating discussions about future research strategy are necessary.

Dedicated to moral & scientific support 2014-1980 of Nobel Laureates: **Australia-**Sir-J.Eccles, **France-**J.-M.Lehn, **Germany-**M.Eigen/K.von Klitzing/H.Michel/E.Neher, **GB-**Sir J.Kendrew/Lord A.Todd, **USA-J**.Deisenhofer-FRG/H.B.Khorana-India/B.Richter/R.Wilson

P2

Molecular and Cell Physiology

P2.1
Regulation of a human stem cell specific microRNA cluster C19MC

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MicroRNAs are small regulatory RNAs of 21-24 nucleotides in length. Their function is fine tuning the post-transcriptional regulation of genes by targeting mRNA molecules via sequence complementarity with their 3'-untranslated region for mRNA decay and/or translation inhibition. During their maturation process, they are transcribed either from their own microRNA locus or as an intronic sequence of a protein coding gene. This primary transcript (pri-miRNA) can encode a single microRNA or a cluster of microRNAs which, in the canonical pathway, undergo further subsequent nucleolytic cleavages by the Drosha/DGCR8 complex and Dicer to form mature microRNA molecules. Our subject of interest is C19MC, which is the biggest human microRNA cluster containing 54 separate miRNA hairpin structures. These microRNAs are currently believed to be expressed simultaneously and in terms of expression pattern, exclusively in stem cells and placenta. Based on these, we would expect similar expression levels for all microRNAs of the cluster. However, our data from TaqMan Low Density Array measurements suggests that the expression of different parts of C19MC regulated differently. Examining a human embryonic stem cell line (HUES9), we found that certain neighboring miRNAs show similar expression patterns, representing a wide range from high expression to total absence, suggesting the presence of differently regulated sub-clusters inside the cluster. Chip-seq data from Encode project strengthens this idea which seems to be further supported by the presence of certain transcription factor binding sites found inside the cluster. Our goal is to prove the concept of sub-cluster regulation with quantitative measurements of the primary transcripts of the microRNA cluster.
Furthermore, we will examine this phenomenon with functional studies of the putative regulatory regions inside of C19MC.

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P2.2
Beneficial effects of hydrogen sulphide treatment of human adipose derived stem cells in a cell-based model of cell therapy

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Purpose: The potential of cell-based therapies in diseases involving ischemia-reperfusion is greatly hampered by the excessive loss of the administered cells in the harsh, oxidative environment. Our earlier studies indicated that preconditioning of human adipose derived stem cells (ASC) with the hydrogen sulfide donor sodium sulfide (NaHS) increased their survival and efficacy in an in vitro model of cell-based therapy for myocardial infarct. Thus we aimed to better understand the underlying mechanism of action.
Methods: ASCs were treated with 0.3 μM (ASC 0.3), 3 μM (ASC-3) or 30 μM (ASC-30) NaHS 4 times with 3 days between treatments whereas a control group (ASC) received vehicle. The effect of endogenous hydrogen sulphide was investigated using the cystathionine-γ-lyase inhibitor

propargylglycin (PAG, 10 mM). Proliferation was followed by microscopical evaluation. Mitochondrial activity was measured at the beginning of the treatments and on the 9th day. Changes are expressed as percentages of the initial value. The antioxidant effect of NaHS pretreatment (3 and 30 μM) was examined after H2O2 treatments (2 hours, 2 mM) by lactate dehydrogenase (LDH) release.

Results: By the 9th day NaHS dose-dependently increased the proliferation of cells (ASC: 234 ± 25%; ASC 0. 3: 331 ± 67% *; ASC-3: 405 ± 61% ****; ASC-30: 471 ± 33% ** **, vs control, * p <0.05, **** p <0.0001). There was no change in the overall mitochondrial activity as measured with resazurin test. PAG treatment decreased the proliferation from the 3rd day (control: 156.2 ± 10.3%; 10 mM PAG: 81.7 ± 4.7%****). LDH-release was reduced when cells were pretreated with 3 μM NaHS (control: 90.3 ± 7.9%; 3 μM NaHS: 67.0 ± 5.7% **; 30 μM NaHS: 89.5 ± 10,2%, p <0.01).

Conclusions: Hydrogen sulfide can dose-dependently increase the proliferation of human adipose derived stem cells. The unchanged overall mitochondrial activity of increased amount of cells indicates a decreased cellular metabolism that may lead to reduced release of reactive oxygen species from the mitochondria. Antioxidant defenses are more efficient after treatment with 3 μM NaHS.

P2.3
Endometrial oestrogen and progesterone receptors localization in the fat sand rat, Psammomys obesus, a diurnal gerbil

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The aim of this work is to characterize the reproductive function of a diurnal rodent, the fat sand rat, considered such as a model for studying several metabolism diseases. In the female, histological, histochemical and cytological aspects of uterus were studied. Preliminary immunohistological results are presented. Paraffin embedded uterine from isolated females were selected throughout the reproductive cycle. After antigen retrieval, indirect immunohistochemistry method with streptavidin-biotin complex was applied. The uterus was the target of both estrogens and progesterone acting by specific receptors ERs (estrogen receptors) and PRs (progesterone receptors). At estrus and diestrus, labeling of ERs was in the cytoplasm of all epithelial cells; in metestrus, labeling became both in the nuclear and the cytoplasm, few unlabeled cells were observed, the stroma was slightly labeled; at proestrus label for both ERs and PRs was localized in the nuclear fraction. During estrus, PRs, were localized in the nuclear fraction of epithelial and stroma cells; in the lining epithelium, predominate cells were labeled while glandular cells remained immunonegative; in the stroma, labeled cells predominated. In metestrus, labeled PRs were nuclear and cytoplasmic in epithelial cells and only nuclear in the stroma. At diestrus, lining and glandular epithelial cells were completely immunonegative; labeled PRs persisted in the stroma. At proestrus, two cases were observed: negative staining of epithelial tissue and positive in the nuclear fraction of stroma cells in some female and nuclear positive staining of the epithelial and stroma cells in other females. The localization of ERs and PRs varied during the cycle, therefore estradiol and progesterone acting in different uterine compartment during the estrous phases deserve to be studed.

P2.4
The effects of angiotensin II on autophagy pathways in H9c2 cells

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He hypertension induced cardiac hypertrophy is one of the most common cause of heart failure and predictor of cardiovascular morbidity and mortality. Several studies show that autophagy plays an important role in the cardiac hypertrophy. It is unclear this self-digest process serve as a survival (remodelling) process or contribute to cell death. We investigated the autophagic and apoptotic processes in H9c2 cell line in response to different concentrations of ATII (angiotensin II) treatment. H9c2 cardiomyoblast cells were treated with low (10-7 M) and high (10-6 M) concentrations, respectively, of ATII to induce hypertrophy. After rhodamine conjugated phalloidin and DAPI staining we examined the alteration of cell size by fluorescence microscopy. Expression levels of autophagic and apoptotic proteins by Western blots were analyzed. To visualize autophagic vacuoles, MDC treatment and tagging LC3B protein immunocytochemistry were carried out and studied with fluorescence microscope. Based on microscopy studies, the cell size was significantly greater in the treated groups indicating the development of hypertrophy. Enhanced LC3BII/LC3BI ratio and an increased level of Beclin-1, Atg 12, and Bcl-2 were found in response to the lower dose of ATII; the levels of these proteins were not significantly altered in the higher ATII treated group. In the higher ATII treated group, enhanced levels of apoptotic proteins were detected. Furthermore, in the higher ATII treated group we observed reduced MDC signal. Taken together, our results suggest an increased autophagic activity, which may help to reduce damaged protein organelles in response to lower dose ATII treatment. However, in higher dose of ATII treatment, the apoptotic activity is enhanced in H9c2 cell line.

P2.5
Arrestin binding of the beta2-adrenergic receptor is regulated via heterodimerization with the angiotensin type 1A receptor

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Increasing number of evidence shows that the signaling originating from G-protein coupled receptors (GPCR) is

strongly regulated by receptorial cross-talk between different GPCRs and this strongly influences receptorial functions under diverse physiological conditions.
We examined whether the angiotensin type 1A receptor (AT1R) could influence the signaling properties of the beta2-adrenergic receptor. The beta-arrestin binding of the B2AR was measured in HEK293T cells by using a BRET-based approach. Strikingly, we found that costimulation with the AT1R agonist angiotensin II (AngII) and the B2AR agonist isoproterenol (ISO) robustly enhanced the beta-arrestin2 binding of the B2AR compared to the ISO stimulation alone. Next we explored the underyling mechanism of this AT1R mediated potentiation of the B2AR beta-arrestin activation. To block the main signaling pathways of the AT1R, we used calcium chelation and specific inhibitors of protein kinase C, Src and ERK. Nevertheless, none of the used inhibitors had any effect on the AT1R mediated increase in the B2AR beta-arrestin binding. Therefore we speculated, that the observed phenomenon is mediated by heterodimerization between B2AR and AT1R. By performing BRET titration experiments we confirmed the existence of the AT1R-B2AR heterodimer. The increase of the AT1R amount in the cells further enhanced the beta-arrestin binding of the B2AR, indicating its dependence on the AT1R-B2AR heterodimer formation. We investigated the effects of different antagonists on the function of the AT1R-B2AR heterodimer. The cotreatment with ISO and the conventional antagonist candesartan had no effect on the B2AR beta-arrestin binding. However, when we costimulated the cells with the AT1R beta-arrestin biased antagonist TRV120023, we experienced, similarly to AngII, an increase in the B2AR beta-arrestin binding.
Our findings reveal a new mechanism of receptorial crosstalk between the AT1R-B2AR heterodimer. Furthermore, the different effects of the conventional antagonist and the beta-arrestin biased antagonist suggest unexpected new possible effects or side-effects of the new clinically tested beta-arrestin biased antagonist drugs.

P2.6
Examination of pituitary adenylate cyclase activating polypeptide (PACAP)-like immunoreactivity in different pathological clinical samples

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a multifunctional neuropeptide with well known neuroprotective and general cytoprotective effects. Earlier we found significantly lower level of PACAP-like immunoreactivity (LI) in both lung and colon tumor samples compared to normal healthy tissue, most probably due to the degeneration of PACAP containing nerve fibers in the tumor. We also showed that PACAP-LI are significantly higher in cardiac samples from ischemic heart diseases compared to valvular abnormalities. In the present study we investigated the PACAP-LI with radioimmunoassay examination from human blood and tissue samples of patients. We collected tissue samples from different urological disorders (kidney tumor, urinary bladder tumor and prostatic hypertrophy) and breast cancer, and we collected blood samples from patients with diabetes, endocrinological disorders, sleep apnea syndrome and ischemic cardiac diseases. We found significantly higher PACAP-LI in breast tumor samples compared to normal mammalian tissue samples. Similarly to our earlier results in kidney tumor samples we found significantly lower amount of PACAP-LI compared with healthy tissue samples. We did not find significant alterations in PACAP-LI between healthy and tumoral urinary bladder and prostate samples. Our results also showed significant correlations with PACAP-LI in the human blood samples and severity of diabetes, sleep apnea syndrome, endocrinological diseases and ischemic cardiac disorder, but further investigations are necessary to describe the exact function of PACAP in different pathological conditions.

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P2.7
The effects of Endothelin-1 on the level of redox proteins in H9c2 cells

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Different types of reactive oxygen (ROS) and nitrogen species (RNS) play a key role in patomechanisms of various types of cardiovascular diseases including ischemic heart failure. The hypertension induced hypertrophy is a common cause of the development of heart failure. Cells are equipped with different antioxidant defense systems, which are capable of neutralizing the ROS and RNS. The antioxidant defense system contains different enzymes or non-enzymatic molecules such as redox proteins. We aimed to investigate the effect of different concentrations of endothelin-1 (ET-1) treatment on the cell size and the levels of redox proteins in H9c2 cell line. At the end of the treatment, cells were stained with rhodamine conjugated phalloidin and 4',6-diamidino-2-phenylindole (DAPI) and the cell size was studied with fluorescent microscopy. To monitor the mRNA level of hypertrophy related atrial natriuretic peptide (ANP), redox related thioredoxin-1 (Trx-1), and glutharedoxin-1 (Grx-1) we have employed RT-PCR. To study the expression of redox related

molecules at protein levels, Western-blot analysis was carried out. At the lower concentration (10-7 M) of ET-1 treated group, a slight but not significant alteration in the cell size was detected; however, at the higher concentrations (10-6 and 10-5M) of ET-1 treated groups, a marked enlargement in the cell size was detected, respectively. Moreover, our RT-PCR results indicate a marked ANP mRNA increment at 10-6 M of ET-1 treated group. We have also detected significantly increased Trx-1 mRNA and protein levels in the 10-6 M ET-1 treated group. In conclusion, the results show that upon hypertrophic stimuli, cells respond with an increased level of mRNA and redox protein, which may represent an adaptive mechanism to reduce the oxidative stress induced by hypertrophic stimuli.

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P2.8

Role of intracellular signalling pathways in the control of transient receptor potential melastatin 3 (TRPM3) channel activity

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TRPM3 is a thermosensitive nociceptor channel involved in the detection of noxious heat. It is activated by the neurosteroid pregnenolone sulphate (PS) and mediates noxious heat sensation and nocifensive behaviour evoked by PS. Recently we described the opening of an alternative ion permeation pore on the channel which further exacerbates pain sensation. In the current study, we aimed at describing the control of TRPM3 activity by various intracellular regulatory factors and signalling pathways. We applied a combined pharmacological and molecular biological approach and investigated the channel activity by electrophysiological measurements and functional imaging on native and recombinant systems. In cell attached and inside out configuration of patch clamp measurements, we found that the composition of the intracellular milieu dramatically influenced the channel activity. By further studying cellular signaling mechanisms potentially involved in the intracellular regulation of TRPM3 we tested the role of adenosine nucleotides, phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2) and various kinases, as well. We found that adenosine 5`-trisphosphate (ATP) crucially influenced the TRPM3 activity. This involves both direct effects and events related to ATP metabolism. We also identified PtdIns(4,5)P2 as a positive regulatory co-factor of TRPM3 that mediates the positive effect of the ATP. Although we showed that PtdIns(4,5)P2 is able to interact functionally with TRPM3, using a wide array of pharmacological inhibitors and molecular biological techniques, we found that protein phosphorylation related processes are even more important in the regulation of TRPM3 in intact cells. Our results highlight the role of the PLC-PKC pathway and the coupled mediators in the regulation of TRPM3 activity.

P2.9

Physiological effects of ophiobolins on inward rectifier ion channels comparing KAT1 channel in plants to Kir2.x channels in animals

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Background: Ophiobolins are sesterterpene-type secondary metabolites, mostly synthetized by some plant pathogenic fungi (e.g. Bipolaris sp.). These microbial metabolites have effects on animals as well on plants but their action on both type of cells are still poorly known. The inward rectifier KAT1 ion channel has a key role in the plant osmoregulation. The inward rectifier potassium current (IK1) determines the resting membrane potential and contributes to the final repolarization in the animal cells. The Kir2.x is pore-forming α-subunit genes underlying the structural base of IK1. Earlier we have shown that the ophiobolin A (OPA) redistributed the Kir2.x ion channels in the plasma membrane as well as in the mitochondrial and nuclear membrane in myocytes.
Aim: In this study we compared the effects of ophiobolins on inward rectifier current ion channels using in vitro cell cultures.
Methods: In this study we detected the special effects of OPA and P1 on KAT1 and Kir2.x ion channels using patch-clamp method. The in vitro model system experiments were performed with HEK293 heterologously expressed KAT1 channels and wild type HEK293 cells.
Results: Kinetic analysis revealed that the KAT1 has time-dependent deactivation. OPA and P1 treatments have a blocking effect with concentration dependence on inward rectifier potassium current. OPA and P1 serve as an external stress factor both on the plant stomatal guard cells as well as on animal cells.
Discussion: These results suggest that the physiologically active KAT1 and Kir2.x channels are crucial for maintain the normal inward rectifier current. The inward rectifier ion channel may serve as a "safeguard function" maintaning the ionic homeostasis balancing the pathological effects in living cells.

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P2.10
NGF-induced neurodifferentiation of PC12 cells is not influencing the expression of Na,K-ATPase genes

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Objective: The rat pheochromocytoma cell line PC12 has become popular model system for the cellular and biochemical analysis of neuronal differentiation. This is because PC12 cells respond to differentiation stimuli into a phenotype resembling symphathetic neurons with an extensive neurite network. Previously it was shown that neurodifferentiation of PC12 cells activated by neuronal growth factor (NGF) is accompanied by an increase in L-type calcium current density and hyperpolarization of the resting membrane potential (1). It is known that the Na,K-ATPase is involved in maintaining stable value of resting membrane potential and, indirectly, also in regulation of intracellular calcium concentration . Therefore the expression of mRNA for 3 catalytic and 2 shaperon isoforms of Na,K-ATPase and possible coupling between Na,K-ATPAase and L-type calcium channels during differentiation of PC12 was analyzed.
Methods: PC12 cells were grown for 9 days. After plating out into 35 mm Petri dishes (day 0) cell differentiation was initiated by supplementing the culture medium with 50 ng/ml of NGF. The expression of mRNA for Na,K-ATPase during differentiation of PC12 was analyzed by RT-PCR method. Results: The genes for all 5 investigated subunits of NKA (α1, α2, α3, β1 and β2) were expressed in PC12 cells. During differentiation of PC12 cells the subunits of NKA were expressed in constant level. When the expression of CaV1.2 L-type calcium channels was suppressed by siRNA specific for the CACNA1C gene the expression of α1, α3, β1 and β2 subunits of NKA was slightly increased, but this effect was statistically insignificant.
Conclusion: Our data suggest that expression of genes for Na,K-ATPase in PC12 cells is not changed significantly during differentiation induced by NGF. Also, Na,K-ATPase seems to be independent of alterations of L-type calcium current induced by NGF. (1) – Lichvárová L, Jašková K, Lacinová Ľ (2012): Gen. Physiol. Biophys., 31, 473 – 478.

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P2.11
Improved methodical approach for quantitative BRET analysis of G protein coupled receptor dimerization

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G Protein Coupled Receptors (GPCR) can form dimers or higher ordered oligomers, which process can remarkably influence the physiological and pharmacological function of these receptors. Quantitative Bioluminescence Resonance Energy Transfer (qBRET) measurements are the gold standards to prove the direct physical interaction between the protomers of presumed GPCR dimers. In this method, the resonance energy transfer (BRET signal) is measured between energy donor and energy acceptor labeled receptors. To distinguish between dimerization and non-specific interactions, the amount of donor is held constant, while the amount of acceptor is increased, and the BRET signal is plotted as a function of acceptor/donor ratio. In these settings, dimerization leads to saturation curve, while non-specific interactions lead to linear relationship. Maintaining constant donor amount is absolutely necessary for the correct interpretation of qBRET curves.
We found in our preliminary experiments that maintaining constant donor amount is almost impossible in transient transfection systems. Based on Monte Carlo simulations, we found that decrease of the donor expression can lead to saturation qBRET curves even if the interaction between receptors is non-specific, thus can lead to a false interpretation of dimerization state. We set up a modified version of qBRET to distinguish between dimerization and non-specific interactions when the results of classical qBRET experiments were ambiguous. Our simulations results were confirmed experimentally using an inducible dimerization system. We used this new method to investigate the dimerization of various GPCRs, and our data have confirmed the homodimerization of V2 vasopressin and CaSR calcium sensing receptors, the heterodimerization of V2 and V1a vasopressin receptors, whereas our data argue against the heterodimerization of these receptors with other studied GPCRs, including type I and II angiotensin, β2 adrenergic and CB1 cannabinoid receptors.

P2.12
Effects of adenosine on human hair follicls and hair follicle deirved outer root sheath keratinocytes

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Adenosine is known to have various physiological functions such as vasodilatation, anti-inflammation, angiogenesis, and tissue protection in vascular or neuronal cells; however, little is known about its function in hair follicular cells. Recent studies strongly suggest that adenosine has an important role in influencing hair follicle (HF) thickness and growth. It was shown that adenosine increased the level of important hair cycle modulator growth factors (endothelial growth factor, fibroblast growth factor-7) on cultured dermal papilla cells via adenosine receptor mediated signaling pathways.
In our present study, we have investigated how human HFs and HF-derived outer root sheath (ORS) keratinocytes respond to adenosine treatment.

Firstly, we identified the expression of the four type of adenosine receptors (A1, A2A A2B and A3), both on mRNA and protein levels, in human HFs and primary cultures of ORS keratinocytes. We found that different concentrations of adenosine did not significantly alter the viability of ORS keratinocytes moreover it did not induce cell death. Furthermore, as revealed by determining expressions of various cytokeratins (RT-qPCR), adenosine caused significant decrease in the level of proliferation marker keratin (KRT)15 however it increased the expression of various differentiation markers (KRT1, KRT10, loricrin and filaggrin), thereby shifting the proliferation-differentiation balance of the cells towards differentiation. Of further importance, adenosine exerted a remarkable anti-inflammatory effect (significant suppression of the basal expression of various pro-inflammatory cytokines, e.g. interleukin [IL]- 1α, IL1β, IL6, IL8 and tumor necrosis factor-α). Finally, using HF organ culture model system our current study revealed that adenosine significantly increased the HF elongation in good accordance with the previous findings.
Collectively, these data suggest that adenosine may function as a promising, novel pharmacological tool for the manipulations of hair growth disorders and inflammatory diseases.

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P2.13
GPCR-induced paracrine transactivation of CB1 cannabinoid receptors in vascular smooth muscle cells modulates calcium signaling and ERK pathways

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Intracellular signaling systems of G protein-coupled receptors (GPCR) are well established, but their role in paracrine regulation of adjacent cells is considered as a tissue-specific mechanism. We have shown previously that AT1 receptor (AT1R) stimulation by angiotensin II (AngII) leads to diacylglycerol lipase activation and diacylglycerol formation, which in turn is converted to 2-arachidonoylglycerol (2-AG), an important endocannabinoid. 2-AG is responsible for the consequent transactivation of co-expressed CB1 cannabinoid receptors (CB1R). In the present study we have tested the hypothesis that GPCR signal-dependent paracrine transactivation effect exists in a physiological situation and can moderate the signaling effects of vascular smooth muscle cells (VSMC). Paracrine transactivation was examined by bioluminescence resonance energy transfer (BRET). HEK293T cells transfected with GPCRs (e.g.AT1R, thromboxane (TP)R, CB1R) during agonist stimulation could activate BRET-based sensors of Gi/o linked with CB1R activation in separate cells. To examine the relevance of this phenomenon to endogenous receptors (AT1R, TPR), VSMCs were prepared from rat aorta. During stimulation of vasoconstrictor GPCRs, BRET signals of HEK cells containing CB1R-Gi/o sensor were detected. Also, GPCR-induced activation of cell calcium signal and ERK phosphorylation was detected. Agonist stimulation augmented BRET signal in transfected cells of both AT1R and TPR, this effect was also observed with AT1R on VSMCs. Inhibition of monoacylglycerol lipase (JZL184) in VSMCs attenuated AngII-induced calcium signal and augmented ERK activation, whereas inhibition of CB1Rs augmented AngII-induced calcium signal and attenuated ERK activation. These effects were not observed with vascular TPR stimulation. These findings verify that VSMCs contain their own conditions to produce 2-AG induced by intrinsic AT1Rs but not sufficiently by TPR. These data suggest that the modulation of endocannabinoid formation and consequent paracrine transactivation of vascular CB1Rs by altering vascular signaling mechanisms can influence vascular adaptation and remodeling.

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P2.14
Role of inositol lipids in the localization of peripheral membrane proteins in mammalian cells

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The plasma membrane (PM) targeting of the peripheral membrane proteins occurs due to variable processes. In most cases these are achieved with posttranslational lipid modifications, such as palmitoylation, myristoylation or prenylation. In several proteins these lipid anchors are enough to reach the proper PM localization. However, in case of other proteins, such as K-Ras, the PM binding is completed via electrostatic interactions between negatively charged phospholipids of the PM and basic amino acids of the proteins. In this study we investigated, how alteration of phosphoinositides (PIs) can regulate the intracellular distribution of peripheral membrane proteins, with special emphasis on those, whom anchoring depends on electrostatic interactions.

Bioluminescence resonance energy transfer (BRET) was applied to follow the lipid-dependent movement of membrane bound proteins in transiently transfected HEK 293T and COS-7 cells. For this, the fluorescent protein, Venus was tagged with various plasma membrane targeting fragments, while luciferase was targeted to the cytoplasmic surface of ER and Golgi, using the targeting sequence of Sac1 and Tgn38, respectively. To validate our BRET results confocal microscopy experiments were accomplished with the targeted Venus proteins. PI(4)P and/or PI(4,5)P2 depletion was performed by hormonal activation of the PLCβ enzyme or by applying a rapamycin-inducible heterodimerization system, in which various PI-phosphatases were recruited to the PM.

We found that joint depletion of PM PI(4)P and PI(4,5)P2 by pseudojanin evoked rapid translocation of Venus from the PM to the ER and mostly to the Golgi, when the CAAX domain of K-Ras or the targeting sequence of Src was used. Stimulation of Gq-coupled M3 muscarinic receptor also resulted in a

significant but transient translocation of Venus. In addition, we were able to show the translocation upon lipid depletion in case of Venus-tagged full length K-Ras. Since the effects of peripheral membrane proteins depend on their membrane localization, this mechanism suggests the potential importance of PIs in the regulation of K-Ras-dependent signalling processes.

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P2.15
Endocannabinoid-mediated modulation of GPCR signaling-induced vasoconstriction and hypertension

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Activation of G protein-coupled receptors (GPCRs) can induce vasoconstriction via calcium signal-mediated or also by other (e.g. Rho-dependent) pathways. Earlier reports have shown that diacylglycerol (DAG) produced during calcium signal generation can be converted to an endocannabinoid, 2-arachidonoylglycerol (2-AG). Our goal was to provide evidence that GPCR signaling-induced 2-AG production and activation of vascular type1 cannabinoid receptors (CB1R) is capable of reducing agonist-induced vasoconstriction and hypertension.
Blood pressure measurements were performed on CB1R knockout (KO) and wild type (WT) mice and also their isolated aortas were examined by myography. Vascular expression of CB1R was demonstrated with immunohistochemistry. Calcium signal generating GPCR agonists such as angiotensin II (AngII), vasopressin or phenylephrine induced dose-dependent vasoconstriction, which was enhanced in CB1R KO, compared to WT mice. In WT mice CB1R agonist WIN55212 induced vasodilatation (by 14.5±6.3%) and vasoconstriction to agonists was enhanced by the blockade of CB1Rs (by 10-30%). These effects were missing in CB1R KO mice. AngII-induced vasoconstriction was also augmented by inhibition of DAG lipase (tetrahydrolipstatin) and was attenuated by inhibition of monoacylglycerol lipase (JZL184), an enzyme that inhibits 2-AG-degradation, suggesting a functionally relevant role for endogenously produced 2-AG. In vivo measurements revealed that blockade of CB1Rs augmented AngII-induced hypertension in WT but not in CB1R KO mice, which suggests that loss of CB1R function augments AngII-induced blood pressure rise in mice.
These data demonstrate that the vasoconstrictor effect of GPCR agonists is attenuated via vascular endocannabinoid formation. It is also suggested that agonist-induced endocannabinoid-mediated CB1R activation can be significant physiological modulator of vascular tone during high contractile states. Thus, the selective modulation of GPCR signaling-induced endocannabinoid release has a therapeutic potential in case of increased vascular tone and hypertension.

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P2.16
Frequency of specific methods for the detection of EGFR in lung tumors

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Epidermal growth factor receptor (EGFR) is a trans-membrane receptor protein and is over-expressed in several tumor types, including NSCLC. It was one of the molecules that were recognized as a potential biomarker for the development of targeted therapies. A Pub Med literature search was performed in order to demonstrate what methods are mostly used in detection of EGFR mutation in lung cancer, with aims to show their distribution through the last 10 years. In this search we took next key words: “mutations”, “epidermal growth factor receptor”, “EGFR”, “non-small cell lung cancer”or “NSCLC. We thus obtained 1,270 articles. Results shows that a lot of methods are used to determine mutations of EGFR in lung cancer. Immunohistochemical analyses in combination with PCR, direct sequencing, FISH or CISH were more frequently used in the period from 2001 to 2005. In the past six years direct sequencing, in combination with PCR alone or with PCR and other advanced techniques, has widely been used to detect EGFR mutation in NSCLC. We wish to point out that certain methods are generally used in the diagnosis of the tumor from biopsy. Other more sensitive methods are recommended to determine the presence of the mutation of EGFR from small tissue quantities.

P2.17
Following the inositol lipid changes upon stimulation of EGF receptor in human HEK 293 fibroblasts

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Phosphorylation of the inositol ring at positions 3, 4 and 5 results in the synthesis of seven different phosphoinositides (PI). Their levels in the plasma membrane (PM), endomembranes and in the cytoplasm can dynamically change upon hormonal stimulation which can influence several cellular processes. Measuring the level of these lipids in living cells can help us to better understand their distinct functions. We recently developed a highly sensitive method which enables us to follow the dynamic change of these lipids in living cells. We performed bioluminescence resonance energy transfer (BRET) measurements between various luciferase-labeled PI-binding domains and a PM-targeted Venus in HEK 293T cells. To monitor the inositol lipid pools the

followings were used: the PH domain of PLCδ1 for PI(4,5)P2, the PH domain of BTK for PI(3,4,5)P3 and an intramolecular IP3 BRET sensor based on the ligand binding domain of the type-1 IP3 receptor. To measure the PI4P level in the PM we used two different PI4P sensors, the 2xPH domain of OSH2, and the 2xP4M domain of SidM. Stimulation of the cells with EGF resulted in a sustained increase of PIP3, a slowly developing IP3 signal and a transiently decreased PIP2 level. Interestingly the distinct PI4P sensors showed different changes, the 2xPH-OSH2 signal continuously decreased meanwhile the 2xP4M signal increased. To further characterize the different PI4P sensors, we used PI4K enzyme inhibitors, and a previously described rapamycin-induced heterodimerization assay, and we found that 2xPH-OSH2 shows a different pool of PIs than the 2xP4M. The increasing signal of 2xP4M could be blocked with 10 μM wortmannin pretreatment but not with 250 nM PIK-93, which indicates that upon EGF stimulation the PI4P synthesis occurs via the activation of PI4KIIIα enzyme. Our highly sensitive approach makes us capable to follow the dynamic inositol lipid changes upon stimulation of the cells with various compounds. Selective measurements of these lipid pools may result in the discovery of differences between stimuli and therefore the role of inositol lipid pools and inositol modifying enzymes during the activation process.

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P2.19
Characterization of the inherited I130N substitution in V2 vasopressin receptor revealed a gain-of-function mutation leading to NSIAD

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Nephrogenic syndrome of inappropriate diuresis (NSIAD) is a recently discovered disease based on the mutation of the V2 vasopressin receptor (V2R). To date the mutation of the 229 phenylalanine and the 137 arginine was identified as a molecular basis of the gain-of-function mutation in this G-protein coupled receptor.
Here we present a newly discovered I130N substitution, identified in a German family. Functional characterization revealed constitutive activity of the mutant V2R. The cAMP concentration was monitored with a highly sensitive Epac-based bioluminescence resonance energy transfer (BRET) sensor. The constitutive activity of the I130N receptor could be inhibited with V2R inverse agonist Tolvaptan and the stimulation of the mutant receptor with AVP resulted a further increased cAMP concentration. Flow cytometry showed a significantly lower plasma membrane expression of the mutant receptor compared to the wild type receptor. The change in the amount of cell surface receptors was found to be due the constitutive internalization of the mutant receptor. This constitutive internalization could also be inhibited with Tolvaptan and was also sensitive to dominant negative dynamin. The mutant receptor was able to bind β-arrestin 2 upon agonist stimulation, although this binding was moderate compared to the wild type receptor.
The hyponatraemia of the patients was found to be the consequence of the I130N substitution in the V2R. The mutation leads to constitutive active receptor, resulting an increased cAMP concentration in the cells and also to constitutive internalization. The mutation generates an active conformation which can be inhibited with Tolvaptan. The internalization processes are dynamin dependent and the inhibition with dominant negative dynamine leads to drop in the cAMP concentration most likely because of the desensitization of the receptors. The inherited mutation has no clinical symptoms in the adult patients, but may have significance in infancy. The complication of the genetic hyponatraemia in infants could be improved with Tolvaptan regarding to the I130N mutation.

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P2.20
Infuence of ɤ-irradiation on properties of Na,K-ATPase in cardiac sarcolemma

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Objective: Previously it was shown that adverse effect of ionizing radiation on the cardiovascular system is beside other factors mostly mediated by reactive oxygen and nitrogen species. One of the structures highly sensitive to radicals is the Na,K-ATPase the main system responsible for extrusion of superfluous Na+ out of the cell which utilizes the energy derived from ATP.
Method: The aim of present study was the investigation of functional properties of cardiac Na,K-ATPase in 20 weeks old male rats 6 weeks after gamma-irradiation by a dose 25 Gy (IR).
Results: Irradiation induced decrease of systolic blood pressure from 133 in controls to 85 mm Hg in IR group together with hypertrophy of right ventricle and hypotrophy of left ventricle. When activating the cardiac Na,K-ATPase with substrate, its activity was lower in IR in the whole concentration range of ATP. Evaluation of kinetic parameters revealed a decrease of the maximum velocity (Vmax) by 40% with no changes in the value of Michaelis-Menten constant (Km). During activation with Na+, we observed a decrease of the enzyme activity in hearts from IR at all tested Na+ concentrations. The value of Vmax decreased by 38%, and the concentration of Na+ that gives half maximal reaction velocity (KNa) increased by 62%.
Conclusion: Impairment in the affinity of the Na+-binding site as indicated by increase of KNa together with decreased number of active Na,K-ATPase molecules as suggested by lowered Vmax values are probably responsible for the deteriorated efflux of the excessive Na+ from the intracellular space in hearts of irradiated rats.

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P2.21

Effects of apocynin, NADPH oxidase inhibitor, on levels of ADMA, MPO, iNOS and TLR4 induced by myocardial ischemia reperfusion

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Aim: In this study, effects of apocynin (Apo) on inflammatory mediators, toll-like receptor 4 (TLR4) and inducible nitric oxide synthase (iNOS), myeloperoxidase (MPO) activity that may represent neutrophil accumulation and oxidative stress-related and endogenous NO synthase (eNOS) inhibitor ADMA levels were analysed in myocardial ischemia reperfusion (MIR) injury.

Methods: In the research, Sprague-Dawley male rats were divided to three groups (sham group, MIR group and MIR+Apo group, n=7 each group). Myocardial ischemia-reperfusion was induced by occlusion of the left anterior dessending coronary artery for 30 min followed by 120 min of reperfusion. In MIR+Apo group, apocynin was given along 15 days 10 mg/kg intraperitoneally. MPO, iNOS, TLR4 and ADMA levels were evaluated with ELISA in myocardial tissue.

Results: While TLR4, MPO ADMA, levels increased in MIR group, they significantly decreased in MIR+ Apo group. iNOS levels was found higher in the MIR than in the sham group and decreased in MIR + Apo group, but not statistically significant.

Conclusion: We first demonstrated that treatment of apocynin significantly changed ADMA, MPO, iNOS ve TLR4 levels in myocardial tissue after MIR injury. Our results suggest that apocynin may be protective in MIR injury by reducing these parameters related with inflammation and oxidative stresss.

P2.22

Regulatory proteins of myocardium in evaluation of cardiotoxicity

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Cardiac troponins T and I (cTnT and cTnI) are becoming serum biomarkers of choice for monitoring potential drug-induced myocardial injury in both clinical and preclinical studies. Troponins can detect presence of anthracycline cardiotoxicity very early, before any impairment of cardiac function can be revealed by common diagnostic approaches. Unfortunately, the timing of cardiac troponin release during anthracycline therapy has not been described.

The aim of the present study was to determine the precise "diagnostic window" of cardiac troponins on the validated model of chronic anthracycline cardiotoxicity in rabbits. The study was carried out on two groups of rabbits: 1) daunorubicin (3 mg/kg, once weekly for 8 weeks), 2) control (saline in the same schedule). All animals were sacrificed 2 weeks after the last dose. Blood samples were obtained before drug administration as well as 2, 4, 6, 12, 24, 48 and 72 hours after the 1st, 5th and 8th drug administration. Plasma concentrations of cardiac troponins were determined using both hs cTnT (Roche) and hs cTnI (Abbott).

The cardiac troponins levels progressively increased in parallel with the increasing number of the chemotherapy cycles, confirming that the risk of cardiotoxicity is dependent on cumulative dose. Unlike acute myocardial infarction, the first troponin raise occurred very early after daunorubicin dose (2 hrs), the maximal values were reached between the 4th and 6th hours and then gradually declined, which is probably given by the release of loosely bound myocyte pool of troponins. Intracellular degradation of cTnI was found only in the case of severe damage of LV (dP/dtmax = 3015.9 mm Hg/s). The plasma cardiac troponins levels correlated with index of contractility dP/dtmax. Histological findings also confirmed gradually increased number of damaged cardiomyocytes (single or later in foci) in relation to increased cardiotoxicity, which process results in breakdown of myofibrils and loss of bound cardiac troponins.

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P2.23

Human serum albumin suppresses the angiotensin-converting enzyme activities in human

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Angiotensin-converting enzyme (ACE) inhibitors represent the 5th most often prescribed drugs. There are reports dating back to 1979 suggesting the existence of endogenous ACE inhibitors, which was overshadowed by the clinical success of ACE inhibitor drugs. We aimed to confirm the existence, to characterize and to identify this endogenous ACE inhibitor. Individuals with DD genotype of ACE gene exhibited a 64% higher serum ACE concentration (median [range], 155.2 ng/mL, [74-288], n=52) compared to individuals with II genotype (94.5 ng/mL [47-194], n=28). However, the difference in ACE activities was only 32% (43.11 U/L [27.3-59.8], and 32.74 U/L [15.6-55.4], respectively). The specific enzyme activities significantly increased by dilution (23.2±0.7U/L, 4-fold dilution, 51.4±0.3U/L, 32-fold dilution, n=3, p=0.001). It was hypothesized that ACE activity is masked by an endogenous inhibitor, which dissociates from the ACE when its concentration decreases upon dilution. Filtering of serum samples through 100 kDa (but not 50 kDa)

filters eliminated the inhibiting factor (after filtering: 56.4±2.4U/L, n=4, control: 26.4±0.7U/L, n=4, p <0.001), indicating the molecular mass of inhibitor between 50 and 100 kDa. Lineweaver-Burk plot indicated non-competitive inhibition of ACE by this endogenous factor. ACE was crosslinked with interacting proteins in human sera. One of the crosslinked products was identified as human serum albumin (HSA). HSA inhibited human purified (circulating) and human recombinant ACE with potencies of 5.7±0.7 and 9.5±1.1 mg/mL, respectively. Effects of HSA on tissue bound ACE were tested on human saphenous vein segments. Angiotensin I evoked vasoconstriction was inhibited by HSA (maximal force with HSA: 6.1±1.3 mN, without HSA: 13.5±2.6 mN), while HSA was without effects on angiotensin II mediated constrictions (with HSA: 18.7±2.2 mN, without HSA: 19.2±3.50 mN). HSA was identified as a potent endogenous inhibitor of the ACE in this study. The ACE activity appears to be almost completely suppressed by HSA when it is present at physiological concentration, in vivo, which suggest angiotensin I to II conversion as a rate limiting step of RAS.

P2.24
Investigation of metabolic processes in cultured melanoma cell lines

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TASK-3 channels are thought to promote proliferation and/or survival of malignantly transformed cells. Our previous results suggested mitochondrial expression of TASK-3 channels so these channels are legitimate proteins that regulate the electrochemical gradient for ATP synthesis and could play crucial role in maintaining the mitochondrial function in hypoxic or impaired metabolic environments.
To investigate the effect of TASK-3 gene silencing on cell proliferation and viability we performed controlled culturing condition changes (reduced glucose concentration and serum deprivation, hypoxic condition). Mitochondrial DNA content, reactive oxygen species (ROS) synthesis and ATP release of the different melanoma cultures were also studied.
Reduction of cell viability (MTT assay) and proliferation (CyQant assay) caused by serum and glucose deprivation as well as in hypoxic culturing condition (4% O2, 96% N2) were significantly larger in TASK 3 knockdown melanoma cells compared to their control counterparts. We also measured the ATP release and mitochondrial DNA content of control and TASK-3 knockdown melanoma cultures using luminescent probe and qPCR, respectively. Both the ATP release and the mitochondrial DNA amount was significantly reduced in TASK-3 deficient melanoma cells. The average ROS release (measured by flow cytometry using the fluorescent dye dihydroethidium) showed no significant difference between control (139.8 +/- 3.8 a.u.) and scrambled transfected cells (147.5 +/- 3.7 a.u.), however this value was significantly lower in TASK-3 knockdown cells (107.2 +/- 4.8 a.u; n=9, p was less than 0.05).
We conclude that reduced TASK-3 expression results in decreased DNA content (number of mitochondria) and mitochondrial activity which presumably cause reduction of the rate of chemical reactions in the mitochondria. It is also suggested that TASK-3 expression in the mitochondria could be essential in supporting the survival and proliferation of malignantly transformed melanoma cells by increasing their hypoxia and starvation tolerance.

P2.25
The bile acid, taurocholic acid activates ryanodine receptor and inhibits SERCA activity

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The earliest critical event of pancreatitis is a long lasting high amplitude rise of intracellular Ca2+ concentration in the acinar cell, which can be triggered by secretagogue overstimulation or high concentration of bile acids. Although, Ca2+-release through ryanodine receptors (RyR) is involved in the process, the significance and the exact mechanism of bile acid`s action on RyR has not been fully elucidated yet. Therefore, we aimed to test whether taurocholic acid (TCA) exerts a direct effect on RyR and SERCA pump. We show that TCA enhanced RyR`s 3H-ryanodine binding in the pathologically relevant 25-500 μM range. We also found that 500 μM TCA triggered robust Ca2+-release from RyR-enriched vesicles, which was prevented by the application of 12 μM dantrolene. Single channel current analysis of RyRs reconstituted into lipid bilayers demonstrated that 200 μM TCA induced a 5-fold increase in channel open probability (Kd=180 μM) Furthermore, we show that TCA suppressed Ca2+-uptake and ATP-ase activity of SERCA- enriched vesicles.
Overall, our data strongly suggest that TCA activates RyR and inhibits SERCA with an allosteric mechanism, which might contribute significantly to TCA-induced pathologic Ca2+-leak from the endoplasmic reticulum in pancreatic acinar cells.

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P2-26
Spatiotemporal analysis of miR-17 and miR-21 during murine kidney ischemia-reperfusion injury

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The gene-expression regulatory role of microRNAs (miRNAs) has a great potential in disease pathomechanism. We investigated miRNA profile and spatiotemporal expression in acute kidney injury (AKI). Ischemia-reperfusion (I/R) is the main cause of AKI an important cause of long-term renal failure due to renal fibrosis. With increasing prevalence, both acute and chronic renal failure is an incrementing, unmet medical need.
After I/R of C57BL/6 mice, AKI was verified by renal histology (tubular necrosis, regeneration), blood urea nitrogen (BUN), renal mRNA, and plasma concentration of the tubular damage marker NGAL. MiRNA profile evaluated 24 hours post-ischemia (Luminex multiplex) was further analyzed during reperfusion (QPCR). Cellular origin of miRNAs was investigated by fluorescence- and magnetic-activated cell sorting with cell-type specific antibodies (tubular cells /LTL/, injured tubular cells / KIM1/, endothelial cells /CD31/, and pericytes /PDGFRβ/).
MiR-21, miR-17-5p, and miR-106a were elevated (3.0, 1.5 and 1.4 fold, respectively, $p < 0.05$) out of the 21 miRNAs successfully profiled on the Luminex multiplex assay. MiR-17-5p expression was elevated early in tubular (LTL+) cells (2.4 fold, $p < 0.05$) and later in injured (KIM1+) tubular cells (3.0 fold, $p < 0.01$). MiR-21 was elevated only later in injured (KIM1+) tubular cells (5.2 fold, $p < 0.01$), in endothelial (CD31+) cells (4.9 fold, $p < 0.05$) and in pericytes (PDGFRβ+, 8.2 fold, $p < 0.05$). Though renal miR-17-5p and miR-21 expressions correlated with each other in the whole kidneys ($p < 0.05$ at each time), we could not detect a direct interaction between them in vitro.
Our results demonstrate that miR-17-5p is activated during the early tubular response to renal I/R injury, while miR-21 is involved in a later phase of AKI. MiR21 seems to play a central role in reperfusion injury regulating regeneration and is known to mediate preconditioning. Similarly to TGFb, miR-21 is essential in recovery from the acute injury, but sustained elevation contributes to fibrosis. Currently we investigate functional inhibition of miR17 and 21 in acute ischemia induced renal fibrosis.

P2.27

Determination of antitumor properties of synthesized chalcone-phosphazenes containing dioxybiphenyl groups against PC-3 and LNCaP cell lines

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Prostate cancer is one of the most prevalent type of cancer in men. The exact solution for the treatment of different types of cancers has not been fully elucideted. Cyclotriphosphazene derivatives have attracted the attention of researchers to be used as potential anti-cancer agents. Chalcones are an important class of natural compounds. Chalcones display a various pharmacological effects, including anti-proliferative, anticancer, antioxidant, anti-inflammatory, or anti-infective activities.
In this study, a new cyclophosphazenes (chemical structures; compound 1; 2,2-(4'-oxy-2-fluorochalcone)-4,4,6,6-bis[spiro(2',2''-dioxy-1',1''-biphenyl)]cyclotriphosphazene(C54H36F2O8N3P3), compound 2: 2,2-(4'-oxy-3-fluorochalcone)-4,4,6,6-bis[spiro(2',2''-dioxy-1',1''-biphenyl)cyclotriphosphazene (C54H36F2O8N3P3), and compound 3; 2,2-(4'-oxy-4-fluorochalcone)-4,4,6,6-bis[spiro(2',2''-dioxy-1',1''-biphenyl)]cyclotriphosphazene (C54H36F2O8N3P3) synthesized in our laboratory was investigated in terms of their mechanism of action and antitumor properties by using androgen-dependent (LNCaP) and independent (PC-3) human prostate cancer cell lines.
Varying concentrations of phosphazenes (1, 5, 25, 50 and 100 μM) was treated with prostate cancer (PC-3 and LNCaP) for 24 h. Antitumor activities of the phosphazene compounds (compound 1, 2 and 3) and were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Compound 1, (1, 5, 50 and 100 μM doses), 2, (1, 5, 25, 50 and 100 μM doses) and 3, (5, 25, 50 and 100 μM doses) reduced cell viability of PC-3 cells ($p < 0.05$). Compound 1, (1, 5, 25, 50 and 100 μM doses), 2, (1, 5, 25, 50 and 100 μM doses) and 3, (1, 5, 25, 50 and 100 μM doses) reduced cell viability of LNCaP cells ($p < 0.05$).
The phosphazene compounds (1, 2 and 3) reduced cell viability of PC-3 and LNCaP cells ($p < 0.05$). The phosphazenes 1, 2 and 3 have antitumor activity on human prostate cancer cell lines (PC-3 and LNCaP).

Key Words: Phosphazene, Chalcone, PC-3, LNCaP, cell viability, MTT assay

P2.28

New synthesized phosphazenes containing chalcone on human prostate cancer cell lines: An in vitro study

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Prostate cancer is the most common type of cancer in males. There is currently no therapy to cure various types of cancer, and hence studies aiming to develop cancer treatment are important and ongoing. In this study, a new cyclophosphazenes (chemical structures; compound 1; 2,2-(4'-oxy-2-chlorochalcone)-4,4,6,6-bis[spiro(2',2''-dioxy-1',1''-biphenyl)]cyclotriphosphazene(C54H36Cl2O8N3P3), compound 2; 2,2-(4'-oxy-2-methylchalcone)-4,4,6,6-bis[spiro(2',2''-dioxy-1',1''-biphenyl)])cyclotriphosphazene(C56H42O8N3P3), synthesized in our laboratory was investigated in terms of their mechanism of action and antitumor properties by using androgen-dependent (LNCaP) and independent (PC-3) human prostate cancer cell lines.
In study, these cell lines were treated with varying concentrations of compound 1 and compound 2 (1, 5, 25, 50 and 100μM) for 24h. Antitumor activities of these compounds were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.
25, 50 and 100 μM doses of compound 1 reduced the viability of PC-3 cells ($p < 0.05$). 50 and 100 μM doses of compound 1 reduced the viability of LNCaP cells ($p < 0.05$). 25, 50 and 100 μM doses of compound 2 reduced the viability of PC-3 cells

($p < 0.05$). 1, 5, 25, 50 and 100 μM doses of compound 2 reduced the viability of LNCaP cells ($p < 0.05$).
Compounds 2 dose-dependently reduced cell viability of LNCaP ($p < 0.05$). Our results indicate that the new phosphazenes have antitumor activity on human prostate cell lines. Our results indicate that the phosphazenes have antitumor activity on PC-3 and LNCaP.

Key Words: Phosphazene, Chalcone, PC-3, LNCaP, Cell viability, MTT assay

P2.29
Function of RasGRP3 in the formation and progression of human breast cancer

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RasGRP3 is a member of the Ras guanine nucleotide releasing protein (RasGRP) family of the Ras-specific guanine nucleotide exchange factors. These proteins play important role in the regulation of the activity of Ras signaling pathway which constitutive activation is demonstrated in many cancer types. In the light of the this potential oncogenic effect, we investigated the putative alteration of expression and potential function of RasGRP3 in the formation and progression of human breast cancer. The RasGRP3 and phosphoRasGRP3 expressions were examined in human invasive ductal adenocarcinoma derived samples and cell lines (BT-474, JIMT-1, MCF7, SK-BR-3, MDA-MB-453, T-47D) both in mRNA (Q-PCR) and protein (Western blot; immunohistochemistry) levels. To explore the biological function of the protein, RasGRP3 knockdown cultures were established. To assess the role of RasGRP3 in the viability of cells, annexin-V/PI staining and MitoProbe TM DilC1 (5) assay were performed. To clarify the function of the protein in cell proliferation and in the development of chemotherapeutic resistance, CyQuant assay was performed. To observe the RasGRP3 function in tumor formation, the Severe combined immunodeficiency (SCID) mouse model was used. To investigate the role of the protein in Ras-related signaling Q-PCR and Western blot experiments were performed. RasGRP3 expression was elevated in human breast tumor tissue samples as well as in multiple human breast cancer cell lines. Down-regulation of RasGRP3 expression in breast cancer cells decreased cell proliferation, induced apoptosis in MCF7 cells, and sensitized T-47D cells to the action of drugs Tamoxifen and trastuzumab (Herceptin). Gene silencing of RasGRP3 reduced tumor formation in mouse xenografts as well. Inhibition of RasGRP3 expression also reduced Akt, ERK1/2 and estrogen receptor alpha phosphorylation downstream from IGF-I insulin like growth factor-I (IGF-I) or epidermal growth factor (EGF) stimulation confirming the functional role of RasGRP3 in the altered behavior of these cells. Taken together, our results suggest that RasGRP3 may have a role in the pathological behavior of breast cancer cells.

P2.30
Investigation of the fate of type I angiotensin receptor after biased activation

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The biased agonism of the type I angiotensin receptor (AT1R) can result in different outcomes such as activation of G protein-dependent and -independent cellular responses. In this study, we investigated whether the biased activation of the AT1R can lead to different regulation of the receptor. We analyzed the β-arrestin binding, endocytosis and the consecutive steps such as early and late phases of recycling of the AT1R in HEK293 cells expressing wild type or biased mutant receptors in response to different ligands. We used Renilla luciferase tagged forms of the receptors and yellow fluorescent protein (YFP) tagged β-arrestin, Rab4, Rab5, Rab7 and Rab11 proteins in bioluminescence resonance energy transfer (BRET) measurements to reveal the events after stimulation. We demonstrated that not only the signaling of the receptor is different upon using selective ligands, but also the long-term fate within the cells is determined by the manner of the stimulation. Our data suggest that not the different internalization routes or calcium signaling but other mechanisms are responsible for the dissimilar rate of the angiotensin II (AngII) induced AT1R endocytosis compared to biased agonists induced AT1-R or AngII induced biased DRY/AAY mutant AT1R endocytosis. Presumably, the main determinant of the fate of the AT1R within the cells is either the manner of the β-arrestin binding to the stimulated receptor or the severely impaired G protein coupling.

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P3

Skeletal, Smooth and Cardiac Muscle Physiology

P3.1
The effect of SERCA 1b shRNA on the differentiation of C2C12 skeletal muscle cells

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The sarcoplasmic/endoplasmic reticulum Ca2+ ATPases(SERCAs) are the main cellular Ca2+ pumps which decrease the sarcoplasmic Ca2+ level by reaccumulating Ca2+ in the sarcoplasmic reticulum. The neonatal isoform

SERCA1b is the major Ca2+ pump in myotubes and young muscle fibers.
Our aim was to study the role of SERCA1b during skeletal muscle differentiation. SERCA1b protein synthesis has been interfered using a specific shRNA sequence. Decreased protein expression was examined in the selected clones using specific antibody in terminally diferentiated myotubes. Clones showing significantly decreased SERCA1b expression(clone C1) were selected for further experiments. Scrambled shRNA transfected and parental cells were used as a control. The main regulatory proteins playing an essential role in skeletal muscle differentiation were examined with Western-blot and RT-PCR analysis. We detected the expression pattern of P-Akt, MyoD, STIM1, CSQ, calcineurin at protein level, while myostatin and MCIP1.4 were identified at mRNA level. Quantitative analysis of the results normalized to actin also confirmed the significant alterations in CSQ, STIM1, P-Akt, and calcineurin expression detected in clone C1 compared to controls. To examine the functional consequences of the decreased expression of SERCA1b, repeated Ca2+-transients were evoked by the applications of 120 mM KCl. The significantly higher [Ca2+]i measured in the 20th and 40th second after the beginning of KCl application(112.3±3.2 nM, and 110.4±3.1 vs. 150.3±6.5 nM and 134.9±4.7, in control and in clone C1 respectively)indicates the decreased Ca2+ uptake capability of the SERCA pumps. This was quantified by extracting the maximal pump rate(453.6±41.01 μM/s vs. 143.9±24.01 μM/s, in control and in clone C1).
SERCA1b is considered to play an essential role in the regulation of [Ca2+]i and ab ovo gene silencing results in decreased skeletal muscle differentiation.

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P3.2
Vascular smooth muscle cell functional network. The difficulties and usefulness of graphic representation

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Vascular smooth muscle cells (VSMCs), by their characteristics of integrating a large array of stimuli and modulating blood pressure and flow are key players in an important number of processes. Their altered functionality serves as an intricate link to an important number of diseases, to mention just hypertension or ischaemic injury. The final point where VSMCs functional abnormalities intervene can be seen at the severe consequences of cardiovascular diseases. The first step in exploring the mechanisms that serve as initiators of altered functioning is an accurate, elaborated, wide representation of the complex inter-relations between various signalling pathways in VSMCs. As the same signalling pathways may serve in health, as well in triggering disease, an integrated graphical representation of as many pathways, with their inter-links, becomes of maximal value.
Methods: We conducted an extended search of specific literature through Internet. We gathered a huge amount of information on the structural constituents and interlinked signalling pathways that determine the characteristics and parameters of cell contraction and relaxation. A key aim of the study was the gathering important data on the methods and tools used in VSMC signalling representation.
Results: We obtained a significant number of VSMC graphical representation of their essential structural and functional elements. Most authors provide despaired, simplified and restricted aspects of the huge machinary of VSMC signaling. The most recent graphical representations could provide useful information on the causes of the disturbed mechanisms, as well as important clues to design targetting therapies.
Conclusions: Knowledge on VSMC physiology and pathology is essential in cardiovascular disease prevention and treatment. An important tool in this process is an accurate and thorough visual representation of signalling pathways, that can be relatively difficult to obtain,if we consider a sophisticated spatial and dynamic network from a unifying perspective. An efficient VSMC graphical representation becomes essential in order to understand its functionality in health and disease and design therapies.

P3.3
Neurokinin A induced contraction of the urinary bladder smooth muscle

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The aim of our study was to elucidate the signal transduction pathways underlying the contractile response of the urinary bladder smooth muscle (UBSM) evoked by the tachykinin neurotransmitter neurokinin A (NKA), in order to better understand the control of micturition and identify potential new pharmacological targets for the treatment of bladder dysfunction.
UBSM strips without urothelium were isolated from adult male wild type, as well as Gαq/11 and Gα12/13 knock-out (KO) mice and investigated in myographs under isometric conditions. NKA was applied on the resting (passive) tension of the UBSM and changes in NKA-induced contraction were determined after genetic deletion or pharmacological inhibition of certain signaling pathways.
Both NKA and the NK2 receptor agonist β-Ala8-NKA(4-10) evoked similar dose-dependent contractions, which were not affected by atropin, but could be diminished by the NK2 receptor-antagonist MEN10376. The contractile effect of β-Ala8-NKA(4-10) was completely abolished in Gαq/11-KO bladders, and also the inositol-trisphosphate-receptor (IP3R) antagonist 2-aminoethoxydiphenyl borate (2-APB) decreased contraction remarkably. In contrast, phospholipase Cβ (PLCβ) inhibitors, U73122 and edelfosine failed to induce any

significant change. UBSM contraction in response to β-Ala8-NKA(4-10) did not differ in Gα12/13-KO mice but was sensitive to the Rho-kinase inhibitor Y-27632. Interestingly, withdrawal of extracellular calcium, or inhibition of L-type voltage dependent calcium-channels by nifedipine both altered smooth muscle contraction adversely.
NKA-induced contraction of UBSM is mediated by NK2 receptors and Gq/11 proteins. Downstream signaling appears to involve both IP3R-mediated intracellular Ca2+-release and influx of extracellular Ca2+ through L-type calcium channels. Surprisingly, PLCβ does not seem to contribute to NKA-signaling, which may indicate the importance of alternative PLC enzymes in the IP3 generation. Finally, Gq/11-mediated activation of Rho-kinase significantly contributes to NKA-induced UBSM contraction, probably via inhibition of myosin phosphatase.

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P3.4
Morphological and molecular changes after application of ionizing radiation on the rat myocardium

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One of the most widely used methods of treating oncological patients is ionizing radiation therapy. Radiation beam causes damage to the cancer cells which can reduce cancerous mass and leads to recovery of the oncological patients. On the other hand, irradiation of nearby tissues like myocardium can cause some damage leading to the heart ischemia and heart failure.

The aim of this work was to study the morphological changes and changes in molecular markers six weeks after irradiation with a single dose of 25 Gy, applied to mediastinal region of Wistar male rats. Irradiated myocardium was examined for Peroxisome Proliferator-activated Receptor alpha (PPAR-alpha) and miRNA gene expression levels. Changes in ventricular myocardium were studied using electron microscopy. Levels of Connexin-43 (Cx-43) expression in irradiated and non-iradiated rat heart tissue were assayed by immunoblotting analysis.
Six weeks after chest irradiation, electron microscopy revealed increased left ventricular capillary density and endothelial cells oedema, activation and/or degeneration of endothelial cells and presence of inflammatory cells. Immunoblotting analysis of left ventricular tissue showed three forms of myocardial Cx43 (two functional phosphorylated and one non-phosphorylated form). In irradiated rats, significantly increased expression of total Cx43 was detected compared to normal control rats indicating that irradiaton has the ability to up-regulate myocardial Cx43 in heart tissues. The irradiated rats exhibited lower expression of PPAR-alpha than the control ones. This indicates a shift in substrate preferences from fatty acids to glucose. Expression of microRNA-15b in irradiated rat myocardium was significantly decreased compared with that in normal hearts. Therefore, it can be assumed that 6 weeks after radiation hearts were probably protected by activation of some adaptive mechanisms.

Despite of deleterious effects of irradiation on the myocardium, the results indicate that protective mechanisms maintaining the physiological function are activated in early phase of radiation injury.

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P3.5
Different characteristics of diabetic cardiomyopathy in rat models

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Purpose: Diabetic cardiomyopathy, a cardiac manifestation of diabetes mellitus (DM), is characterised by specific structural, molecular and functional alterations of the myocardium. Upon this concept we investigated whether type-1 or type-2 diabetes lead to different alterations in cardiac function or histological and molecular changes.

Methods: Our experiments were carried out in a rat model of type-1 (streptozotocin induced) and type-2 DM (Zucker Diabetic Fatty rats). Left ventricular (LV) function was characterised using a pressure-volume (P-V) conductance catheter system. Load independent indices of LV contractility (preload recruitable stroke work (PRSW)) and indices of LV relaxation (time constant of LV pressure decay(Tau)) and stiffness (LV end-diastolic pressure (LVEDP)) were calculated, respectively. In addition to our functional measurements TUNEL assay was carried out to evaluate degree of apoptosis. Myocardial gene expression analysis was performed by qRT-PCR. Cardiac remodelling was investigated by histological and immunohistological techniques.

Results: In comparison to the control, type-1 DM resulted in decreased LV systolic performance: decreased systolic pressure, maximal dP/dt and PRSW (45.39±2.45 vs 76.44±4.06 mmHg). Type-2 DM was associated with increased LV stiffness (LVEDP: 9.4±0.5 vs 7.7±0.4 mmHg) while systolic indices were altered only to a lower extent. We found cardiac hypertrophy and degeneration with histomorphological examination. More pronounced nitro-oxidative stress, DNA damage and cardiac fibrosis were observed in type-1 DM compared to type-2 DM. Overexpression of c-fos and c-jun and downregulation of eNOS were seen in type-1 diabetic rats. On the other hand TGF-β1 and ANF mRNA-levels were significantly higher in type-2 diabetic model.

Conclusions: Diabetic cardiac alterations are characterised by decreased systolic performance and impaired relaxation in type-1 diabetic rats, while diastolic dysfunction was more pronounced in type-2 DM. In the background of diabetic

cardiomyopathy different processes can be identified in the two models.

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P3.6
Beneficial effects of Allium ursinum herbal extract on hyperthrophic hearts

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Heart failure and atherosclerosis-related syndromes are the leading causes of death in middle-income countries with obesity and hypertension as risk factors. Knowledge about the role of the right ventricle historically has lagged behind the role of the left. Partly as a consequence, right-sided heart failure is considered to be incurable. The most common cause of this condition is the elevated pulmonary arterial pressure. Many recent studies suggest that the antioxidant, antiplatelet and ACE inhibitory effects of the herbal agent Allium ursinum (AU) may have value in treatment of pulmonary hypertension. Sprague-Dawley rats were divided into 4 groups: I.: Control (C); II.: Allium ursinum-control (CAU); III.: Pulmonary hypertensive (PAH); IV.: PAH+Allium ursinum-treated (Tr). PAH was induced by means of a single dose of MCT (60 mg/kg, i.p.). The animals received normal or Allium ursinum extract-enriched chow for 8 weeks. Echocardiographic measurements were obtained on the 0. and 8. weeks. Functional parameters, systolic and diastolic function of the left and right ventricle were measured. Isolated working heart method was used to determinate cardiac functions ex vivo after ketamine-xylazine anaesthesia followed by thoracotomy on the 8th week. Our data demonstrated that Allium ursinum extract improved cardiac functions in both test groups compared to PAH group. Doppler echocardiography showed that S wave and E/A ratios were significantly greater in the treated groups. Right ventricle function (TAPSE) were improved in animals treated with AU compared to controls. Isolated working heart measurements showed that aortic flow and coronary flow were significantly higher in treated groups. Our results suggest that Allium ursinum has clearly beneficial effects in right ventricle hyperthrophy.

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P3.7
Effects of methionine-enriched diet on the rat heart and aorta

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Methionine is a sulfur-containing essential amino acid. Its metabolism is closely related to those of homocysteine, well-known risk factor. The aim of the current study was to determine the effects of chronic methionine nutritional overload on the rat heart and aorta simultaneously. Male Wistar albino rats were randomly divided into control and experimental group and have been fed either with standard laboratory chow or methionine-enriched diet (double content of L-methione) for 30 consecutive days. At the end of this period, the hearts and aortas were removed from rats. Sections 5μm thick were stained with haematoxylin and eosin (H&E), Masson's trichrome and observed under light microscopy. Oxidative stress parameters (malondialdehide, superoxide dismutase and catalase activity) were determined in heart tissue spectrophotometricaly. None hystopathological changes were observed in the hearts and aortas of rats on standard diet. One month of methionine-enriched diet in experimental group caused changes mostly in subendocardial region of the heart wall. There are diffusely distributed cardiomyocytes with condensed homogenous cytoplasm and increased acidofila what can be seen on slices stained with H&E or Masson's trichrome. In some of these cells can be seen nuclear pyknosis. In surrounding cardiomyocytes can be noticed vacuolisation of cytoplasm. This kind of methionine overload did not caused histopathological changes in the wall of aorta in the same animals. None of the analyzed oxidative stress parameters were significantly affected by the applied treatment. It could be concluded that incipient changes caused by methionine nutritional overload used in this study in rats predominantly affected cardiomyocytes and did not change the oxidative stress parameters significantly.

P3.8
Contribution of carbon monoxide on vascular tonus in different vascular beds and segments. A descriptive study

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Aims: Carbon monoxide (CO) is regarded as an important mediator in regulation of vascular tonus, released from endothelium and vascular smooth muscle. Although CO is produced via heamoxygenase (HO) enzymes (HO-1 and HO-2) endogenously, it is not stated which vascular beds it is produced and extend of its effects. The primary aim of this descriptive study is to investigate contribution of CO to vascular tonus in different organs and different sizes of vessel segments. Furthermore, this study shows the mechanisms of vasodilation caused by CO in these vessels.

Materials and Metods: 100 Wistar Albino rats, aged 6 to 8 months and 300 to 350 g in weight, were used as control group. Arteries isolated from the rats, were mounted on organ baths or wire myrograph. CO response of thoracic and abdominal aorta and mesenteric, renal, gastrocnemius muscle, gracilis muscle, heart, lung and brain vascular beds were studied endogenously and exogenously. All vessel segments were treated with HO inhibitor in order to assess the endogenous CO contribution to vascular tonus and were contracted with seratonin (SER) or phenylephrine (PHE) before and after the inhibitor treatment. The CO releasing molecules, tricarbonyldichlororuthenium (II) dimer (CORM) vasodilatory response, with the effect mechanism of CO was examined in the presence of cGMP inhibitor, 1H-[1,2,4]Oxadiazolo[4,3-a] quinox-aline-1-one (ODQ), and non-specific potassium channel inhibitor tetraethylammonium (TEA), following CO exogenous vasodilatory response to CORM. Additionally, HO-2 protein expression was studied with western blot analysis in isolated vessel segments.

Results and Discussion: Although CO was shown to contribute to regulation of vascular tonus in all feed arteries ($p < 0.05$) except gracilis vascular bed; there was no effect in resistance arteries except pial artery ($p < 0.05$). It was shown that there was no relationship between HO-2 protein level and CO contribution to endogenous vascular tonus. While the vasodilatory effect of CO in vessels smaller than 600µm in diameter is found to be via potassium channels, in vessels bigger than 600µm in diameter is through both potassium channels and cGMP pathway.

P3.9

Impact of ion currents on beat-to-beat variability of action potential duration in canine myocytes

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Objective: Recent studies suggest that the short term beat-to-beat variability (SV) is a better predictor of cardiac arrhythmias than the measurement of repolarization prolongation alone. The aim of our work was to study the underlying ion currents with specific activators and blockers. INa, IKATP and ICa,L was activated by veratridine, lemakalim and BAY K8644, respectively, while IKr, IKs, IK1, INa, ICa,L was blocked by dofetilide, HMR-1556, BaCl2, tetrodotoxin (or lidocaine) and nisoldipine, respectively.

Method: Action potentials were recorded with conventional sharp glass microelectrodes on enzimatically dispersed canine ventricular cells. SV was determined from 50 consecutive beats. Values are expressed as mean ± S.E.M., compared by ANOVA.

Results: We found significantly increased action potential duration (APD) and SV in the case of dofetilide (ΔAPD +80 ms, ΔSV +5.70 ms, n=6, $p < 0.05$), HMR-1556 (ΔAPD +13 ms, ΔSV +0.96 ms, n=11, $p < 0.05$), BaCl2 (ΔAPD +125 ms, ΔSV +3.40 ms, n=6, $p < 0.05$) and veratridine (ΔAPD +82 ms, ΔSV +5.71 ms, n=19, $p < 0.05$), while significantly decreased APD and SV with lemakalim (ΔAPD -119 ms, ΔSV -1.38 ms, n=8, $p < 0.05$), tetrodotoxin (ΔAPD -33 ms, ΔSV -0.83 ms, n=13, $p < 0.05$) and lidocaine (ΔAPD -41 ms, ΔSV -1.02 ms, n=10, $p < 0.05$). Nisoldipine shortened the APD, but failed to decrease the SV (ΔAPD -110 ms, ΔSV +0.36 ms, n=13, $p > 0.05$). BAY K8644 lengthened the APD in a concentration-dependent manner, nevertheless after 20 nM BAY K8644 the SV stayed unaltered (ΔAPD +49 ms, ΔSV -0.07 ms, n=7, $p > 0.05$), but 200 nM BAY K8644 caused a moderate increase in the SV (ΔAPD +99 ms, ΔSV +2.29 ms, n=12, $p < 0.05$).

Conclusion: Since the SV is somewhat dependent upon the APD, result of each drug was compared to the previously measured APD-SV correlation, which was measured by injecting constant electrotonic pulses. Our results suggest that the ion currents playing critical role on the negative feedback regulation of APD have the major role reducing the SV (i.e. ICa,L, IKs and IKr). Thereby inhibition of these currents can be proarrhythmic in susceptible patients.

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P3.10

Expession and estrogen-dependent up-regulation of Transient Receptor Potential Ankyrin 1 (TRPA1) and Vanilloid 1 (TRPV1) ion channels in the rat endometrium

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Transient Receptor Potential Ankyrin 1 (TRPA1) and Vanilloid 1 (TRPV1) cation channels localized predominantly on capsaicin-sensitive peptidergic sensory nerves play essential roles in pain, hyperalgesia and neurogenic inflammation. They are activated by a variety of noxious stimuli, chemical irritants and cold or heat, respectively. Besides sensory nerves, both receptors have been described on epithelial and immune cells. Estrogen-induced TRPV1 up-regulation in the human uterus suggests its potential involvement in pain during the reproductive cycle. Since there are no data regarding TRPA1 expression in the endometrium and little is known about TRPV1 regulation, we investigated estrogen- and progesterone-dependent alterations of these channels in the rat endometrium.

Different groups of sexually premature 4-week-old and adult 4-month-old female rats were treated with subcutaneously implanted wax pellets containing synthetic estrogen analog diethylstilbestrol (DES, 100 µg), progesterone (4 mg) and their combination for 8 or 12 days, respectively. Ovariectomy was performed in separate groups of 4-month-old animals (n=5/group). TRPA1 and TRPV1 mRNA levels were measured in the endometrium layer with quantitative PCR, while the localization of the receptor proteins was determined with immunohistochemistry on paraffin-embedded uterus sections. Both TRPA1 and TRPV1 were detected in the rat endometrium

at mRNA and protein levels as well, showing their remarkable local, non-neuronal expression. DES treatment resulted in a 5-fold and 7-fold significant up-regulation of TRPV1 mRNA in young and adult rats, respectively, which were absent if progesterone was added simultaneously. DES also induced significant elevation of TRPA1 mRNA in both groups. Progesterone by itself did not alter the levels of either channel in either group. In young rats, weak TRPV1 and A1 staining were observed in the epithelium, while in adult animals it was detected in the stroma and the glands with weak expression in the epithelium. Further investigations are in process to elucidate the functions of TRPA1 and TRPV1 in conditions related to pain and inflammation.

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P3.11
The short term beat-to-beat variability of action potential duration depends on the the length of action potential and intracellular calcium

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Prolongation of QT interval is regarded as an indicator and risk factor for arrhythmia. On the basis of the latest experiments short term beat-to-beat variability of repolarization (SV) is proposed to be a better predictor for arrhythmia development than the prolongation of repolarization alone. Since the exact mechanism of SV is still unclear we aimed to investigate the mechanisms underlying the SV.

Our measurements were performed on canine isolated ventricular myocytes on 37 °C, action potentials were recorded using conventional sharp microelectrodes. To quantitatively describe SV the duration of 50 consecutive action potentials (APD) were measured and plotted on Poincaré diagram. To assess the APD-SV relationship we modulated the APD in two independent ways: the stimulation frequencies were changed or current injections were applied.

We observed that APD and SV decreased simultaneously with increasing stimulation frequency. At low stimulation frequencies the action potentials were lengthened while SV increased steeply. The APD90 and SV values were 153±5 ms and 2.4±0.2 ms; 244±8 ms and 3.5±0.2 ms; 308±13 ms and 6.3±0.4 ms at 3.3 Hz, 1.0 Hz and 0.2 Hz, respectively (n=8). Similar APD changes were obtained after the current injection (-600 − +70 pA). However the values of SV did not show complete overlap with frequency modulation experiments, because the SV reduced to a greater extent in case of APD shortening current injections than at higher stimulation frequencies. We supposed that this difference is caused by the accumulation of intracellular Ca2+. Supporting this, the pharmacological modification of intracellular Ca2+ level by BAPTA (Ca2+ chelator) or A23187 (Ca2+ ionophore) altered the APD without any change in the value of SV.

In conclusion, the increase in APD exponentially increased SV independently from the way the APD changes were elicited. Our results suggest that beside the APD changes, the alteration of intracellular Ca2+ contributes to modification of SV, as well.

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P3.12
Exogenous nicotinamide adenine dinucleotide (NAD+): effects and mechanisms of action on the mammalian heart

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OBJECTIVES. Nicotinamide adenine dinucleotide (NAD+) is well known compound, playing central role in cellular metabolism and energy turnover. Recently, NAD+ has been regarded as potential neurotransmitter, since releasing from nerve endings was demonstrated. Effects of extracellular NAD+ as regulatory compound is not completely investigated, especially in the hearts. This study is aimed to the investigation of the NAD+ effects and mechanisms of action in a mammalians heart.

METHODS. Multicellular preparations of left atria (LA), right atria (RA), sinoatrial node (SAN), right ventricular wall (RV) were dissected from male Wistar (200-250 g) rats hearts and perfused in standard conditions with Tirode solution. Also, multicellular preparations of rabbit Purkinje fibers (PF) were used in experiments. Action potentials (APs) were recorded with use of standard microelectrode technique under control conditions and after NAD+, ATP or adenosine administration (1-100 mcM). APs duration at level of 90% repolarization (APD) were estimated. APs were recorded in electrically paced (LA, RA, RV, PF, SS=300 ms) or spontaneously active preparations (SAN).

RESULTS. NAD+ (10 and 100 mcM) induces significant ($p(T)<0.05$) decreasing of APD in rat LA (to 77±3% and 65±2 % in respect to control APD, n=8), RA (78±4% and 66±3%, n=5) and RV (57±6% and 70±5%, n=6).

Also, NAD+ induces shortening of APs in PF (91±3%, 100 mcM, $p(T)<0.05$, n=5). Administration of NAD+ caused decreasing of rate of spontaneous firing, slow diastolic depolarization and APD in rat SAN.

APDs decreasing after 1-100 mcM NAD+ administration were similar to those, induced by ATP or adenosine (1-100 mcM). NAD+- induced APs alternation, in contrast with adenosine, was not suppressed by P1-purinoreceptor antagonist theophylline (100 mcM, n=8). P2 antagonist suramine (10 mcM, n=10) completely attenuated APs shortening caused by 10-100 mcM NAD+ in rat preparations ($p(U)<0.05$).

CONCLUTIONS. Exogenous NAD+ have significant influence on the bioelectrical activity of pacemaker, atrial and ventricular myocardium. Metabolite independent, direct NAD+ action via P2-purinoreceptros is suggested.

P3.13
Characteristic of ischemic preconditioning under conditions of simulated hyperglycemia hyperglycemia

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The aim of our study was to characterize ischemic preconditioning (PC) during ischemia/reperfusion injury in isolated rat hearts perfused under conditions of simulated acute hyperglycemia (HG).
Experiments performed on rat hearts perfused according to Langendorff by Krebs-Henseleit solution with standard glucose concentration (11 mmol/L) present „normoglycemia NG“ and with elevated glucose (22 mmol/L) present HG. Non-PC controls and hearts preconditioned by two cycles of 5 min coronary occlusion/5 min reperfusion were exposed to 30 min ischemia/120 min reperfusion. The severity of I/R injury was characterised by determination of the size of infarction (IS, expressed in % of area at risk size) and the amount of heart-type fatty acid binding protein (h-FABP, a marker of cell injury) released from the hearts to the effluent. Samples for Western blot analysis (WB) were taken from hearts before and after exposure to global 30 min ischemia/40 min reperfusion. Activity of PI3K/Akt pathway was expressed as P-Akt/Akt ratio. Levels of BAX were measured after I/R.
Under NG conditions, significantly smaller IS and lower total released amount of h-FABP in the PC group than in the controls were observed. Under HG conditions, in the PC group IS was significantly larger and released h-FABP was higher than in the controls. Significant increase of P-Akt/Akt prior to I/R and its decrease together with reduced BAX was observed in PC group under NG conditions. Only insignificant increase of P-Akt/Akt prior to I/R without its decrease and no significant changes of BAX at the end of I/R was observed in PC group under HG conditions.
Prosurvival antiinfarct effect of PC was confirmed in standard NG conditions. Decreased P-Akt/Akt after I/R indicates probable negative feedback regulation which prevents chronic PI3K/Akt activation. Higher IS and h-FABP released from PC hearts point out to negative effect of PC on heart resistance and only insignificant P-Akt/Akt increase without its decrease and no significant changes of BAX indicate attenuated cell signaling and antiapoptotic activity under HG conditions.

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P3.14
Nicotinic acetylcholine receptors containing the α7-like subunit mediate contractions of muscles responsible for space positioning of the snail tentacle

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Three recently discovered tentacle muscles are crucial to perform a variety of special movements of the upper tentacles of terrestrial snails. These muscles receive central and peripheral excitatory cholinergic innervation however lacks inhibitory innervation. Here, we investigate the pharmacology of acetylcholine (ACh) responses in tentacle muscles to determine the properties of the ACh receptor (AChR), the functional availability of which was assessed using isotonic contraction measurement. Using a broad spectrum of nicotinic and muscarinic agonists and antagonists, we provide the first demonstration that ACh-elicited contractions in the tentacle muscles (M1, M2 and M3) are attributable to the activation of nAChRs that contain the α7-like subunit. Contractions could be evoked by nicotine, carbachol, succinylchloride, TMA and the selective α7-nAChR agonist choline chloride and PNU-282987, and blocked by several nAChR selective antagonists such as mytolon, hexamethonium, succinylchloride, d-tubocurarine, hemicholinium, DMDA, α-Bungarotoxin (αBgtx) and α-Conotoxin IMI. The specific muscarinic agonist oxotremorine and arecoline failed to elicit contractions. At the same time, atropine, scopolamine, strychnine and levetimide effectively blocked the ACh elicited contractions, while the specific muscarinic antagonist orphenadrine did not. Based on these pharmacological properties we conclude that the ACh receptors of the flexor muscle are Na+ and Ca2+ permeable nicotinic receptors that contain the α7-like subunit. Immunodetection and PCR experiments confirmed the presence of α7- or α7-like AChRs in muscle cells, and α4-AChRs in nerves innervating the muscle. These results support the conclusion that the slowly desensitizing αBgTx-sensitive responses obtained from flexor muscles are produced by activation of α7- like AChRs.
We conclude that the nAChRs in flexor muscles of the snail tentacle more closely resemble a vertebrate neuronal receptor than a muscle or electroplaque receptor. This is the first demonstration of an obligatory role for a functional α7-like nAChR in the molluscan periphery.

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P3.15
Selective Na+/Ca2+ exchanger inhibition prevents Ca2+ overload induced triggered arrhythmias

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Background and purpose: A crucial role for augmented Na+/Ca2+ exchanger (NCX) activity in cardiac arrhythmogenesis is often suggested, however, in related studies the antiarrhythmic efficacy of NCX inhibition was apparently controversial. Feasible explanations could be the unsatisfactory selectivity of NCX inhibitors and/or experimental model

dependence of the degree of Ca2+i overload. In the present study, using NCX inhibitors SEA0400 and the more selective ORM10103, we evaluated the efficacy of NCX inhibition against arrhythmogenic Ca2+i rise in conditions when [Ca2+]i was augmented via activation of the late sodium current (INaL) or inhibition of the Na+/K+ pump. **Experimental approach:** Action potentials (APs) were recorded from canine papillary muscles and Purkinje fibers by microelectrodes. NCX current (INCX) was determined in ventricular cardiomyocytes utilizing the whole cell patch clamp technique. Ca2+i transients (CaTs) were monitored with a Ca2+-sensitive fluorescent dye, Fluo-4.
Key results: Enhanced INaL increased the Ca2+-load and AP duration (APD). SEA0400 and ORM-10103 suppressed INCX and prevented/reversed the ATX-II induced [Ca2+]i rise without influencing APD, CaT or cell shortening. The APD lengthening effect of ATX-II was neither reduced. ORM-10103 significantly decreased the number of strophantidine-induced spontaneous diastolic Ca2+ releases, however, SEA0400 failed to restrict the veratridine-induced augmentation in Purkinje-ventricle APD dispersion.
Conclusions and implications: Selective NCX inhibition – presumably via blocking revINCX – is effective against arrhythmogenesis caused by [Na+]i-induced [Ca2+]i elevation, without influencing the AP kinetics. Therefore, selective INCX inhibition, by significantly reducing the arrhythmogenic trigger activity caused by the perturbed Ca2+i handling, should be considered a promising antiarrhythmic therapeutic strategy.

P3.16
Chronic L-DOPA administration decreases relaxation responses of corpus cavernosum tissue of rabbit

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Dopamine is a crucial central neurotransmitter, which plays a fundamental role on the autonomic and somatic components of penile reflexes in animals and man as well. Similar to the erectile responses of dopamine, systemic administration of L-DOPA (dopamine precursor) induces yawning and penile erection in some species. In this study, possible effects of L-DOPA on NO-dependent and -independent non-adrenergic non-cholinergic (NANC) relaxation responses mediated by electrical field stimulation (EFS), postsynaptic nitric oxide (NO)/guanylate cyclase/cGMP pathway and endothelium-dependent relaxation were investigated. Thirty-two adult albino male rabbits of two- and four-week-treatment groups, each were divided into three as control (saline-injected), 3 mg/kg/day (low dose) and 12 mg/kg/day (high dose) L-DOPA-injected groups. After thirty daily intraperitoneal injection treatments, rabbit corpus cavernosum tissues were placed in organ bath chambers containing Krebs solution. Isometric contractions of cavernosal smooth muscle were recorded via force displacement transducers. The EFS-mediated NANC responses were obtained in presence of guanethidine (10-6M) and atropine (10-5M). In two-week treatment with high dose, L-DOPA decreased NO-dependent NANC relaxation responses while there was no change in the low-dose of two-week and four-week treatment groups. NO-independent NANC relaxation responses in two-week-groups decreased, the responses in four-week-groups were unchanged. Relaxation responses to carbachol had no difference among all groups except high-dose administration of four-week-group. Relaxation responses of SNP and sildenafil were increased in all the treatment groups when compared to the controls. These results suggested a desensitization of both central and peripheral dopaminergic pathways. On the other hand, increase in SNP and sildenafil induced responses indicated either a sensitivity in NO/guanylate cyclase/cGMP pathway or an inhibition of PDE activity following the L-DOPA administration.

P3.17
Cannabinoids and muscle weakness – Investigating the function of CB1 receptors in mammalian skeletal muscle

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The presence of CB1 cannabionid receptors (CB1R) has been shown in skeletal muscle, but it is yet to be cleared whether they have any significance in the regulation of muscle contractions. Muscle contractions are evoked by the elevation of intracellular Ca2+ concentration ([Ca2+]i) during a process called excitation-contraction coupling. CB1-mediated signaling can interefere with this process in several ways. CB1-knockout (CB1-KO) mice showed hypoactivity, however it is questionable whether this was solely originated by effects on the central nervous system or impairment of skeletal muscle function also contributes to this. It was also shown that treatment by cannabinoid agonists attenuates the contractions of frog skeletal muscle. Our aim was to study the role of CB1R in mammalian skeletal muscle, and the effects of cannabinoid drugs on Ca2+-transients.
Running ability (average and maximal speed, distance) of control and CB1-KO mice was tested by activity-wheel-tests and in vivo muscle force of the animals was tested by grip-tests and hang-tests. Ca2+ transients evoked by KCl-depolarization in the presence of cannabinoid agonists were studied on enzymatically isolated flexor digitorum brevis (FDB) fibers of control and CB1-KO mice.
CB1-KO mice performed worse in all the behavior tests compared to control. Depolarization-evoked Ca2+-transients were significantly higher in FDB fibers isolated from CB1-KO mice (847.8±98.2 nM, n=47) compared to control (375.6±59.9 nM, n=32, $p < 0.01$). On control FDB the second transients after the CB1 agonist WIN55,212 treatment were significantly smaller than in untreated fibers.
On the basis of the [Ca2+]i measurements we can conclude that CB1R-mediated signaling contributes to the regulation of skeletal muscle contractions, but as the main cause of the worse muscle performance of CB1-KO mice the effects mediated by the absence of CB1R in the central nervous

system can neither be ruled out. These results can contribute to the identification of the side effects of medically used cannabinoid drugs on skeletal muscle.

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P4

Cardiovascular Physiology

P4.1
Poly (ADP-ribose) polymerase (PARP) activation in chronic heart failure correlates with the level of cardiac dysfunction

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Multiple lines of evidence suggest that reactive oxygen and nitrogen species are generated in cardiomyocytes during various forms of heart failure and cardiomyopathies. PARP is a eukaryotic nuclear enzyme participating in DNA repair and activated in response to DNA damage induced by oxidative- and nitrosative stress. Upon binding to damaged DNA, PARP catalyzes the cleavage of NAD+ into nicotinamide and ADP-ribose polymers. Overactivation of the enzyme, by depleting NAD+, causes energy deficit, and finally necrotic cell death. In this study we aimed to measure PARP activation in human recipient heart samples and to investigate the correlation between the enzyme activation and the severity of the heart failure. Human left ventricular (anterior wall) tissue histological sections were taken from 25 explanted end-stage failing hearts (NYHA class III-IV.) and were prepared for PAR immunhistochemistry. PARP activity was analyzed in the epicardial and endocardial surface using MBF ImageJ software in order to measure the ratio of positive cell area compared to total cell area. Ejection fraction, inflammatory markers, liver and kidney function parameters were also documented. The PAR positivity that shows PARP activity was significantly higher in the endocardial side of the samples than in the epicardial part (32.42±13.25 vs. 20.84±12.83; $p < 0.0001$). The epicardial enzyme activation negativly corraleted with ejection fraction (EF) ($R=-0.41$, $p=0.043$), and positivly corraleted with the gamma-glutamil transpeptidase (GGT) level ($R=0.42$, $p=0.035$). The endocardial enzyme activation shows negative correlation with the heart rate ($R=-0.57$, $p=0.005$), and positive correlations with the GGT ($R=0.46$, $p=0,021$) and the aspartat aminotransferase levels ($R=0.47$, $p=0.023$). The current study provides the first evidence of a significant difference between the epicardial and endocardial PARP activation in human failing heart samples, with a negative correlation of ejection fraction and epicardial PARP activation. Further studies are required to explore the complex function of PARP activation in the pathomechanism of chronic heart failure and its possible role as a new therapeutic target.

P4.2
Remodelling of coronary artery network during quercetin supplementation

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Objective: Polyphenols, including quercetin, present in human diet, have various physiological effects. Short term vasodilatory actions on coronary arterioles have been demonstrated in our laboratory, but no information is available concerning their long term effects on microvascular networks.
Methods: Male Wistar rats were divided into two groups. In group Q, animals were treated with 30 mg/kg quercetin per os (n=9), while group C was kept in parallel (n=10) for 8 weeks. Animals were sacrificed, and left desceding coronary artery was prepared in a standard manner with its ramifications, down to about 80 μm inner diameter. Orifice was cannulated, and continuous saline flow, and pressure was maintained during recording. Pictures of the network were digitally analyzed.
Results: Quercetin treatment reduced hemodynamically disadvantageous components in the network: branching angles under 45° or over 105° were less frequent in group Q (9% vs. 24%), like multiple branching (1/13 vs. 1/10). Segmental tortuosity decreased (2.0±0.3% vs. 3.5±0.4%, $p < 0.05$), similar to the frequency of broken courses (1.0±0.5 vs. 3.1±0.4 pc, $p < 0.05$). At bifurcations, asymmetry of daughter branches decreased (1.69±0.08 vs. 2.26±0.24, $p < 0.05$). Overall vascularization of exposed network slightly increased (23.4±3.1 mm vs. 28.3±4.1 mm, ns.) due to a significant lengthening in 50-100 μm and in 150-300 μm inner diameter range. Wall was thickened in up to 400 μm arterioles ($p < 0.05$), but not above 400 μm.
Conclusions: Chronic administration of quercetin resulted in reduced number of hemodynamically disadvantageous sections of coronary networks. We assume that it can delay non-benefitial remodelling caused by e.g. long term hemodynamic stress, or ageing.

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P4.3

Angiogenic and positive inotropic effects of apelin fragments on human pluripotein stem cell-derived cardivascular cells

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Background: Regulatory role of apelin have been proven in the cardiovascular physiology. However, this has not been investigated in human stem cell derivatives.
Aims: Our aim was to investigate the effects of different apelin fragments on human cells. 1) the angiogenic activity of apelin (AP13, pyrAP13, AP36) in human embryonic stem cell derived endothelial cells (hESC-EC), 2) the inotropic effect of apelin (pyrAP13) in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM).
Results: The mRNA levels of apelin and APJ receptor were comparable in hESC-EC and HUVEC. Upon in vivo implantation of cells into immunosuppressed nude rats, apelin mRNA levels were increased 103-fold (n=3). In response to AP13, AP13pyr and AP36, we found comparable changes in angiogenic factor angiopoietin-2 mRNA levels (0.29±0.08; 0.56±0.21; 0.46±0.2) in coronary artery endothelial cells (HCAEC:0.44±0.01, 0.59±0.38, 0.86±0.55,) and in venous HUVEC: 0.68±0.4, 1.1±0.39, 1.51±0.54 vs control, all n=4). High content microscopy showed an increase in cell number and proliferation of hESC-EC after 24h (n=6, AP13 p=0.0051, pyrAP13 p=0.0011). Apelin also reduced necrosis in hESC-EC (as shown by Topro3; n=6, AP13 p=0.0029, pyrAP13 p=0.0166). Using Matrigel tube formation assay, we showed that pyrAP13 increased both number and length of hESC-EC tubes (n=4, p <0.001). Impedance data on xCELLigence platform showed that a key feature in angiogenesis, adhesion of hESC-EC were increased in response to apelin fragments (AP13 0.78±0.06, pyrAP13 0.81±0.05, AP36 0.72±0.02, n=3; p <0.001). Our pilot data with calcium transients showed that frequency of action potentials of hiPSC-CMs were increased by pyrAP13 (0.1nM, n=3).
Conclusions: We showed that apelin has key role in vascular maturation and may have a proangiogenic effect in differentiated hESC-EC. Apelin also shares positive inotropic effect on cardiomyocytes in vitro. Due to their angiogenic affinity and paracrine effects, use of stem cell-derived cardiovascular cells could be considered as potential therapeutic approach in regeneration therapies.

P4.4

Consumer investigation and toxicological analysis of sour cherry seed kernel extract

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INTRODUCTION: The incidence of the cardiovascular diseases is increasing to include progressively younger sectors of the human population, particularly in affluent nations. Previously, we demonstrated that sour cherry seed extract (SCSE) strongly protects the heart against ischaemia/reperfusion injury. Now we aimed to elicit whether SCSE has any potential side effect managing a consumer investigation on healthy volunteers or toxic effect on long term-treated rats.
MATERIALS AND METHODS: Following ethical licensing we involved 10 non-smoker volunteers between ages of 18 and 70 to the investigation. Treated group received 250mg/day SCSE for 14 days along their normal menu, while control group received placebo. On days 0 and 14 physical examination, electrocardiogram (ECG), exercise ECG and laboratory blood tests were carried out. In addition, volunteers were requested to fill in a list of queries regarding to their quality of life (SF36). Toxicological analysis was carried out on Sprague-Dawley rats. The animals were fed by SCSE containing (0.05 and 1%) rodent chow for 6 months, while control group received a regranulated chow with gelatin. After treatment the organs were subjected to histology-toxicological analysis.
RESULTS AND CONCLUSION: Consumer pilot investigation: blood parameters remain in normal range. We found no significant discrepancy on neither ECGs compared to 0 and 14 days. SCSE treated group declared positive changes of their quality of life after two weeks especially on their social activity and their general state of health. No histological changes were found between treated and control group animals. We concluded that using SCSE in these doses on humans are safe and do not cause any pathological changes on long term treated rats.

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P4.5

Carbon monoxide pollution induces heme oxygenase-1 in ischaemic rat heart

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INTRODUCTION: One of the constituents of both smoke and urban pollution is carbon monoxide which is reported to be associated with cardiac dysfunction. Despite the implication of promoting oxidative stress, the underlying mechanisms remain today still unknown. We aimed to study whether the HO-1 (heme oxygenase-1), the inducible isoform of heme oxygenases to different stressors is involved in these machanisms or not.
MATERIALS AND METHODS: Wistar rats were randomly assigned to a control group and CO (carbon monoxide) group. CO concentration of 30 ppm during a 12-hour period completed with five peaks of 100 ppm (1 h each), to reproduce

environmentally relevant variations in air quality. For the remaining 12 hours, the CO exposed group was inhaled filtered air (<1 ppm CO). Experiments were performed 24 h after the last CO exposure to avoid acute CO effects. Each heart was subjected to 30 min ischemia followed by 120 min of reperfusion. Heart rate (HR) was continuously monitored during isolated heart experiments. Coronary effluent was collected through reperfusion to check tissue damage by measuring lactate dehydrogenase (LDH) activity. After the end of reperfusion, infarct size measurement and Western blot analysis were carried out.

RESULTS AND CONCLUSION: Carboxyhemoglobin level measured 24 hours after the last CO exposure and it did not significantly differ from those of the control group. In the CO group, HR was increased indicating that the inhalation of this air pollutant could be arrhythmogenic. LDH activity and infarct zone were also elevated in the CO group. We found an elevated level of HO-1 in the CO polluted samples, which shows that this stressor is also a potent inducer of this enzyme.

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P4.6
Angiotensin converting enzyme-2 as biomarker of human hypertension and systolic heart failure

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Angiotensin converting enzyme 2 (ACE2) is member of the renin-angiotensin system (RAS) and is considered as an enzyme catalyzing Angiotensin II conversion to Angiotensin 1-7. Growing evidence exists for a role of ACE2 in cardiovascular disease. Here we tested its involvement in hypertension and systolic heart failure.

A single centre prospective clinical study was performed involving: (i) hypertensive patients (n=239) with preserved ejection fraction (EF >50%), (ii) patients with moderate to severe systolic heart failure (NYHA II-IV, n=100) and (iii) a healthy cohort (n=45). Left ventricular end-diastolic (EDD) and end-systolic diameter (ESD) as well as EF were measured by echocardiography. Serum ACE2 activity was determined by a fluorescence substrate.

A remarkable elevation of serum ACE2 activity was present in hypertensive patients with preserved left ventricular EF, compared to normotensive, healthy people (healthy: 16.2±0.8 UF/mL, hypertensive: 24.8±0.8 UF/mL; P <0.0001). Serum ACE2 was further elevated in patients with systolic heart failure (heart failure: 30.2±1.7 UF/mL; P <0.0001). Serum ACE2 activity correlated with the clinical status of heart failure. Serum ACE2 activities negatively correlated with EF in hypertensive patients (r=0.198; P=0.002) similarly to heart failure patients (r=0.46; P=0.0001). In contrast, no correlation was present between EF and sACE2 activity in healthy individuals (r=0.04; P=0.793). While sACE2 activities positively correlated with NT-proBNP levels in heart failure patients (P <0.01, r=0.52) there was no such correlation in individuals with normal left ventricular systolic function.

Serum ACE2 activity was confirmed as a biomarker in hypertension and systolic heart failure. Our data suggest that serum ACE2 is involved in the pathomechanism of human hypertension and systolic heart failure.

P4.7
Free radicals in civilization diseases – Friends or foe of endogenous protective processes in the myocardium

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Background: Till 1990 the studies dealing with free radicals were focused predominantly on disclosing the loci and conditions of their generation, to find out what everything and to what extent they may damage and how may they be detoxicated. However, in last decades they started to accumulate the studies indicating that the primary damage to the heart caused by free radicals may consequently trigger numerous compensatory reactions indicated as endogenous protective mechanisms (EPM). Radicals may even bear signals inducing expression of hypoxic genes involved in EPM.

Aim of the study: Demonstration of deteriorative and also of the EPM-inducing effects of free oxygen radicals on the heart affected by a typical civilization disease, the diabetes mellitus (DIA).

Material and Methods: Male Wistar rats (n= 360), 220±20 g b.wt., water and food ad libitum, light regimen 12/12 D/N. 8 days DIA induced with streptozotocin (65 mg/kg, i.p.), isolated perfused Langendorff heart, calcium paradox with 5 min Ca deprivation. Heart sarcolemma isolated with hypotonic shock, heart mitochondria isolated with differential centrifugation. Estimations: sarcolemmal Na+/K+-ATPase, mitochondrial Mg2+-ATPase, mitochondrial and sarcolemmal membrane fluidity by means of DPH fluorescence spectroscopy, conjugated dienes in mitochondrial membranes. Genes: HIF 1 and 2α, VEGF, GLUT 1, iNOS. Metabolic variables: glucose, glycohemoglobin, triacylglycerols, cholesterol, insulin.

Results: DIA is accompanied with persisting production of free oxygen species. The latter are inducing damage to subcellular membrane systems and jeopardizing the heart function. On the other hand, many radicals-induced alterations are directly or indirectly triggering EPM which are alleviating effectively the course of the disease.

Conclusions: Free oxygen radicals are participating considerably in the DIA-caused damage to the heart which mitigate effectively the deteriorative effect of DIA on the heart. Hence, the answer to question friend or foe sounds: both.

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P4.8

Beta-adrenergic stimulation reverses the IKr-IKs dominant pattern during the cardiac action potential

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β-Adrenergic stimulation differentially modulates different K+ channels and thus fine-tunes cardiac action potential (AP) repolarization. However, it remains unclear how the proportion of IKs, IKr, and IK1 currents in the same cell would be altered by β-adrenergic stimulation, which would change the relative contribution of individual K+ current to the total repolarization reserve. In this study, we used an innovative AP-clamp sequential dissection technique to directly record the dynamic IKs, IKr, and IK1 currents during the AP in guinea pig ventricular myocytes under physiologically relevant conditions. Our data provide quantitative measures of the magnitude and time course of IKs, IKr, and IK1 currents in the same cell under its own steady-state AP, in a physiological milieu, and with preserved Ca2+ homeostasis.
We found that isoproterenol treatment significantly enhanced IKs, moderately increased IK1, but slightly decreased IKr in a dose-dependent manner. The dominance pattern of the K+ currents was IKr > IK1 > IKs at the control condition, but reversed to IKs > IK1 > IKr following β-adrenergic stimulation. We systematically determined the changes in the relative contribution of IKr, and IK1 to cardiac repolarization during AP at different adrenergic states. In conclusion, the β-adrenergic stimulation fine-tunes the cardiac AP morphology by shifting the power of different K+ currents in a dose-dependent manner. This knowledge is important for designing antiarrhythmic drug strategies to treat hearts exposed to various sympathetic tones.

P4.9

Interaction of Ca-sensitiser levosimendan and different catecholamines in chronic heart failure: Experimental studies

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Background: Ca2+-sensitiser levosimendan (LEV) and catecholamines (CAs) are widely used in the treatment of acutely decompensated chronic heart failure. However, the haemodynamic and arrhythmogenic effects of co-administration of these inotropic agents are less characterised. The present study aims to evaluate above effects of LEV administered together with dobutamine (DOB), dopamine (DA) or norepinephrine (NE) in a canine heart failure (HF) model.
Methods: HF (n=12) was induced by chronic right ventricular tachy-pacing (240/min) until acute cardiac decompensation. In the first group continuous infusion of LEV (0.1 g/kg/min iv.) combined with 10-10 minutes infusion of increasing doses of different CAs. In the second group CAs were given in same doses without backround infusion of LEV. Pressure and contractility parameters, charecteristics of monophasic action potential, number of ventricular premature beats and ventricular tachycardias were conitnously recorded.
Results: LEV alone did not alter mean BP and LVEDP significantly. However, dP/dtmax and dP/dt min were increased by approximately 50%. There was a further increase in dP/dtmax with combination of LEVO and CAs, maximal effect was observed with LEV+DA 16 μg/kg/min 16 (+73±19%, p <0.001). In the cathecolamines only group the basal hemodynamic parameters (BP, LVEDP, dP/dtmax, dP/dtmin) did not differ significantly from the respective values in Group I. Moreover, CAs without LEV exerted cardiovascular responses very close to those in LEV+CA group. As a result of LEV infusion left ventricular MAPD50 decreased significantly (214±8 vs 242±9 msec, p <0,01), which was further shortened by addition of NA. However, malignant ventricular arrhythmias or increase in VES occurrence could not be observed in both groups.
Conclusion: Co-administration of levosimendan and different catecholamines elicited similar improvement in cardiac contractility compared to catecholamines alone. The combination of inotropic agents was not accompanied by malignant arrhythmias, despite the shortening of MAPD50 during LEV infusion.

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P4.10

Two sides of the same coin: Integrative role of the calcium-activated chloride channels in the ventricular myocardium

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According to the recent findings TMEM16A mediates calcium-activated chloride current (ICl(Ca)) in the heart. This current takes part in the early repolarization of action potential (AP); however, if Ca2+ concentration remains elevated, it can contribute to the formation of delayed afterdepolarizations.
The aim of our work was to study the role and distribution of TMEM16A in various parts of ventricular myocardium.
Electrophysiological studies were performed in enzymatically dispersed canine cardiomyocytes using conventional sharp microelectrode, whole-cell patch-clamp and AP-clamp techniques. ICl(Ca) was dissected by perfusion of a selective blocker, anthracene-9-carboxylic acid (9-AC, 0.5 mM). Distribution pattern of TMEM16A in different regions of

ventricular myocardium was evaluated by Western blot analysis and confocal microscopy.
Inhibition of ICl(Ca) increased AP duration (APD) in midmyocardial and subendocardial cells (+24.34±3.26 ms and +15.85±2.14 ms at 1 Hz, n=15 and n=7, respectively), while decreased that in myocytes derived from the subepicardial layer (-13.18±7.50 ms at 1 Hz, n=7). Application of 9-AC increased the beat-to-beat variability of APD. In spite ofdifferent effect on APD, similar current amplitudes were recorded in every cardiac region. TMEM16A expression was most abundant in the t-tubules and its density was identical in the whole ventricular wall. In contrast, AP-clamp experiments revealed a larger outward current in subepicardial myocytes than that measured in subendocardial cells. ICl(Ca) amplitude was about twice as much compared to control during beta-adrenergic activation (10 nM isoproterenol), suggesting its calcium-sensitivity (1.15±0.11 pA/pF in control, n=13 and 2.43±0.11 pA/pF in isoproterenol, n=4). Blockade of this current prevents AP shortening during beta-adrenergic activation. Moreover, early afterdepolarizations (EAD) occurred after ICl(Ca) inhibition, especially during sympathetic activation at low heart rates.
ICl(Ca) decreases the apparent transmural and apico-basal temporal difference in repolarization, diminishes the extent of beat-to-beat variability and has a protective role against generation of EAD.

P4.11
Using the pulse transit time for calculation of systolic and diastolic pressure

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The aim of this paper was to present an algorithm to estimating the blood pressure using the pulse transit time -PTT as a more convenient method of measuring the blood pressure. After measuring ECG and pressure pulse, and photoplethysmography, the PTT was calculated from the acquired signals. This system measure indirectly the systolic pressure and the diastolic pressure composed using the statistic method. In comparison between the blood pressure indirectly measured by proposed algorithm estimating the blood pressure and real blood pressure measured by conventional sphygmomanometer, the systolic pressure indicates the mean error of ± 3.00 mmHg and the standard deviation of 2.50 mmHg. The signal was aquire using LabView and the algoritm structure was realized in MatLab. Park and Lass estimated the blood pressure using time interval between the R-peak of electrocardiogram (ECG) and the characteristic points of the pulse wave signal, such as the base line point of pulse wave, maximum point, and point indicating 50% of the pulse wave amplitude. After recording the ECG, pressure pulse and photoplethysmography signals from the 10 subjects without cardiovascular disease, the PPT with respect to each cardiac period as for each pulse wave was calculated and averaged. The systolic blood and the diastolic blood pressure were estimated using the derived blood pressure estimating formula. A model formula estimating the systolic and the diastolic blood pressure are proposed using PTT calculated from the pulse pressure and PPG measured simultaneously. As the statistic significance is represented below 0.10 in the systolic and the diastolic blood pressure, a model formula of R-squared regression turned to have a significant feasibility. Although the root mean square error-RMSE of 5.00 in the systolic blood pressure was higher than that of 3.0 in the diastolic blood pressure, in the systolic blood pressure was 4.50, representing the significance of calculated R-squared regression formula.
The results of the estimated the blood pressure by algorithm proposed in this research indicated an excellent performance.

P4.12
In vitro effect of apelin on contractions and endothelial-independent relaxation in the human internal mammary artery

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Recent evidence from preclinical studies indicates that apelin-13, a neuropeptide ligand of the orphan receptor (The apelin receptor also known as the APJ receptor) APJ, has a significant cardiovascular effect. In the present study, we therefore investigated effect of apelin-13 (10μM) on NE (10-9–10-4M) evoked maximal contractile responses and sodium-nitropruside (SNP) (10-9–10-4M) induced endothelial-independent vasodilatation in human internal mammary artery (IMA).
This study used in the human IMA from the distal portion, excluding the bifurcation, were obtained from patients undergoing coronary artery bypass graft (CABG) surgery. Tissue bath containing Krebs-Henseleit solution at 37 0C and pH 7.4, constantly bubbled with 95% oxygen and 5% carbondioxide. The IMA strips were allowed to equlibrate under 2g tension and isometric contractions were measured by force displacement transducer. Control contractions were recorded for 120 min and single dose of apelin were added to the tissue bath.
Treatment with apelin (10 μM) did not cause any significant change in basal tension of IMA rings. Dose-dependent contractile responses to cumulatively added NE (10-9–10-4M) was observed which was significantly reduced by application of apelin (10μM, $P < 0.01$, n=7). And, cumulatively added SNP (10-9–10-4M)-induced vasodilatation was significantly reduced by apelin (10 μM) ($P < 0.01$, n=7).
These results demonstrated that acute treatment with apelin cause vasorelaxing effect and apelin interacts with endothelium-independent realization of IMA which has been used as a bypass graft to the coronary vessels

P4.13
Role of store operated calcium and L-type calcium channels in coronary artery hypercontraction after ischemia and reperfusion process

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Rational and Aims: Calcium antagonists are used in interventional cardiology to prevent coronary vasoconstriction or to overcome the no-reflow phenomenon after primary angioplasty therapy. Coronary hypercontracture is originated by the alterations in intracellular calcium handling after ischemia and reperfusion (IR), which have an exacerbated response to agonist released by endothelium such as Endothelin. Recent evidences have highlighted the new role of store operated calcium channels (SOCC) in intracellular calcium homeostasis in coronary artery. The aim of this study was to evaluate the role of SOCC and L-type Ca2+ channels (LTCC) in coronary artery vasoconstriction originated by in vitro simulated ischemia and reperfusion protocol.
Methods: We used left anterior descendent coronary arterial rings (ADA) isolated from Wistar rats, to study the contractility as well as intracellular Ca2+ concentration ([Ca2+]i) under a simulated IR protocol.
Results: First, we observed that coronary artery tone barely increased after a period of ischemia and, this small change was not maintained during reperfusion. Second, we used Endothelin to mimic the effect of agonist released during "no-reflow" phenomenon. We found that Endothelin addition during ischemia promote small [Ca2+]i and coronary artery tone increase. Meanwhile, these effects were significantly potentiated during reperfusion. Interestingly, the resulting Endothelin-induced vasoconstriction and [Ca2+]i increase were successfully abolished by the inhibitors of SOCC, Gadolinium, and LTCC, nifedipine.
Conclusions: Our data confirm that hypercontraction of coronary artery induced by Endothelin after a process of ischemia-reperfusion involve the activation of LTCC and SOCC.

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P4.14
Vasoactive actions of lysophosphatidic acid

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We aimed to analyze the vasoactive effect(s) of lysophosphatidic acid (LPA) in order to better understand its role in cardiovascular homeostasis. Thoracic (TA) and abdominal aorta (AA) segments were isolated from adult male wild type and knock out mice deficient in LPA1 or LPA2 receptors, endothelial NO synthase (eNOS), cyclooxygenase-1 (COX1) or thromboxane receptors (TP). Isometric tension changes of the vessels were determined in myographs. The endothelium was mechanically removed in some segments. Expression of LPA receptors in freshly isolated mouse aortic endothelial cells (MAEC) and the vascular smooth muscle (VSM) were analyzed by quantitative real-time PCR. The vasoactive effects of LPA depended on the integrity of the endothelium: it induced relaxation in intact vessels (both TAs and AAs), whereas in the absence of endothelium or eNOS vasocontraction developed which was more pronounced in the AA. PCR analysis revealed the presence of mRNA encoding the 1, 2, 4, and 5 subtypes of LPA receptors in MAEC while in VSM LPA1 was predominantly whereas LPA3 and LPA4 expressed at lower levels. The LPA1–3 agonist VPC31143 mimicked whereas the LPA1,3 antagonist Ki16425 inhibited all effects of LPA. In accordance, genetic deletion of LPA1 but not that of LPA2 abolished LPA-induced vasorelaxation. Inhibition of the protein kinase B/Akt pathway by wortmannin and MK-2206 failed to influence the effect of LPA, while inhibition of phospholipase Cbeta by U73122 or edelfosine abolished LPA-induced vasorelaxation. Surprisingly, inhibition of COX by indomethacin and genetic deletion of either COX1 or TP abolished LPA- and VPC31143-evoked vasocontraction in endothelium denuded AA segments. Our results indicate that in intact vessels LPA induces LPA1- and PLCbeta-dependent activation of eNOS and consequent relaxation of the VSM. In contrast, in the absence of endothelium or eNOS LPA induces vasoconstriction by activating VSM COX1 and autocrine/paracrine release of a prostanoid, presumably thromboxane A2. This latter effect of LPA may contribute to the altered vascular reactivity in pathophysiological states associated with endothelial dysfunction.

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P4.15
Cardiovascular effects of beta-carotene are lost when it was applied at high concentration

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Nowadays, there is a growing interest in compounds derived from plants as potential drug raw materials. This great interest due to the fact that substances of natural origin frequently are less toxic and have less side effects. One of the most studied compounds is beta-carotene (BC). Several clinical studies can be found having investigated the cardiovascular effects of BC, but all these results are rather controversial. There is increasing body of evidence showing that beside the well known antioxidant properties, under strong oxidative circumstances BC could be prooxidant either. We investigated the effects of long term, low- and high-dose BC treatment in ischemic/reperfused hearts isolated from healthy and ZDF rats. Rats were treated with various daily doses of BC for 4 weeks, and then hearts were isolated and subjected to 30 min

of global ischemia followed by 120 min of reperfusion. Blood glucose levels were measured before, at half time and the end of the treatment. During the experiments heart functions were registered. At the end of reperfusion the infract size (IS) and heme oxygenase-1 (HO-1) expression were measured. The results show that low dose treatment significantly improved postischemic recovery, which was reflected in decreased IS. Interestingly, when BC was applied at high concentration, the observed protective effects were lost. Although, BC treatment increased HO-1 expression, we did not observe a better heart function and/or decreased IS in case of high dose treatment. The glucose tolerance test showed concentration independent decrease in blood glucose levels in diabetic rats, however; BC treatment had no effect on it in intact animals. The observed controversial effects would be the result of formation of harmful oxidative products of BC during reperfusion.

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P4.16
Effects of Apelin-13 on arterial blood pressure in the epileptic male rats

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In this study, it was aimed to investigate effects of apelin-13 (a high affinity ligand for the G-protein-coupled receptor APJ) on the arterial blood pressure in penicilline-induced epileptiform activity in male rats. Adult male Wistar rats were divided into control and experimental groups. Apelin-13 (50 µg) was intracerebroventricularly (icv) infused 30 min after application of penicillin to the experimental group while control group received icv saline alone. Epileptic activity and arterial blood pressure were recorded "online" by PowerLab data acquisition (LabChart; AD Instruments) unit for a period of two hours. C-fos expression in the rostral ventrolateral medulla (RVLM), supraopticnuclei (SON) and paraventricular nucleus (PVN) was determined imunohistochemically in frozen brain sections. Independent sample T-test was used for statistical analysis of the results. It was found that apelin-13 significantly reduced the spike frequency values of epileptiform activity. Our immunohistochemical findings indicate that the increase in mean arterial pressure evoked by apelin-13 administration on penicillin-induced epileptiform activity in rats was not associated with increased activation of c-fos in the RVLM and SON. However, c-fos expression in the PVN was found significantly higher in the experimental group compared to control group ($p < 0.05$). In conclusion, these findings suggest that PVN may have a role in the central control of arterial blood pressure increase after apelin-13 administration in penicillin-induced epileptic acitivity.

P4.17
Combined effects of chronic partial occlusion and gravitational load on saphenous vein: A new venous varicosity model in rat

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The precise underlying mechanisms leading to venous varicosity disease still remain unclear. A new varicosity model was developed in rat in which the chronic partial occlusion of the saphenous vein simulated the hemodynamic changes (increase in pressure, decrease in flow) that may lead to the development of varicose veins. It was demonstrated previously that a remarkable collateral vessel network developed in the vicinity of saphenous vein in response to 4-week occlusion. After 8- and 12-week occlusion the size of the network was significantly enhanced. The combination of venous occlusion with 4-week orthostasis did not change the extension of the collateral network applied in the 4-8 and 8-12-week of collateral development. In this study we aimed to elucidate the effects of immediate chronic orthostasis righ after the occlusion of saphenous vein on the properties of collateral venous network (0-4 weeks). m) were placed to the left saphenous veins of male Wistar rats, while the right saphenous veins were considered as controls. After wound healing the rats were placed into tubelike cages (45°, head-up tilt position) for 4 weeks. At the end of orthostasis Batson 17 plastic fluid was injected into a popliteal side branch which refilled the saphenous vein and its collateral vessels.
After consumption of the hind limb tissues (2 weeks, 10% KOH) the collateral network became visible. Surface of frontal projection of the casting was measured (Leica QWinV3 softver) to elucidate the size of the network.µSilicon clips (500 Combination of venous occlusion with gravitational load induced significantly greater collateral network compared with occlusion in itself (occlusion: 10±3 vs. occlusion+tilt: 205±60 µm2×10-6). Already after 4 weeks tortuous, dilated venous segments appeared, resembling human varicosity specimens.
Our results have shown that in our new varicosity model the magnitude of collateral venous network induced by partial chronic occlusion of rat saphenous vein was significantly enhanced in response to chronic gravitational load applied in early phase of collateral network development.

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P4.18

PPAR-gamma agonists treatment affected radical and cell signaling, antioxidant response and blood pressure of hypertensive rats

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The PPAR gamma (Peroxisome Proliferator-Activated Receptor) - nuclear receptor regulated lipid metabolism and cellular signaling. Activation of PPAR gamma agonist - rosiglitazone was found to affect neurogenic hypertension. Application of PPAR gamma agonists influenced also contractility and relaxation of blood vessels in hypertensive animals and regulated expression of genes involved in antioxidant response. Our study was focused on radical signaling triggered via angiotensin 1 receptor (AT1R) - NADPH-oxidase subunit p22phox - superoxid production, antioxidant response of SODs, HO-1 and involvement of Akt and beta - catenin in signaling pathway after treatment with PPAR gamma agonists. PPAR gamma agonist pioglitazone (PIO 10 mg/kg/day) was administered by gavage to young and/or adult spontaneously hypertensive rats (SHR) during two weeks. Moreover combination with alpha-lipoic acid (LA 16mg/kg/day) has been administrated to aduld SHR. Superoxide were detected by chemiluminiscence.
Gene expression was observed by qPCR and protein level by western blot. Enzymes activity of SOD and NOS was determined by UV VIS or radioactively using [3H]L-arginine. Administration of PIO significantly slowdown blood pressure increase in young rats. A significant increase in PPARgamma and SOD2 mRNA was observed in braistem (BS). Main changes were observed mainly in young rats. PIO treatment had tissue-dependent effects on SOD activities and increased activity was observed only in left ventricle (LV). The treatment of young SHR with PIO differentially affected also the levels of other intracellular signaling components (Akt kinase, beta-catenin) in the BS and LV. While Akt kinase was increased only in the BS, beta-catenin level, deeply involved in the response to oxidative stress, was down-regulated in BS and up-regulated in LV. Moreover additional treatment of PIO with lipoic acid in adult rats improved several measured parameters. It seems that beta-catenin level and antioxidant SOD response can be important agents mediated PIO effects in the brainstem and the left ventricle.

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P4.19

Interactions between the enzymes matrix metalloproteinases 2 and 9 and regulatory T-cell immunity in the pathogenesis of atherosclerosis

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Changes in immune and inflammatory responses play a crucial role in the development and progression of atherosclerosis, as an autoimmune, chronic progressive inflammatory disease. Immunological activity can be induce by many risk factors in very different age and include vascular dysfunction and atherogenesis of medium and large-sized arteries. Some evidence suggests that the vascular inflammation in atherosclerosis is modulated by autoimmune responses against self-antigens such as oxidized low-density lipoprotein (ox-LDL) in the vascular wall. The members of the matrix metalloproteinase (MMP) family play a crucial role in angiogenesis and vascular remodeling and are involved inthe pathogenesis of vascular diseases such as atherosclerosis, varicose veins, hypertension, abdominal aortic aneurysm, preeclampsia, etc. Alterations in vascular tone are usually a result of changeable endothelial cell function, as well as increasing activity of neurohormonal stimuli or altered sensitivity of vascular smooth muscle.
The aim of our study was to examine the values of enzyme matrix metalloproteinase-2 and 9 in urine in patients with atherosclerosis of carotid arteries, undergoing surgery, and compare to controls (healthy volunteers).
Patients and methods: we analyzed 40 patients with atherosclerosis who were undergoing the surgical procedure. The method of enzyme immunoassay (ELISA) was used to determine enzymes expression of matrix metalloproteinase-2 and 9 (MMP-2 and 9).
Results: The patients with atherosclerosis had a statistically significantly increased level of MMP-2 and 9 in the urine in comparison with healthy volunteers.
Conclusion: Our data has showed a large increase in the enzyme MMP-2 and 9 in the urine of atherosclerotic patients, which can be an easy marker for the monitoring of the development of atherosclerosis.

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P4.20

Protective effects of Quercetin from oxidative/nitrosative stress under intermittent hypobaric hypoxia exposure in rat heart

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Background: Exposure to high altitude in hypobaric hypoxia (HH) is considered to be a physiological oxidative/nitrosative stress. Quercetin (Que) is an effective antioxidant and free radical scavenger against oxidative/nitrosative stress. The aim of this study is to evaluate the cardioprotective effects of Que in animals exposed to intermittent hypobaric hypoxia (IHH) and therefore exposed to oxidative/nitrosative stress.

Materials and methods: Adult male Wistar rats were exposed to short-term (2 days) or long-term (4 weeks; 5 days/week) IHH in a hypobaric chamber (5500 m, 8h/day, 380 mmHg, 12% O2 and 88% N2). Half of the animals received natural antioxidant Que (30 mg/kg body weight) daily, before each IHH exposure; the remaining rats received saline solution. Control rats were kept under normobaric normoxia (Nx) and treated in a corresponding manner. One day after the last exposure to IHH, the oxidative/nitrosative stress parameters were determined in heart tissue homogenate: the free radicals (malondialdehyde, MDA and carbonylated proteins, CP), nitrite plus nitrate (NOx) production, activity of antioxidant enzymes (superoxide dismutase, SOD and catalase, CAT) and inducible nitric oxide synthase (iNOS) protein expression.

Results: In heart tissue homogenate, MDA and CP levels, NOx production and iNOS expresion of IHH-exposed rats had increased significantly ($P < 0,005$) and SOD and CAT activities had decreased significantly compared to those of the Nx-exposed rats (control groups). Expression of iNOS, NOx production, lipid peroxidation and protein carbonilation had decreased significantly ($P < 0,005$) in Que-treated IHH-exposed rats compared with IHH-exposed rats (control groups). However, Que administration increased significatly ($P < 0,05$) SOD and CAT activities of the heart tissue in the IHH-exposed rats.

Conclusions: These results suggest that the Que provides substantial antioxidant protection against hypobaric hypoxia-induced oxidative/nitrosative damage in cardiac tissue.

Key words: cardioprotection, intermittent hypobaric hypoxia, Quercetin, oxidative/nitrosative stress

P4.21

Autophagy and ventricular fibrillation in isolated rat hearts

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Pathomechanisms of many diseases are promoted by disruption of cellular homeostasis through the accumulation of toxic detritus produced by altered cellular metabolisms. The activated processes, including autophagy, degrade toxic aggregates, enabling cells to maintain structural, biochemical and functional integrity. Autophagy involves enclosure of protein aggregates and damaged organelles in pre-autophagosomic vesicles which fuse with lysosomes, maturing into autolysosomes that digest the enclosed material.

The objective of the study was to determine whether electrically-induced ventricular fibrillation (VF) can elicit autophagic processes in the absence of ischemia in the myocardium. Isolated working rat hearts were aerobically perfused at 37 oC, and VF was induced by pacing (20 Hz, 1200 beats/min) for 1, 3, 5, 10 min, respectively, followed by 120 min of VF-free aerobe perfusion. Since there were no significant changes in coronary flow rates, the changes in autophagic markers could not be attributed to a pacing-induced ischemic event. In additional studies, hearts were paced by 10 Hz (600 beats/min), to demonstrate whether ventricular tachycardia could also elicit autophagic processes. The deterioration of the cardiac function (heart rate, aortic flow, coronary flow, cardiac output, stroke volume) was registered with the increase of the duration of the VF. Increased expression of autophagic proteins including LC3BII/LC3I ratio, Beclin-1, Atg5, Atg7, and Atg12 were detected in hearts subjected to VF for 1, 3, and 5 min. However, a decline of the autophagic protein levels was observed in hearts subjected to 10 min of VF. Parallel to the VF-induced autophagic processes, an increased expression of apoptotic signaling was detected indicating by the level of cleaved caspase-3, and the TUNEL positive apoptotic cells.

The results suggest that during a prolonged duration of VF the autophagic process is unable to control the stress and may induce apoptotic activity.

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P4.22

Dietary supplementation of zinc increases acethylcholine induced relaxations of isolated rat carotid arteries

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Objective: Zinc (Zn) and selenium (Se) are important part of antioxidant enzymes which protects against reactive oxygen species (ROS). It has been suggested that altered levels of Zn and/or Se in liver and kidney can contribute or correlate to the impaired antioxidant defense leading to endothelial dysfunction. However, there are few data available regarding the effects of dietary Zn and Se on the vasomotor responses of vessels. Thus we hypothesized that changing dietary content of Zn and Se will alter vasomotor responses of isolated rat carotid arteries.

Methods: Male Sprague Dawley rats (n=33) were fed with 4 types of rat chows for 10 weeks: a) low Zn-low Se group (n=8) (normal chow) b) high Zn-high Se (N=5); c) high Zn-low Se group (N=10); d) low Zn-high Se group (N=10). Carotid arteries (CA) were isolated and their isometric tensions were measured by DMT wire Myograph system. Responses of noradrenaline pre-contracted CA to ACh (10-9-10-5 M) in the absence or presence of L-NAME. Statistical significance was set at $P < 0.05$ (SigmaPlot v12, Systat Software).

Results: Compared to the LSn/LZn (Ach 10-9: -0,11±0,03mN, Ach 10-8: -0,23±0,04mN, Ach 10-7: -0,47±0,07mN, Ach 10-6: -0,76±0,11mN, Ach 10-5: -1,10±0,16mN, $p < 0.05$) ACh-induced relaxations were significantly greater in LSe/HZn (Ach 10-9: -0,36±0,06mN, Ach 10-8: -0,63±0,08mN, Ach 10-7: -1,15±0,13mN, Ach 10-6: -1,68±0,18mN, Ach 10-5: -2,02±0,22mN, $p < 0.05$) and in HSe/HZn(Ach 10-9: -0,17±0,10mN, Ach 10-8: -0,34±0,13mN, Ach 10-7: -0,72±0,18mN, Ach 10-6: -1,15±0,23mN, Ach 10-5: -1,52±0,24mN, $p < 0.05$) group compared to that of LSe/LZn. Presence of L-NAME blocked ACh-induced vasorelaxation in all four groups of rats.

Conclusions: Data suggest: 1) changes in dietary Zn and Se substantially affects arterial relaxations to ACh, 2) presence of high level of Zn is responsible for the improved endothelial function and 3) the underlying mechanisms likely include increased NO bioavailability.

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P4.23

Role of the transient receptor potential vanilloid-1 (TRPV1) in the development of hydrogen chloride (HCl)-induced vasomotor response in isolated rodent carotid arteries

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It was shown earlier that acidic products released during anaerobic tissue metabolism are involved in the regulation of local blood flow. However, the mechanism, through which reductions of local pH evoke the change in vascular tone, is not fully elucidated. Among TRP channels located on vascular elements, TRPV1 plays an important role in sensing of environmental changes (e.g., pH, temperature, etc.). In the present study we hypothesized that TRPV1, which is abundantly expressed in the vascular wall and can be activated by acidic stimuli, plays a role in the vascular responses to low pH. Carotid arteries were isolated from Wistar rats, from mice genetically lacking TRPV1 channels (KO) and from wild-type mice. Isometric wall tension was measured with a myograph. Vessels were precontracted with 10-5 M phenylephrine (PhE), then isometric changes were measured in response to increasing concentrations of HCl (10-6–10-3 M). Functional integrity of the vessels (endothelium-dependent and -independent relaxation) was verified after each experiment.

In rat carotid arteries, PhE caused a significant (~5 mN) isometric contraction, and addition of HCl caused dose-dependent relaxation, which was significant already at 10-6 M (-0.3±0.0 mN, $p < 0.05$), and reached a maximum of -1.4±0.1 mN at 10-3 M. Capsaicin elicited significant contractions in carotid arteries isolated from wild-type mice, whereas it was ineffective in that of TRPV1 KO mice, confirming the lack of functional TRPV1 channels. Interestingly, HCl (10-6–10-3 M) caused a significantly greater arterial relaxation in TRPV1 KO mice as compared to controls ($p < 0.05$ for each concentration). Based on these results, we can conclude that acidic environment results in arterial relaxation, the magnitude of which is limited by TRPV1 channels and that changes in local pH contributes to regulation of vasomotor tone to maintain optimal tissue blood flow.

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P4.24

The effects of NMDA receptors modulation on cardiodynamic parameters in isolated rat heart

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The N-methyl-D-aspartate (NMDA) receptor is a member of the ionothopic glutamate receptor family. NMDA receptors are mainly expressed in central nervous system (CNS) where play important roles in processes such as learning and memory, but they are also located in heart on cardiomyocytes. Homocysteine (Hcy) is sulfur amino acid which acts as NMDA receptor agonist, and disocilpine (MK-801) is NMDA receptor noncompetitive antagonist. DL-Hcy thiolactone is one of the most toxic Hcy metabolites, and some of his negative effects are due to NMDA receptor overstimulation. The hearts of male Wistar albino rats (n=24, 12 in each experimental group, age 8 weeks, b.m. 180–200 g, coronary perfusion pressure 70 cmH2O) were excised and perfused in Langendorff apparatus. The experimental protocol for the first group included the application of DL-Hcy TLHC (10 microM), combination of DL-Hcy TLHC and MK-801(10 microM + 50 microM), and MK-801(50 microM), successively in durations of 5 minutes. Application of MK-801continued with recovery period of 10 minutes. In the second group of experiments we first applied MK-801, followed by combination of DL-Hcy TLHC and MK-801, and in the end DL-Hcy TLHC, in same concentrations. Using sensor in the left ventricle we registered the next parameters of myocardial function: maximum and minimum rate of pressure development in the left ventricle (dp/dt max and dp/dt mix), systolic and diastolic left ventricular pressure (SLVP and DLVP) and heart rate (HR). Before the ending of application of each substance, coronary flow (CF) was measured flowmetrically. In the first experimental group administration of DL-Hcy TLHC induced significant decrease of dp/dt max, SLVP, HR and CF in comparison with control conditions. Combination of DL-Hcy TLHC and MK-801 did not cause any significant change in observed cardiodynamic parameters. MK-801 caused reduction of dp/dt max, dp/dt min, SLVP and HR values in relation to the combined use of substances.
After recovery period all cardiodynamic parameters, except DLVP and HR, reached the initial values. In the second experimental group application of MK-801 induced reduction of dp/dt max, dp/dt min, SLVP, HR and CF.

P4.25

Functional crosstalk between L-type Ca2+ and Orai1 channels and their regulation of vascular tone

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Rationale: Vascular smooth muscle cells (VSMC) contracts in response to an increase in intracellular Ca2+ concentration ([Ca2+]i) owing to Ca2+ release from sarcoplasmic reticulum (SR) and Ca2+ entry through voltage dependent and independent ion channels. Voltage-dependent CaV1.2 L-type Ca2+ channels (LTCC) are considered the main route for calcium entry in VSMC. However, recent studies have determined the relevant role of store-operated Ca2+ channels (SOCC) in vascular tone regulation.
Objective: The aim of this study was to characterize the crosstalk between orai1-dependent SOCC and LTCC and their regulation of vascular tone.
Methods and Results: We used wild type and aorta smooth muscle-selective conditional CaV1.2KO knockout (CaV1.2KO) mice to study aorta rings contractility, intracellular Ca2+ mobilization and membrane potential changes in isolated VSMC. We found that serotonin (5-HT) and the inhibition of sarco-endoplasmic reticulum calcium ATPase (SERCA) with thapsigargin induced aorta vasoconstriction and the increase of [Ca2+]i in VSMC, which were sensitive to LTCC and SOCC inhibitors indicating the participation of both channels in vessels contraction. 5-HT and thapsigargin-induced aorta contraction and [Ca2+]i elevation were significantly attenuated in CaV1.2KO arterial rings compared to wild type. Interestingly, Orai1 antibody delivery by aorta transfection inhibited significantly 5-HT-induced aorta contraction. We determined that CaV1.2KO and Orai1 co-localize uniformly in sub-membrane compartments of freshly isolated VSMC. Furthermore, store depletion by thapsigargin induced a progressive depolarization that was inhibited by nifedipine.
Conclusions: These data suggest a functional interaction between SOCC and LTCC in VSMC, indicating that upon agonist stimulation vessel contraction involves Ca2+ entry due to a co-activation of SOCC and LTCC.

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P4.26

Shift in Na+/Ca2+ exchange balance modulates the inotropic consequences of the NCX inhibition

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Inhibitors of the Na+/Ca2+ exchange (NCX) may exert a positive inotropic action by modifying the balance of the cellular Ca fluxes, which may present a possible new way in the treatment of heart failure. However, recent data are not consistent regarding the inotropic effects of the available NCX inhibitors. In this study we analyzed this problem by applying ORM10103 (ORM), a new selective NCX inhibitor. Experiments were done on isolated dog cardiac myocytes. Ca2+ transient (CaT) was measured by fluorescent dye in field

stimulated cells. Measurement of membrane currents and cell contractions were carried out in patch clamp configuration. At physiologic ion concentrations, ORM (3, 10 μM) did not influence CaT in field stimulated cells. In the modified balance after augmentation of the Ca2+ influx via NCX ([Na+]o 70 mM), application of ORM decreased CaT. In contrast, after increasing Ca2+ efflux ([Ca2+]o 0,5 mM, forskolin 200 nM) ORM elevated the CaT. In patch clamp experiments in presence of 5 mM [Na+]i and -10mV plateau potential ORM inhibited a complex inward current, which was paralelled by increased contractions. However, at 20 mM [Na+]i and +30mV application of ORM increased the inward current during the pulse, suggesting outward current inhibition, which was followed by decreased contractions. Our results suggest that accentuated consequences of inhibition of augmented direction of NCX can be expected. In heart failure cellular balance of Ca2+ and Na+ may differ from physiologic state, therefore our results underline the necessity of using disease specific experimental models in the study of inotropic actions of the NCX blockers.

P4.27
Cardiovascular manifestations of complement activation-related pseudoallergy following administration of liposomal nanomedicines

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Complement (C) activation-related pseudoallergy (CARPA) is a hypersensitivity reaction to intravenous therapies with liposomal and many other nanomedicines that are recognized by the immune system as foreign. CARPA is characterized by severe cardiopulmonary changes and sometimes anaphylactic shock that can limit the use of reactogenic drugs. The most sensitive species for modeling CARPA is the pig; however, the underlying mechanisms of CARPA can also be studied in rats. The present study compares the hemodynamic and immunological responses of these two species inducing CARPA by i.v. bolus injections of the reactogenic liposomal nanodrug AmBisome. AmBisome caused significant consumption of C3, indicating C activation, along with massive parallel changes in blood pressure, white blood cell, platelet counts and in plasma thromboxane B2 levels, indicating CARPA in both pigs and rats. Pigs responded to AmBisome with severe (3-fold) pulmonary hypertension and systemic hypotension (up to 50%). The major response of rats was systemic hypotension (40% after 5 min), as well as leukopenia followed by leukocytosis and thrombocytopenia. These effects were similar in the two species in terms of kinetics, but significantly differed in the doses that caused major hemodynamic changes (~ 0.01 and ~ 22 mg phospholipid (PL)/kg in pigs and rats, respectively). These observations highlight fundamental differences in the immune mechanisms of porcine and rat CARPA, rats being much less sensitive than pigs; however, the causes of these differences remain unclear. Measurements of local (e.g. pulmonary) cardiovascular parameters, as well as identifying cellular mediators involved in the hemodynamic responses might contribute to better understanding of the phenomenon.

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P4.28
Autonomic function and cold-induced vasoconstriction in nicotine dependent young people

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The health of people living in the North appears to be a result of general impact of the discomfort cold climate and other environmental factors combined with individual effects of the lifestyle. In this respect tobacco smoking in the North significantly increases health risk due to well-known hazardous impact on the cardiovascular system and other functions of the organism. The present study was focused on the evaluation of cold-induced vasoconstriction and autonomic function in nicotine dependent young people. Ten tobacco smoking people (7 m, 3 f) of 20-22 years old studying at the Petrozavodsk State University (Republic of Karelia, Russia) volunteered to participate to our study. The reference group consisted of 31 age-matched non-smoking students (14 m, 17 f). Fagerström Test for nicotine dependence revealed the weak degree of addiction to nicotine in the investigated group. The local cold test showed the impaired skin temperature restoration in compare to the reference group ($p < 0.01$) which is considered as an increased cold-induced vasoconstriction. Autonomic balance been estimated by the analysis of skin sympathetic responses and by temporal and power spectrum parameters of heart rate variability (HRV) was characterized by the prevalence of parasympathetic activity in both groups. Yet, vagotony as well as the cardiorespiratory synchronization were found to be significantly less in nicotine dependent group. Thus, the decreased adaptive capability according to HRV analysis in even low nicotine dependence was documented. Besides, the increased cold-induced vasoconstriction at early stage of nicotine dependence found in this study did not involve neurogenic mechanisms to large extent, but rather originated due to increased vascular sensitivity to humoral vasoconstrictors and/or endothelial dysfunction. Such a vascular reactivity in nicotine dependent people appears to be a premorbid sign of cardiovascular disorders because of the similarity of observed increased cold-induced vasoconstriction to the mechanisms of Raynaud's phenomenon which is known as a premorbid sign in cold regions.

P4.29

Beneficial effects of Allium ursinum herbal extract (AUHE) on hypercholesterolemic hearts

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Atherosclerosis is the most frequent death in the human population. The present study characterized the complex cardiovascular effects of AUHE in hypercholesterolaemic rabbit model. The experiments were performed on 18 New Zealand white rabbits.
Animals were divided into three groups, which were: (i) normal cholesterol free rabbit chow (C), (ii) 2 % cholesterol-containing chow (HC), (iii) 2% cholesterol + 2% AUHE containing chow (AUHE), administered for 8 weeks. Rabbits were evaluated echocardiographic (EF, FS, E/A, TAPSE) and serum parameters (TotalChol, HDL, LDL, CK, LDH, GOT, ApoA, ApoB). After the 8-week treatment period the following haemodinamic variabless were estimated using an isolated working heart model: aortic flow (AF), coronary flow (CF) and aortic pressure (AoP). AUHE treatment was observed to improve cardiac functions. Post-ischaemic values of aortic flow in treated animals were significantly greater compared to the HC group ($p < 0,05$). Echocardiographic measurements showed improved ejection fraction (EF) and fractional shortening (FS) functions in animals receiving AUHE diet, while E/A rations showed no significant differences. Interestingly, better right ventricle functions were measured in treated animals (higher TAPSE values). Total blood cholesterol levels in in the HC goup exhibited dramatic increases following the 8-week period. Cholesterol levels in the AUHE group were significantly lower compared to the HC group ($p < 0,05$). AUHE also had notable beneficial effects on the other serum parameters (GOT, LDH, CK). Our data demonstrates that AUHE has complex cardioprotective effects on hypercholesterolaemic hearts presumably through the HO-1 pathway. Future studies will characterize the molecular mechanisms contributing to effects of this herbal agent.

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P4.30

Innovation of education of cardiovascular physiology with respect to clinical practice

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Cardiovascular physiology represents one of the most important, but also the most difficult part of physiology. The aim of this project is to deepen students' knowledge in this field, to link theoretical data with clinical practice and to teach students how to interpret both clinical and experimental hemodynamic data using modern diagnostic methods and technologies (computer simulation, clinical imaging methods).

The used computer program CorVascSim (Aplysia, Sweden) represents closed-loop real-time simulation model of hemodynamics and oxygen transport in the cardiovascular system, which includes the behavior of the four cardiac chambers, interatrial and interventricular septum, pericardium, intrathoracic pressure changes, heart valves, intracardiac shunts and vascular system. The possibility to change the model parameters one by one facilitates better understanding in educational sessions. The model is presented from a normal cardiovascular physiological point of view, but many pathological scenarios are also shown, e.g. systolic heart failure, diastolic heart failure, valve malfunction, intracardiac shunts and extra-corporeal circulatory support. Furthermore, the echocardiographic material has been prepared to illustrate the cardiac cycle, the effect of inspiration on venous return (the Valsalve maneuver), the effect of gravity on cardiovascular system, etc. The voting equipment, TurningPoint (AV Media) is used not only for the examination of students, but also for evaluation of the teaching, which contributes to the effective participation of all students on practical classes.

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P4.31

Comparison of ishemic and omeprazole preconditioning on oxidative stress in isolated rat heart

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Proton pump inhibitors efficiently supress gastric acid secretion and they are widely used in treatment of disorders where its excessive secretion is present. Althoug they are often presribed class of medications, their protective effects on reducing myocardial damage by reperfusion injury are still not enough investigated. The aim of the study was to evaluate the effects of preconditioning induced by omeprazole compared to the effects of ischemic preconditioning on oxidative stress in the isolated rat hearts. The rat hearts (total number =24, 12 for each experimental group) isolated from male Wistar albino rats, age 10 weeks and average body mass 250g were retrogradely perfused according to Langendorff technique at constant perfusion pressure of 70 cm H2O. Oxidative stress markers (TBARS, NO, O2- and H2O2) were measured spectrophotometrically in coronary venous effluent. In control group, hearts were submitted to preconditioning which includes five-minute ischemia and ten-minute reperfusion after which was induced the 20-min ischemia and established

thirty-minute reperfusion. In experimental group, hearts were perfused with omeprazole (100 μM) during 5 min, then recovered 10 min and after that submitted to twenty-minute ischemia and thirty-minute reperfusion. Samples of coronary venous effluent were collected in following periods: after stabilization, every minute during reperfusion, and every 5 minutes during reperfusion. In the ischemic preconditioning group there was no significant increase in the release of nitrite which was continued during the first three measuring time of reperfusion after that, values were moved closer to those values before ischemia. A similar trend occurred with the other tested parameters. In the group with omeprazole preconditioning, there was a significant decrease in the values of nitrites and also no changes in dynamics of other oxidative stress parameters.
Our results suggest that ischemic preconditioning does not change production of free radicals during reperfusion. Preconditioning with omeprazole, also does not promote oxidative stress and may be of interest in pharmacological pretreatment of ischemic heart.

P4.32
Asynchronous activation of calcium and potassium currents by beta-adrenergic stimulation in mammalian ventricular myocardium

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During cardiac adaptation L-type Ca2+ (ICa,L) and various K+ currents are activated via beta-adrenergic receptor stimulation. The aim of the present work was to study the timing of activation of Ca2+ and K+ currents in isolated canine ventricular cells in response to exposure to isoproterenol (ISO). The type of β receptor responsible for the changes was also examined.
Action potentials (AP) were recorded by sharp microelectrodes, while ion currents (ICa,L, IKs and IKr) were measured in whole cell configuration of the patch-clamp technique in either conventional voltage clamp or AP voltage clamp modes.
ISO (10 nM) greatly increased ICa,L and IKs currents and to a smaller extent IKr too. These changes were manifested as the elevation of plateau potential and the reduction of AP duration (APD) in subepicardial and midmyocardial cells and were absent in case of the blockade of both β1- and β2-adrenoceptors. In subendocardial cells the APD did not change only an elevation in the plateau potential was observed. The ISO-induced plateau shift and ICa,L increase developed faster than the shortening of AP and stimulation of IKs and IKr. The AP shortening observed in control conditions was mainly mediated via β1-adrenoceptors as in case of the selective blockade of β2-adrenoceptors the shortening increased. Moreover, selective the β2 stimulation led to the prolongation of the AP and resulted in a faster response as the latency of APD change reduced. The elevation of the plateau potential was preserved in the selective stimulation of β1 receptors, while its magnitude was much less in case of selective β2 stimulation. The plateau potential increase was mainly mediated by β2-adrenoceptors as is case of a selective β1 stimulation the latency of plateau shift was much greater compared to control condition as well as in selective β2 stimulation. Inhibition of phosphodiesterases decreased the differences observed in the turn on of the ISO induced plateau shift and AP shortening.
ISO-induced activation of ICa,L is turned on faster than the stimulation of IK in canine ventricular cells due to the involvement of different adrenergic pathways and compartmentalization.

P4.33
The effects of Tarantula cubensis extract on renal ischemia/reperfusion injury in the rats

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Objective: Free radicals and nitric oxide (NO) have played a crucial role in the pathophysiology of renal ischemia/reperfusion injury. The aim of the present study was to investigate the effects of the homeopathic medicine Tarantula cubensis extract, regeneration, resorption and demarcation effects, in the tissue damage during ischemia/reperfusion injury of kidney.
Material and Methods: Thirty two male Wistar Albino rats divided into four groups: Group 1 saline solution and Group 2 received Tarantula cubensis extract (1 ml/kg) subcutaneously. Group 3 subjected to bilateral renal ischemia (60 minutes) followed by reperfusion (24 hours) and saline subcutaneously injected 30 min before induction of ischemia. Group 4 is also subjected to bilateral renal ischemia followed by reperfusion and Tarantula cubensis extract (1 ml/kg) 30 min before induction of ischemia. At the end of the reperfusion period (24 hour), the rats were sacrificed. The kidney malondialdehyde and glutathione levels, plasma urea, creatinine levels and alanine aminotransferase (ALT), aspartate aminotransferase (AST) activities were determined. In the kidney tissue, immunochemical parameters such as inducible nitric oxide synthase (iNOS), endothelial nitric oxide synthase (eNOS) and hypoxia-inducible factor 1-alpha (HIF-1α) were determined and histopathological examination was also performed.
Results: Ischemia/reperfusion application increased urea and creatinine levels ($p < 0.01$) and decreased eNOS activities ($p < 0.01$). The kidney tissue malondialdehyde levels increased ($p < 0.05$). In this group, necrosis and cast formation and iNOS and HIF-1α levels were also higher ($p < 0.01$). Tarantula cubensis extract application increased the ALT and AST activities ($p < 0.05$).
Conclusion: We failed to find any protective effects of Tarantula cubensis extract on other investigated parameters. Moreover, Tarantula cubensis extract via increasing ALT and AST activities may further worsen the liver injury in this model. Therefore, we suggest that Tarantula cubensis extract may have not any beneficial effects under these conditions kidney ischemia/reperfusion injury model.

P4.34

Ellagic acid prevents cardiac fibrosis and attenuates high blood pressure in chronic nitric oxide-deficient hypertensive rats

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Objective: We investigated antihypertensive and antifibrosis effects of ellagic acid (EA) on NG-nitro-L-arginine methyl ester (L-NAME)-induced hypertensive rats.
Methods: Male Sprague-Dawley rats were given L-NAME (40 mg/kg/day) to induce hypertension, and simultaneously treated with EA 15 mg/kg/day for 5 weeks (L-NAME+EA group), or a vehicle (L-NAME group). Age-matched rats served as a control group. After 5 weeks of treatment, rats were anaesthetized with peritoneal injection of pentobarbital-sodium (60 mg/kg) and systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and heart rate (HR) were evaluated. Rats were scarified by exsanguinations and the heart was isolated. The left ventricle was excised and weighed. The relative heart weight or left ventricular weight (LVW) per body weight (BW) was determined as ventricular hypertrophy index. The paraffin sections of left ventricle were stained by Picrosirius red. Fibrosis was quantified using polarisation microscopy and ImageJ analyses software.
Results: EA significantly reduced SBP, DBP, MAP and HR of L-NAME treated rats when compared to those of the L-NAME group (164.62±4.92 vs. 199.39±6.14 mmHg, 111.23±3.68 mmHg vs. 140.40±3.95 mmHg, 133.08±4.20 mmHg vs. 164.30±4.61 mmHg and 368.03±3.12 bpm vs. 424.78±6.19 bpm, $p < 0.05$, respectively). After 5 weeks of L-NAME administration, LVW/BW ratio was significantly increased when compare to those in the control groups (2.65±0.07 mg/g vs 2.17±0.04 mg/g, $p < 0.05$). Administration of EA caused significant decrease in this ratio when compared to the L-NAME-induced hypertensive groups (2.32±0.09 mg/g vs 2.65±0.07 mg/g, $p < 0.05$). The amount of fibrosis in the left ventricle of L-NAME group was significant increase compared to control (4.00±0.28 % vs. 1.42±0.20 %, $p < 0.05$), whereas in L-NAME+EA group, the amount of fibrosis in the left ventricle was lesser than that in L-NAME group (1.54±0.13 % vs. 4.00±0.28 %, $p < 0.05$).
Conclusion: In conclusion, these data suggest a cardio-protective role of the EA against L-NAME-induced hypertension.

P4.35

Pituitary adenylate cyclase-activating polypeptide (PACAP) induces location- and age-related relaxations of isolated arteries

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Introduction: Pituitary adenylate cyclase activating polypeptide (PACAP) is a well-known neuropeptide with widespread organ and tissue distribution, which also has vasomotor effects. However, less is known regarding the organ specific and age related vasomotor effects of PACAP, which could be important for better understanding its physiological roles.
Hypothesis: We hypothesized that the vasomotor effects of PACAP depend on the tissue origin of the vessels and aging substantially modulates its actions.
Methods: Thus carotid (CA) and basilar arteries (BA) were isolated from young (2 months old), middle age (12 months old) and old (30 months old) rats. Their vasomotor responses were measured with an isometric myograph (DMT-610M) in response to cumulative concentrations of PACAP1-38 (10-9 M - 10-6 M).
Results: PACAP1-38 induced 1) a significantly greater concentration-dependent relaxation in CA compared to that of BA of young, middle age and old rats; 2) relaxations of CA significantly decreased, whereas it did not change substantially in BA, as a function of age; 3) sodium-nitroprusside (SNP) - induced relaxation did not change after PACAP1-38 administration in any conditions.
Conclusions: These findings suggest that PACAP1-38 has greater vasomotor effect in peripheral arteries than in cerebral arteries and aging has less effect on PACAP-induced relaxation of cerebral arteries than in peripheral arteries suggesting that PACAP could be a vasoprotective substance in old age.

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Keywords: PACAP1-38, isolated arteries, aging, cerebral and peripheral circulation, dilation

P4.36

Combined modulation of IK, ATP and IKr to reduce reverse use-dependency and repolarization heterogeneity

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Objective: The effects of activation of the cardioprotective outward IK, ATP current, that could ideally limit the excessive repolarisation prolongation of IKr blockers and could also decrease heterogeneity of repolarisation in various types of cardiac tissues, were investigated in our present study. These beneficial effects could contribute to a new and safer therapeutic option for the management of ventricular arrhythmias.

Methods: Action potential measurements were carried out in dog and rabbit Purkinje fibre and ventricular muscle preparations by conventional intracellular microelectrode technique. For IKr block dofetilide (50 and 300 nM in dogs; 12.5 nM in rabbits) and for IK, ATP activation pinacidil (1 and 3 uM in dogs; 20 uM in rabbits) were used.

Results: The repolarization was significantly prolonged at all applied stimulation frequencies by dofetilide and combination of dofetilide+pinacidil as well. In rabbit preparations, the action potential duration, primarily at slow stimulation frequency (40/min), was increased at lesser degree by the combination of dofetilide+pinacidil than with dofetilide alone (46.1 ± 7.3 ms (31.6 %) vs. 59.2 ± 7.6 ms (41.5 %) in left ventricular muscle and 67.2 ± 14.8 ms* (32.6 %) vs. 97.2 ± 21.5 ms (47 %) in Purkinje fibres; *p <0.05). Slight reduction of repolarization heterogeneity was observed by application of combination of dofetilide+pinacidil compared with dofetilide. Results obtained from dog preparations showed similar results.

Conclusions: The proarrhythmic reverse use-dependent effects of IKr blockers and the increased repolarization heterogeneity may be reduced by simultaneous activation of IK,ATP. For this aim, cardioselective IK, ATP activators, devoid of reflex tachycardia due to extensive vasodilating effects, would be preferred.

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P4.37

Effect of swimming exercise on CO pathway of resistance and conduit arteries in chronic NOS inhibition induced hypertensive rats

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The blood pressure reducing effect of regular physical activity has been demonstrated in various experimental hypertension models including the nitric oxide synthase (NOS) inhibition model. Carbon monoxide (CO) another endogenous gas known to dilate blood vessels in a manner similar to nitric oxide. CO may show a compensatory effect under the conditions in which NO production is reduced or not present. However, in hypertension model with NOS inhibition, the effect of the exercise to heme oxygenase/carbon monoxide (HO/CO) system in vascular tissues is not known. Hypertension was induced by oral administration of a nonselective NOS inhibitor L-NAME for 6 weeks. Training protocol in exercise groups was performed as swimming. Systolic blood pressure, as measured periodically by the tail-cuff method, was significantly decreased by the training protocol in exercising hypertensive rats. Citrate synthase activity in red vastus lateralis increased significantly exercise groups. The vasoreactivity of vessel was evaluated by wire and organ bath studies. Endogenous CO relaxation responses of vessel rings were not different between the groups apart from the increase in thoracic aorta in exercise group. Thoracic aorta HO-1 expression was significantly higher in both exercise groups. There was no difference between the groups in vasodilation in responses to exogenous CO in vessel rings. While CO demonstrated its effect in thoracic aorta by means of both sGC and K+ channels, it did so by means of K+ channels in resistance arteries. In conclusion, the lack of a positive effect of regular physical activity on relaxation responses in NOS inhibition hypertension model proves that HO/CO system does not function in compensatory manner in this hypertension model.

P4.38

Hemodynamic effects of Isatin on isolated perfused heart

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Aim: Isatin is an endogen indole that has been determined inhibitory effects on natriuretic peptides by reducing cGMP production. It has been reported anti-inflammatory and anti-tumor effects of isatin. The aim of this study was to invesitigate hemodynamic effects of isatin on ischemia and reperfusion in rat heart.

Material and Method: Male Wistar rats (250-350 gr) were used in this study. Normal saline (2,5 ml/kg) in K group (n=10) and isatin (50 mg/kg) in I group (n=10) were applied intraperitoneally to rats 30 minutes before ischemia. Isolated hearts were harvested in a Langendorff model. Krebb's Henseleit solution perfused for 15 minutes in each group. ANP (0.1 µM/L) was added to the perfusion solution in ANP group (n=7) and I-ANP group (n=8). Low flow ischemia (30 minutes) and reperfusion (60-minutes) were applied in all groups. Left ventricular developed pressure, maximum and minimum rate of pressure development (+dP/dT and -dP/Dt) were recorded during the experiments. cGMP levels were measured in perfusion solution before and after ischemia. Cyclooxygenase-2 (COX2) mRNA and NF-KB mRNA levels

of left ventricle tissue were also determined 30 minute after administration of isatin (n=10) or normal saline (n=10).
Results: Infarct size was larger in the I group (19,7±7,1 %) than ANP (8,8±6,0 %; p <0.01) and ANP-I (9,1±4,2 %; p <0.01) group. Developed pressure was lower in I group (18,9 ± 17,1 mmHg) than ANP group (68,9± 55,5 mmHg; p <0.05), and I-ANP group (80,8± 53,2 mmHg; p <0.05) in reperfusion (60 minutes). Decreased -dP/Dt values were observed 60 minutes after reperfusion in I group (-474 ± 277) than ANP group (-1527± 894; p <0.05), and I-ANP group (-1319±674; p <0.01). Higher cGMP values were found in reperfusion in ANP (1,22±0.07 pm

P4.39
Cardiovascular target-organ damage in women during menopause

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Abstract Introduction: Menopause is often accompanied by degenerative processes such as arteriosclerosis that suggest an acceleration of aging triggered by estrogen lack. Diseases of the cardiovascular system, especially of the coronary blood vessels, are among the leading causes of death in menopausal women. The present study was designed to evaluate some of the arteriosclerotic risk factors: LDL-CH, LDL-CH / HDL-CH index of arteriosclerosis, triglicerydes, total cholesterol, plasminogen activator inhibitor type 1 antigen (PAI-1 Ag) and factor VII of coagulation, in women during menopause.
Matherial and methods: The study comprised a number of 120 women divided into three groups. The control group included 40 healthy women in their reproductive period. The perimenopausal group consisted of 42 women, with FSH level under 25mU/ml, and with anamnestic data of irregularity of menstrual cycle. The postmenopausal group encompassed 38 women, regarding lack of cycle for more than 12 months. Hormone level was determined with RIA method. Lipid level was determined with standard colorimetric-spectrophotometric method, the concentration of PAI-1 Ag was determined by using immuno-enzymatic method and the concentration of f. VII was determined by method of plasma deficiency.
Results: Statistical analysis has shown that there was a significant increase of total cholesterol, triglycerides, LDL-CH, LDL-CH / HDL-CH index, PAI-1 Ag and f. VII in both perimenopausal and postmenopausal examines in comparison with the control group (p <0,001).
Conclusion: This study favours the view that decrease in estradiol level and associated increase in LDL-CH, LDL-CH / HDL-CH index, triglycerides, total cholesterol, PAI-1 Ag and f.VII seen in perimenopausal and postmenopausal women may be responsible for the increased risk of atherosclerotic and thromboembolic complications in women during menopause.

Key words: estrogens; menopause; cardiovascular diseases; arteriosclerosis.

P4.40
Evaluation of Urotensin-II and Urocortin as biomarkers of myocardial damage in an animal model of acute myocardial infarction

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Background: Urocortin (Ucn) and Urotensin-II (UII) are potent vasoactive peptides that contribute to the physiopathology of several cardiovascular diseases. Ucn expression increased has been demonstrated in heart submitted to ischemia and reperfusion process; meanwhile UII has been linked to atherosclerosis and coronary artery disease.
Aims: We sought to determine levels of UII and Ucn and their specific receptors in an animal model of acute myocardial infarct (AMI) in order to evaluate their possible use as sensitive biomarker of myocardial injury.
Methods: We used a rat model of AMI by occlusion of the left anterior descending coronary artery. Blood and tissues samples were taken from Sham wistar rats and from two groups of animals that were sacrificed three days or six weeks after AMI. Ucn and UII levels were examined using highly specific ELISA. Heart tissue samples were also processed to determine the expression of receptors of Ucn (CRF-R2) and UII (UTS2R) by Western blotting.
Results: First, we have found a significant increase in blood levels of UII and Ucn on the third day after AMI, while six weeks after surgery both peptides levels return to baseline. Moreover, we have determined a significant enhancement of in CRF-R2 protein expression in the infarcted myocardium of animals at six weeks after AMI. However, the UTS2R receptor expression barely changed under the same conditions.
Conclusion: Our preliminary data confirm that Ucn and UII could be a sensitive biomarker of early myocardial injury followed an AIM, even though their receptors are differentially expressed 6 weeks after AMI.

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P4.41
Distinct effect of crowding stress on cardiac ischemic tolerance in borderline and spontaneously hypertensive male and female rats

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Introduction. Genetic predisposition and social stress represent important risk factors in etiology of hypertension associated with impaired myocardial response to ischemia and reperfusion (I/R), which can differ in male and female heart as

sex is an important determinant of cardiovascular morbidity and mortality in human population. Tolerance to I/R injury persists even in the hearts of animals with predisposition to hypertension and may be modified by cardiac adaptation to stressful conditions.
The study aimed to examines the impact of chronic stress on response to I/R in borderline/spontaneously hypertensive male and female rats (BHR/SHR) in comparison with its effects in normotensive (WKY) counterparts.
Methods and Materials. Male and female 5-week-old BHR, SHR and WKY were exposed to 2-week crowding stress (CS; livingspace 200cm2/rat). Unstressed animals had livingspace 480cm2/rat. Langendorff-perfused hearts of all experimental rats were exposed to 30-min global ischemia and 2-h reperfusion for the evaluation of reperfusion-induced ventricular arrhythmias, infarct size (IS) and recovery of contractile function.
Results. Tolerance to reperfusion-induced ventricular arrhythmias was decreased in hearts of non-stressed BHR of both sexes. Opposite effect was seen in hearts of non-CS SHRs. Interestingly, exposure to CS significantly decreased the total duration of VT in BHR males and females as well as WKY females. Stressed SHR animals of both sexes exhibited higher susceptibility to VT. Non-CS BHR male and female hearts showed decrease in lethal injury. Reverse effect was observed in non-CS SHRs. IS was not affected by CS in any of the groups. Exposure the rats to the mild CS resulted in statisticaly important decrease in NOS activity in left ventricle of BHRs as well as SHRs of both sexes. In WKY animals no change in NOS activity was seen after crowding.
Conclusions. CS modifies parameters of I/R in a distinct way. Inherited predisposition to hypertension may be responsible for differences in arrhythmogenesis and adaptive potential in animals exposed to social stress, which appear to be sex-dependent.

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P4.42
Impaired baroreflex-function is not related to deteriorated carotid elasticity in schizophrenic patients

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Background: Arrhythmia-related sudden cardiac death is a common late complication in schizophrenic patients. Impaired short-term blood pressure regulation, indicated by reduced baroreflex-sensitivity (BRS), was a risk factor for sudden cardiac death in various diseased states. Reduced BRS was found in schizophrenic patients, but the underlying mechanism is not clear. Stiffening of the baroreceptor vessel wall may result in decreased activity of the baroreceptors and blunt the baroreflex. We investigated the distensibility of a baroreceptor vessel wall – such as the common carotid artery - and tested the hypothesis that reduced BRS is associated with increased carotid artery stiffness in schizophrenic patients.
Subjects, methods: 24 first-episode schizophrenic patients (28±5 years) and 28 healthy, age- and gender-matched control subjects were examined. Diastolic diameter and pulsatile distension of the common carotid artery were measured by echo wall-tracking, carotid pulse pressure was registered by tonometry. Based on these data, distensibility coefficient (DC) was calculated. The BRS was determined by analysing, simultaneous ECG and beat-to-beat blood pressure recordings.
Results (mean±SD): DC showed marked reduction in schizophrenic patients compared to control subjects (3.9±0.9* vs. 5.2±1.3 10-3/Hgmm). As expected, BRS was significantly decreased in patients (9.9±7.1* vs. 22.9±7.9 ms/Hgmm). No relation was found between DC and BRS in patients, whereas the two parameters showed positive correlation in control subjects (r=0.31*). (unpaired t-test, Pearson's correlation test, * $p < 0.01$)
Discussion: In schizophrenic patients, carotid artery distensibility was markedly reduced but it was not related to diminished BRS. The underlying mechanism for increased carotid artery stiffness is yet unclear. In schizophrenic patients, elevated plasma homocysteine level and increased oxidative stress may contribute to the decrement of carotid distensibility. Our results suggest that decreased carotid elasticity does not contribute to the impairment of baroreflex-function substantially. We presume that reduced BRS may be related to neuronal damage within the baroreflex arch.

P4.43
Conduction of excitation in the rat atria and pulmonary veins under normal condition and after octanol application

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OBJECTIVE. Much attention is paid to the pulmonary veins (PVs) myocardium as a region responsible for atrial fibrillation triggering. Arrhythmogenic properties of PVs are partially associated with abnormal propagation of excitation wave. Decreased density and conductance of gap junction (GJ) were demonstrated in PVs in several studies. Functional role of altered GJ in suppression of conduction velocity (CV), formation of conduction blocks and PVs arrhythmogenity in is not completely clear. The aim of this study is to investigate conduction of excitation in rat PVs myocardium in condition of gap junction uncoupling.
METHODS. Rats (male, 250-300 g) were anaesthetized (urethane, 1.5 g/kg i.v.), multicellular preparations of left atrium (LA) and PVs of left lung lobe were dissected. Tyrode superfused LA and PVs preparations were treated with potential sensitive dye di-4-ANEPPS (5 μM) in presence of 2,3-butanedione monoxime (1 g/l) for excitation mapping. Optical signals were captured with use of CCD camera (WuTech Instruments). CV estimation and isochronic maps reconstruction were performed with use of Cardioplex software (RedShirtImaging). Conduction of excitation in LA and PVs was estimated both in normal condition and after

uncoupling agent octanol (1,6 mM, up to 30 min) administration.

RESULTS. Rat LA and PVs demonstrate similar CV (63,3±4,5 (n=6) and 61,5±4,2 (n=8) cm/c, respectively). Octanol significantly suppress CV both in rat LA and PVs, but decreasing of CV in pulmonary veins after 6 min of octanol application was less prominent than in LA (40,8±4,7 and 28,4±2,9 cm/c, respectively, p(U) <0.05). Octanol was unable to induce single local conduction blocks in rat LA or PVs preparations. Prolonged administration of uncoupling agent finally leads to total suppression of LA and PVs conduction and excitability. Duration of period of excitability preserving during octanol perfusion was similar in LA and PVs (12,7±3,8 and 11,3±3,4 min, respectively).

CONCLUSIONS. Rat PVs probably less affected to octanol induced GJ uncoupling than atrial myocardium. Role of GJ uncoupling or intrinsic altered GJ in PVs conduction disturbances, particularly in rat, remains discuss

P4.44

Assessment of myocardial protection with new biochemical markers during on-pump coronary bypass surgery

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Objectives: Myocardial protection during on-pump coronary bypass surgery is the most significant issue which may cause mortality and morbidity in the early postoperative period. We tried to investigate this method with new biochemical markers that predict ischemia sensitively in the very early term.

Material and Methods: Thirty patients who need elective coronary artery bypass surgery according to AHA/ACC guidelines were taken to this study after taking the informed consent. After harvesting the left internal mammarian artery (IMA) and great saphenous vein standard cardiopulmonary bypass was initiated in all patients with standard cannulation. Antegrade and retrograde cannulas were both inserted during the cannulation. Blood samples were taken from the coronary sinus via retrograde cardioplegia cannula. Ischemia modified albümin, troponin I, creatin phosphokinase- MB (CK-MB), intracellular adhesion molecule-1 (ICAM-1), brain natriuretic protein (BNP), tumor necrosing factor alpha (TNF- α), and interleukin- 1 (IL-1) were investigated in all blood samples taken before and after the cardiopulmonary bypass. All patients had transthoracic echocardiography before and after the operation for ejection fraction (EF) comparison.

Results: There were 24 male and 6 female patients with a mean age of 62.27± 9.15 year. The post-cardiopulmonary bypass levels of TNF- α, IMA, CK-MB, and Troponin I levels were elevated when compared with the preoperative levels, however, these were statistically not significant (p >0.05). IL-1 levels were decreased in the post-cardiopulmonary bypass levels, however this was statistically not significant (p >0.05). Post-cardiopulmonary bypass ICAM-1 levels were significantly decreased (p <0.05). Echocardiography showed that ejection fractions were protected after cardiopulmonary bypass.

Conclusion: This study showed that antegrade-retrograde cardioplegia regimen during the cardiopulmonary bypass is still a safe method in the era of new, sensitive and early term biochemical ischemia markers. Moreover, echocardiography showed that the ejection fractions were also protected in the postoperative phase which is a clinical evidence of this study.

P4.45

The effect of curcumin on mechanical function and monophasic action potential in isolated rat hearts

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Curcumin is a natural phenolic compound which exhibits anti-oxidant, anti-inflammatory and anti-carcinogenic properties. It is possible that curcumin affects cardiovascular functions, but in isolated perfused rat hearts, little is known about the role of curcumin on mechanical function. Furthermore, the action of curcumin on action potential has not been investigated. Therefore, we studied the possible effects of curcumin on left ventricular developed pressure (LVDP; an index of cardiac contractility), maximal rate of pressure development (+dP/dtmax; another index of cardiac contractility), heart rate, left ventricular end-diastolic pressure (LVEDP), monophasic action potential (MAP) amplitude, MAP duration at 90% repolarization, maximum upstroke velocity (dMAPdtmax) and maximum downstroke velocity (dMAPdtmin).

The hearts were isolated under sodium thiopental (50 mg/kg) anesthesia and perfused with modified Krebs-Henseleit solution under constant pressure conditions. After stabilization, curcumin at doses 0.1, 1 and 10 μM was infused to the hearts for 30 min.

All doses of curcumin markedly decreased LVDP and +dP/dtmax. Curcumin at a dose of 10 μM also significantly decreased heart rate. Curcumin at doses of 1 and 10 μM markedly increased LVEDP. Furthermore, curcumin (1 and 10 μM) decreased MAP amplitude with a concomitant increase in MAP duration but curcumin at all doses had no effect on dMAPdtmax and dMAPdtmin

There are sufficient evidences from this study that curcumin possesses a negative inotropic action. Our results might suggest that curcumin at higher doses (≥1 μM) increases LVEDP with a negative chronotropic effect. The high doses of curcumin also may decrease MAP amplitude and may increase MAP duration.

P4.46

Estrogens prevent impairment of Ca2+-sequestration and efficiently improve ischemia tolerance of the diabetic heart

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The increased vulnerability of the cardiac tissue to the ischemic insult in chronic estrogen deficiency is incompletely understood. Our earlier studies showed that depressed Ca2+-sequestration developing in ovariectomized rats contribute substantially. The aim of the present study was to assess the alterations of cardiac Ca2+i-transients in estrogen deficiency coexisting with diabetes (DM) and delineate their influence on ischemia tolerance.

Adult female rats were either surgically castrated (OVX) or sham-operated. A subgroup of them received estrogen replacement therapy (ERT: 450 μg/bwkg estradiol propionate i.m. weekly) for 10 weeks. Diabetes (DM) was induced by a single injection of streptozotocin (70 mg/bwkg) 4 weeks after the operation. After the 10-week ERT treatment period hearts were isolated and mounted on a Langendorff-perfusion system where coronary flow, left ventricular pressure and Indo-1 surface fluorometry based Ca2+i-transients were recorded. Hearts were subjected to global ischemia (I) for 30 min, after which they were reperfused (R) for 30 min.

OVX did not influence the resting cardiac performance; however pump function was impaired by DM. The hearts of OVX, DM and OVX+DM groups exhibited slower Ca2+-removal from the cytosol, which was prevented by ERT in both the DM and non-DM OVX groups. The hemodynamic restitution of sham-operated DM hearts during reperfusion was similar to that of controls. However, OVX and OVX+DM hearts were characterized by marked ischemic contracture, weaker contractile performance, slower Ca2+-release and -removal during reperfusion as compared to control. ERT restored these parameters to control levels.

DM and OVX both individually impair myocardial Ca2+-transport. In DM this entails depressed resting cardiac pump function. OVX enhances the vulnerability of hearts to ischemia which can be prevented by estrogen replacement. Impaired cardiac function associates with decreased ischemia tolerance when estrogen deficiency and DM coexist. However, ERT successfully improves postischemic hemodynamic restitution in this condition as well, which is, at least in part, due to its protective effect on the Ca2+i-transients.

P4.47

Sphingomyelinase induced vasorelaxations in db/db mice depend on nitric oxide and hydrogen sulfide signaling

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Purpose: Sphingomyelinase (SMase) induces changes of vascular tone with an initial contraction and subsequent relaxation in aortic rings of wild type mice. Sphingolipid metabolites have been implicated as important regulators in diabetes. We aimed to investigate the vascular effects of SMase in a mouse model of diabetes and to elucidate the involved mediators and signal transduction pathways.

Methods: Thoracic aorta segments were isolated from adult db/db and non-diabetic littermate mice. The effect of 0.2U/ml SMase was investigated after precontraction with 0.1μM phenylephrine under isometric conditions in myographs. Vascular segments were also tested in the presence of the following inhibitors: L-NAME (100μM), SQ 29,548 (1μM), D-erythro-MAPP (50μM), Wortmannin (0.1μM), MK2206 (1μM) and propargylglycin (PAG, 10mM). Results are expressed as percentage changes compared to the tone of precontraction.

Results: SMase evoked an initial contraction in control vessels (13.8±6.3%) followed by relaxation to the original tone, while it caused a marked relaxation in the diabetic vessels (-21.9±7.1%). L-NAME administration further increased the contraction in the control (25.3±7.6%) and contraction occured in the diabetic vessels as well (36.8±3.1%). SQ 29,548 led to relaxation in both groups, but these were more pronounced in the db/db group (-25.1±6.3% vs. -63.1±14.9%). Co-administration of the inhibitors caused no change in vascular tone after SMase treatment. The use of D-erythro-MAPP, Wortmannin and MK2206 did not alter the vasoactive effects of SMase in any groups, but PAG decreased the SMase induced vasorelaxation (-22.0±4.9%, $p < 0.05$) in diabetic vessels.

Conclusion: SMase induces biphasic changes in the tone of db/db mice derived vessels and these effects are mediated by thromboxane A2 and endothelial nitric oxide. Importantly, the relaxations are enhanced in diabetic vessels, which is a surprising and novel phenomenon and it could be blocked by PAG. This leads to decreased hydrogen sulfide levels generating higher levels of cGMP, thus relaxation. Our results indicate a novel possibility to enhance vasorelaxation in diabetes by SMase via hydrogen sulfide signaling.

P5

Respiratory Physiology

P5.1

Physiological, pulmonary and immunological effects of negatively charged Waterfall-Nanoaerosol

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Traditionally, numerous beneficial health effects have been attributed to waterfalls in the alpine regions of Austria. They produce a charged nano aerosol which is formed within micro seconds after the ionization of primary ions, due to hydration and cluster ion formation processes. Waterfalls mainly produce negatively charged ions, referred to as Lenard ions. Due to the waterfall wind, the negatively charged particles, atomized by thermophoretic processes, drift away from the waterfall, whereas the positively charged droplets quickly sink to the ground. This causes surplus of negatively charged air ions in the proximity of the waterfalls, which can be of the order of several 10.000 ions/cm3 air. We have conducted research on the physicochemical properties of waterfall aerosol and their effects on human physiology, allergic asthma and stress/burnout prevention in a series of randomized controlled clinical trials: In comparison, to a control location in the open countryside, exposition to a waterfall creates a parasympathetic tonus: The heart rate slows down and the better synchronization of abdominal and thoracic breathing as well as deeper inhalation improves blood circulation in the lungs. Overall, the transport of oxygen in the blood is facilitated, increasing oxygen saturation. The nitric oxide exhaled (FeNO) is specifically reduced at waterfalls that produce very small and transpirable nanoaerosols (peak at 5,5nm). In belong of asthma bronchiale, waterfall induced immune modulation is characterized by a change in the ratio of allergic/anti-allergic biochemical messengers, the induction of anti-inflammatory messengers as well as the production of anti-allergic regulatory T-cells. Exposure to a waterfall improves pulmonary function by 30% with a measured effective sustainability of 2 month and an alleviation of asthmatic symptoms up to 4 month compared to a control group. Waterfalls enhance the mucociliary clearance, boost the immune response against and oral vaccine and reduce physiological and psychological stress by acting on the hypothalamic-pituary-adrenal axis and their succeeding endocrine and immune pathways.

P5.2

The endocannabinoid system of human bronchial epithelium

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The endocannabinoid system (ECS) derives its name from the Cannabis sativa plant, since the first receptors of the ECS were originally discovered when research focused on the distinct natural compounds responsible for the effects attributed to the plant. Since the identification of CB1, the first receptor to specifically bind cannabinoids, research in the past several decades has greatly expanded the ECS. As such, the ECS is now considered to be a novel signaling network encompassing a wide range of receptors, endogenous lipid mediators and the enzymatic apparatus required for the synthesis and degradation of said molecules. Recent research has also highlighted the important regulatory function of endogenous cannabinoids, namely anandamide and 2-arachidonoyl glycerol (AEA and 2-AG), in non-neuronal tissues, however there is scant evidence pertaining to lung tissue about their effect and possible local production/degradation.
The aim of our current study was the investigation of the expression of the members of the ECS on human bronchial epithelium.
Using immunohistochemistry we investigated the expression of classical and novel metabotropic cannabinoid receptors, as well as the expression of synthesizing and degrading enzymes.
Our results show that CB2 as well as the "novel" receptors GPR55, GPR18 and GPR119 are expressed on the bronchial epithelium. Interestingly, CB2 was found to localize along the ciliary border, while the other three receptors showed a more diffuse staining pattern. CB1 positivity was not detected in the bronchial epithelium.
Of DAGLα and DAGLβ, which produce 2-AG, only α shows a strong expression in the epithelial cells, while β is weakly expressed. NAPE-PLD, the synthesizing enzyme for AEA, is expressed mainly in the basal epithelial cells. FAAH, which degrades AEA, is expressed in a similar fashion. MAGL, which degrades 2-AG, on the other hand is expressed most strongly on the apical portion of the cells.
These results provide an interesting glimpse into the possible role of the ECS in human bronchial epithelium.

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P5.3

Anti-inflammatory effect of apelin/ APJ receptor system on ovalbumin induced allergic lung disease

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Introduction: Published data sustain the regulator effect of apelin (AP) on immune response.

Objective: To validate the effect of apelin/ APJ receptor system on inflammatory response in pulmonary allergic disease.
Methods: Ovalbumin (OVA)-sensitized rats were used. The OVA-induced increase of specific airway resistance (sRaw) and accumulation of inflammatory cells on bronchoalveolar lavage fluid (BALF) were assessed from rats treated with vehicle, apelin 13 (AP13) or apelin antagonist F13A by intratracheal instillation before OVA challenge.
Results: AP13 administration reduced the increase of sRaw by more than half as compared with vehicle. F13A significantly increase the total number of cells on BALF as compared with vehicle and amplified the sRaw augmentation as compared with AP13 treated rats.
Conclusion: These results sustain the involvement of apelin/ APJ receptor system in allergic pulmonary inflammation and could provide an interesting concept for developing suitable therapeutic approaches.

Key word: apelin, lung, allergic inflammation, rats

P5.4
Effect of radical stress on NO production in rats exposed to chronic hypoxia

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At the onset of exposure to chronic hypoxia rats have increased NO concentration in expired air and signs of lung tissue oxidant stress. Oxidant stress induces NO production and NO interacts with superoxide yielding peroxynitrite. In addition, whether NO synthase produces NO or oxygen radicals depends on its dimerization state which is redox dependent. This all has pulmonary vascular effects and may participate in the mechanism of hypoxic pulmonary hypertension (1).
In the present study we focused on the effect of oxygen radicals (ROS) on expired NO, plasma nitrites and nitrates (NOx) and nitrotyrosine (peroxynitrite marker) concentrations in adult Wistar rats. Periods of 0, 1, 4 and 21 days of hypoxia (FiO2 =0.1) were studied. Nitric oxide and NOx were measured by chemiluminiscence, nitrotyrosine by ELISA. Two groups of hypoxic rats were used. The first one, experimental (n=24), was given ROS inhibitor N-acetylcystein (NAC) in drinking water (20g/l). The second group, control (n=25), was given just tap water.
Hypoxia increased the exhaled NO levels, with the highest values detected on the 4th day of hypoxia (14.5±1 vs 5.6±0.4 ppb in normoxia, $P < 0.05$). The hypoxia-induced elevation of NO concentration in exhaled air was significantly inhibited by NAC (8.1±0.2 ppb on day 4, $P < 0.001$). A similar pattern of changes was observed in plasma concentration of NOx (with the exception that it was already high after 1 day of hypoxia).
These findings imply that elevated ROS are needed for increased NO production during the first days of chronic hypoxia. NO synthase uncoupling and lower NO conversion to peroxynitrite also may play a role.

1. Hampl V, Herget J Physiol Rev 2000;80:1337-1384.

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P5.
N-acetylcysteine effectively diminished meconium-induced oxidative stress

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Since inflammation and oxidative stress play a key role in the pathophysiology of neonatal meconium aspiration syndrome, various anti-inflammatory drugs have been used in the therapy.
This study evaluated therapeutic potential of antioxidant N-acetylcysteine in modulation of meconium-induced inflammation and oxidative lung injury. Oxygen-ventilated rabbits were intratracheally given 4 ml/kg of meconium (25 mg/ml) or saline (Sal). Thirty minutes later, meconium-instilled animals were treated by intravenous N-acetylcysteine (10 mg/kg, Mec+NAC) or were left without treatment (Mec). All animals were oxygen-ventilated for additional 5 h. Total and differential leukocyte counts in the blood were determined at baseline, and at 1, 3 and 5 h after the treatment. After sacrificing the animals, left lung was saline-lavaged and total and differential leukocyte counts in the bronchoalveolar lavage fluid were determined. Strips of the right lung were used for biochemical analyses and for estimation of the wet-dry ratio. In the lung tissue homogenate, thiobarbituric acid-reactive substances (TBARS), dityrosine, lysine-lipid peroxidation (LPO) products, and total antioxidant status (TAS) were determined. In isolated lung mitochondria, TBARS, dityrosine, lysine-LPO products, thiol group content, conjugated dienes, and activity of cytochrome c oxidase were estimated. To evaluate effects of meconium instillation and NAC treatment on the systemic level, TBARS and TAS were determined also in the blood plasma. Participation of eosinophils in the meconium-induced inflammation was detected by eosinophil cationic protein (ECP) in the plasma and lung homogenate. Meconium instillation increased oxidation markers and ECP in the lung and decreased TAS. NAC treatment reduced ECP and most of oxidation markers in the lung and prevented a decrease in TAS in the lung homogenate compared to Mec group. In the plasma, NAC decreased TBARS and ECP, and increased TAS compared to Mec group.
Concluding, N-acetylcysteine diminished meconium-induced polymorphonuclear activation and oxidative lung injury.

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P5.6

Administration of exogenous surfactant: Global and regional lung functional changes in a rabbit model of surfactant deficiency

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Background: Administration of exogenous surfactant is recognized as an effective therapy for respiratory distress involving surfactant deficiency. We aimed at characterizing the short-term effects of exogenous surfactant instillation on the respiratory mechanics and on the distribution of regional lung ventilation by using functional imaging technique in an animal model of respiratory distress.

Methods: Regional specific ventilation (sV') was measured using K-edge subtraction synchrotron CT imaging during xenon washin in anaesthetized, mechanically ventilated rabbits (n=8). Based on regional density, lung regions were classified as poorly-aerated, normally aerated or hyperinflated. A functional category was defined within each class based on the distribution of sV' in normal conditions. Airway resistance (Raw), respiratory tissue damping (G) and elastance (H) were measured by forced oscillations to assess global respiratory mechanics. Measurements were made before and after surfactant depletion by whole lung lavage, immediately and 30 min after intratracheal instillation of exogenous surfactant (beractant, 70 mg/kg).

Results: Following lavage, surfactant instillation improved Raw (-29±21%), G (-12.8±7.6%) and H (-23.2±11.6%; $p < 0.05$ for all, respectively). Increases in the poorly aerated regions with high sV' were also observed (from 20.9±11.8% to 34.5±19.1%, $p<0.001$), which was partly due to a reduction in the atelectatic lung areas ($p < 0.001$), but can also be attributed to the decreased aeration in previously normally-aerated regions with high sV' ($p=0.013$).

Conclusions: These findings suggest that surfactant treatment improves central airway and tissue mechanics in this model of surfactant deficiency. However, surfactant therapy may also deteriorate the regional lung function when local aeration was normal prior to its administration. The significance of these findings stems from the perception that improvement of the global respiratory mechanics following surfactant administration may be associated with regional worsening of respiratory distress via local mechanical and functional heterogeneities.

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P5.7

Questions in differential diagnosis of bronchial asthma, chronic obstructive pulmonary disease and overlap syndrome

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The overlap syndrome (OLS) shares common features with bronchial asthma (BA) and chronic obstructive pulmonary disease (COPD), inducing considerable challenge for differential diagnosis that is mandatory for treatment. In the present study we attempted to delineate the three entities based on the results of examinations. After informed BA, COPD or OLS outpatient were recruited at the Department of Pulmonology between August 15 2012 and October 15 2013. Routine laboratory tests and whole body plethysmography were made. The smoking habits were quantified as boxes smoked/year. The quality of life (QoL) was measured with the St. George Respiratory Questionnaire. Overall 167, 74 and 20 AB, COPD and OLS patients were included, respectively (134 male, 127 female, mean age: 52.12±15.10 years). The mean age of the OLS patients was significantly higher than that of the BA-patients (46.64±14.90 AB vs. 60.80±8.65 OLS $p < 0.001$). Significant differences were found in smoking habits (12.81±11.76 OLS vs. 3.74±8.38 AB $p < 0.001$ and 12.81±11.76 OLS vs. 22.65±2.46 COPD $p=0.047$). Comparison of OLS and AB groups showed differences in CRP (2.83±3.56 AB vs. 5.41±6.73 $p=0.009$), procalcitonin (0.1±0.046 OLS vs. 0.0007±0.1 AB $p=0.021$), fibrinogen (3.91±1.15 vs. 3.33±0.64 $p < 0.001$). Plethysmography results showed significant differences between the OLS and AB patients concerning FVC, FVC%, FEV1, FEV1%, FEV1/FVC, FEV1%/FVC%, FEF25-75%, IVC, IVC %, RV%. Significant differences were also for QoL indices. No significant differences were found however in plethysmography and quality of life parameters of the OLS and COPD groups. In conclusion, BA and OLS may be easily differentiated while distinction from COPD needs the identification of further parameters.

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P5.8

Respiratory effects of acute blood loss and subsequent fluid resuscitation with colloid or crystalloid solutions

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Introduction: Acute haemorrhagic shock and consecutive replacement with autologous blood affect lung mechanics (Bayat, S. et al. J Appl Physiol 2011; 111:458-64). However, blood is not the only choice for fluid replacement of acute haemorrhage. We aimed at comparing the respiratory mechanical changes following fluid resuscitation with autologous blood (n=8, Group B), colloid (HES 6% 130/0.4, n=8, Group CO) or crystalloid solution (0.9% NaCl, n=9, Group CR) after acute haemorrhage.
Methods: Anaesthetised, ventilated rats were used in the present study. With targeting a loss of 5% of total blood volume in each manoeuvres, the rats were bled in 6 sequential steps, which was then replaced stepwise by equal amount of autologous blood, colloid or crystalloid solution. Following each step, airway resistance (Raw), tissue damping (G) and elastance (H) were determined by forced oscillations. The extent of lung oedema was assessed from lung histology by measuring the size of perivascular oedematous area.
Results: Decreased Raw was observed in all groups following blood loss (-20.3±1.9[SE]% vs. control, $p < 0.05$), which was normalized only by replacement with colloid (5.5±3.8% vs. control, NS), but was not affected by blood (-21.7±2.9% vs. control, $p < 0.05$). Crystalloid had an intermediate effect on reversal (-8.4±4.9%, vs. control, NS). G and H exhibited increments following both blood loss and replacement, with H being significantly higher in groups CO (37±6.6%, vs. control, $p < 0.05$) and CR (40±4.4%, vs. control, $p < 0.05$) than in Group B (23±3.5%, vs. control, $p < 0.001$). Histological analysis revealed greater extent of perivascular oedema in groups CR and CO than in group B ($p < 0.05$).
Conclusion: We conclude that airway properties are normalized following fluid resuscitation with colloid solution, while a persistent airway dilation remains after blood replacement with crystalloid. Conversely, a deterioration of respiratory tissue mechanics is present following both colloid and crystalloid fluid replacement strategies due to the development of perivascular oedema.

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P5.9

The involvement of local renin angiotensin system in obesity augmentation of pulmonary allergic disease

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Objective: Published data sustain the obesity as a risk factor not only for cardiovascular diseases and metabolic disorders but also for lung diseases. This study aims to investigate the interaction between obesity and local (pulmonary) RAS during ovalbumin – induced allergic airway disease.
Methods: Obese resistant (OR) and obese prone (OP) rats were feed with moderately high-fat diet (4.41 kcal/gm; 31.8% fat kcal) and sensitized to ovalbumin. Both, OR and OP rats were divided in losartan (LOS) treated (by intratracheal instillation) or vehicle treated rats. The specific airway resistance (sRaw) increase in response to acetylcholine and bronchoalveolar lavage fluid (BALF) cellularity of LOS treated and vehicle treated rats were comparatively evaluated.
Results: After sensitization and challenge, the airway reactivity to ACh was increased on both OP and OR rats by at least 1.25 times. The sRaw increase by acetylcholine was 121.15% higher on OP vs OR rats. LOS instillation decreases the sRaw in response to acetylcholine on OP rat by 12% and erases the significance of OP vs. OR rats sRaw variation difference. Evaluation of BALF number revealed a decrease in total cellularity as result of intratracheal LOS administration only on OR rats but a decrease of neutrophils on both OP and OR rats.
Conclusion: In our experimental model, the obesity augmentation of OVA sensitization induced airway hyperreactivity indicated by sRaw variation is mediated at least partially by activation of pulmonary (local) RAS. Even more, our data indicated that blocking of local RAS could have an inhibitory effect on pulmonary allergic inflammation progression.

P5.10

Predictive mouse model of chronic cigarette smoke-induced pulmonary and cardiac pathophysiological alterations

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Chronic cigarette smoke exposure leads to lung inflammation, structural damage and pathophysiological alterations in the vasculature and cardiac muscle. The complexity of these mechanisms are still unclear due to the lack of predictive animal models. We describe and characterize a chronic obstructive pulmonary disease (COPD) model in a longitudinal follow-up study using integrative functional, in vivo imaging and histopathological approaches. Two-month old male C57BL/6 mice were exposed to whole body cigarette smoke for 6 months (2/day, 5 days/week; Kentucky research cigarette 3R4F), mice without smoke exposure served as controls. Functional respiratory parameters were investigated by whole body plethysmography, cardiac functions with echocardiography before and after every month. MicroCT images were acquired monthly and histopathological evaluation of the lung was performed. Respiratory frequency and peak expiratory flow significantly decreased from the first month, but airway hyper-responsiveness did not develop. On echocardiography, deceleration time significantly diminished already from the second month, ejection fraction and tricuspidal annular plane systolic excursion decreased from the fourth month, indicating diastolic and systolic dysfunction of both ventricle. Aerated lung volume and structure thickness,

as markers of emphysema gradually increased reaching significant differences in the second and fourth months. In the first month perivascular/peribronchial edema, minimal peripheral atelectasis and neutrophil/macrophage infiltration were detected, in the second one accumulation of all inflammatory cells, from the third one increasing atelectasis and emphysema, irregular bronchiolar mucosa and increased mucus production were observed. Long-term cigarette smoke-induced characteristic functional and morphological changes in the mouse lung and heart are duration-dependent and similar to the human conditions. Therefore, this is a good predictive model for translational research to analyze the pathophysiological mechanisms (cytokines, enzymes, receptors), identify key mediators and potential new drug targets in COPD.

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P5.11

Cigarette smoke-induced upregulation of the Transient Receptor Potential Ankyrin 1 ion channel in the mouse lung and in a human pulmonary tissue 3-dimensional model

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The Transient Receptor Potential Ankyrin 1 (TRPA1) ion channel is localized on capsaicin-sensitive peptidergic sensory nerves and several non-neural cells, e. g. fibroblasts, epithelial and smooth muscle cells in the lung, but functional data are contradictory. TRPA1 is activated by chemical irritants, including unsaturated aldehydes and nicotine in cigarette smoke which implicates a role in the pathogenesis of airway diseases, such as chronic obstructive pulmonary disease (COPD). We investigated the expression and cigarette smoke-induced alterations of TRPA1 in the mouse lung and human pulmonary cell culture.
Chronic bronchitis was elicited by 2x1 hours whole-body cigarette smoke exposure daily for 3 months in C57Bl/6 mice. The whole lungs were examined and epithelial cells, fibroblasts and leukocytes were isolated using Fluorescence-Activated Cell Sorting (FACS) for TRPA1 mRNA detection with qPCR. 3-dimensional (3D) human tissue models containing fibroblasts, epithelial cells and macrophages were exposed to cigarette smoke for 15 minutes, 1 day and 4 days. Lung sections stained with hematoxylin-eosin. The number of inflammatory cells was assessed with flow cytometry in the bronchoalveolar lavage fluid (BALF). TRPA1 was expressed in the whole mouse lung and upregulated after a 2-month smoke exposure.
On the histological slides remarkable lymphocyte and macrophage accumulation occured by this time and the inflammation became chronic, as confirmed by BALF analysis. Leukocytes and epithelial cells expressed TRPA1 channel mRNA. TRPA1 expression minimally increased in the epithelial cell- and fibroblast-containinig human lung model after 4 days, but significantly elevated in the macrophage-containing cultures. Upregulation of TRPA1 gene expression in leukocytes and lung tissue suggests its activation by cigarette smoke and a possible function in chronic bronchitis. Corresponding results in human 3D tissue model imply translational relevance of our murine findings.

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P5.12

Impact of altered ventilation pattern on capnography phase III slope in patients undergoing elective heart surgery

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Rationale: While some degree of ventilation and perfusion heterogeneities exists even under physiological conditions in the lungs, this further deteriorated during cardiac surgeries due to the prolonged supine position, compromised left ventricular function, mucus secretion in the airways and the intermittent positive pressure ventilation. The changes of the capnography phase III slope (SIII) reflect alterations in the ventilation and perfusion heterogeneities. However, the SIII is currently assessed by anesthesiologist only on subjective bases. We developed a software with the aim of characterizing quantitatively how the alterations in the ratio of inspiration to expiration (I:E) affect SIII values and whether this change can be attributed to altered alveolar heterogeneities.
Methods: Mainstream capnogram measurements were performed with open and closed chest conditions in adult, anaesthetized and mechanically ventilated patients undergoing heart surgeries (n=38) by randomly changing the I:E ratio (1:2-1:1 and 1:3-1:2) within a recording. The SIII was calculated by the slope of regression line applied to the last 60% of III phase of time-based capnography curve.
Results: When the chest was intact, SIII increased from 0.74±0.094[SE] Hgmm/s to 0.80± 0.1 Hgmm/s ($p=0.02$), and from 0.75±0.12 Hgmm/s to 0.86±0.12 Hgmm/s ($p <0.001$) by changing the I:E ratio from 1:3 to 1:2 and from 1:2 to 1:1, respectively. Changes in ventilation pattern had no significant effect under open chest conditions.
Conclusions: Under closed chest conditions, real differences in SIII were found by changing the ventilation pattern. The decreases in SIII with increasing inspiratory time reflect reopening of atelectatic alveoli at the end of the longer inspiration, and/or improving the compromised ventilation perfusion mismatch in the dependent lung regions. These processes cannot be detected when the chest is open, because the elevated functional residual capacity may not allow manifesting the effect of longer inspiratory period.

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P5.13
MDR1 C3435T allele and genotype frequency in chronic obstructive pulmonary disease

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Background: P-glycoprotein (P-gp/MDR1), a member of the ATP-binding cassette (ABC) transporters super family, encoded by the ABCB1/MDR1 gene, is one of suggested respiratory tract protection components. Presence of MDR1 polymorphisms and altered P-gp expression may be important for pathogenesis of reduced lung inflammatory response on cigarette smoke exposure, as well as for the severity of chronic obstructive pulmonary disease (COPD) and lung cancer pathogenesis and treatment efficacy.
Objectives: To investigate the allele and genotype frequency of MDR1 C3435CT genetic polymorphism in patients with COPD, in order to get better insight into pathogenesis of this important disease.
Patients and methods: 38 patients (27 males, 11 females) with COPD and eighty healthy control subjects (66 males, 14 females), originating from South-East Serbia, were genotyped for MDR1 C3435T genetic polymorphism. For determination of MDR1 C3435T genotype, a multiplex mutagenically separated PCR was performed. Results were compared between clinical and control group and statistical significance was tested.
Results: Genotype frequency of MDR1 C3435T in COPD patients was 0.34, 0.54 and 0.13 for CC, CT and TT respectively, compared to 0.17 for CC, 0.64 for CT and 0.19 for TT in the control group.
Discussion and conclusions: Although this was only a pilot study with small number of patients, a statistically significant difference was found between COPD patients and control group considering genotype frequency of MDR1 C3435T genetic polymorphism. Patients with COPD had higher frequency of CC genotype and C allele when compared to the control group, which is rather contrasting to the previous literature data of other authors. Further research with bigger group of COPD patients is essential in order to elucidate the role of MDR1 3435 polymorphisms in pathogenesis of COPD.

Key words: MDR1 C3435T, COPD, respiratory diseases, P-glycoprotein

P5.14
Lipopolysaccharide-induced fever elicits changes in lung surfactant proteins

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The aim of the study was to prove the hypothesis that fever induced by lipopolysaccharide (LPS) elicits the changes in lung surfactant proteins, potentially related to thermal tachypnea. Fever was induced by intraperitoneal administration of LPS at a dose 100 μg/kg to adult rats; controls received saline. Respiratory parameters, arterial blood gases and pH and colonic body temperature (BT) were recording during 5 hours. After sacrifice the animals, surfactant proteins (SP) A, B, C and D were evaluated in lung tissue homogenate (LT) and bronchoalveolar lavage fluid (BALF).
Monophasic thermic response (at 300 min 38.7±0.2 vs. 36.4±0.3 °C, $P < 0.05$) and an increase in minute ventilation due to changes in breathing rate and tidal volume were observed after LPS administration. Animals with fever had higher levels of SP-A and SP-D in LT ($P < 0.05$ and 0.01), and higher SP-D in BALF ($P < 0.01$) than controls. SP-B increased in LT and SP-C in BALF of animals with LPS (both $P < 0.05$ vs. controls).
LPS-induced fever evokes changes in all surfactant specific proteins. Alterations of proteins related to lung defense (SP-A, SP-D) might be a part of general inflammatory response to pyrogen. Changes in proteins related to surface properties (SP-B and SP-C) probably reflect the need to stabilize the lungs in thermal challenge.

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P5.15
Meconium-induced oxidative damage and surfactant/budesonide therapy in experimental meconium aspiration syndrome

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Objectives: Meconium aspiration syndrome (MAS) is a serious life-threatening condition in the neonates. MAS therapy is based on the support ventilation and administration of exogenous surfactant into the lungs. However, aspirated meconium initiates local inflammation followed by lung edema, increased expression of inflammatory markers and huge oxidation damage of lung tissue mediated by activated neutrophils. These processes may inactivate the surfactant and reduce the effect of therapy. Thus, adding of anti-inflammatory agent budesonide into the surfactant therapy should suppress the development of inflammation and oxidative damage and contribute to better efficiency of the therapy in experimental MAS.
Methods: New Zealand rabbits with meconium-induced respiratory failure were divided into groups according to the therapy (n=6 each): without therapy (Mec), with surfactant therapy (Mec+Surf), with budesonide therapy (Mec+Bud), and with combined therapy (Mec+Surf+Bud), or were given saline instead of meconium (Control). After sacrificing the animals, lung edema (lung wet/dry weight ratio, W/D), and oxidative damage (TBARS for lipid oxidation, 3-nitrotyrosine for protein oxidation, dityrosine and lysine-lipoperoxidation products for fluorescence detection of protein and lipid oxidation) in lung homogenate were determined.
Results: Combination Surf+Bud reduced W/D ratio and TBARS ($p < 0.05$ vs. Mec, Mec+Bud, Mec+Surf), reduction in 3NT was not significant. Dityrosine formation decreased in both treatment groups compared to Mec, but significantly only

for Mec+Surf+Bud group. Lysine-LPO fluorescence decreased in both Mec+Surf and Mec+Surf+Bud vs. Mec and in Mec+Surf vs. Mec+Surf+Bud ($p < 0.05$).

Conclusion: Adding budesonide to surfactant therapy showed superior effects on lung edema and oxidative damage in the lung homogenate and plasma compared to given monotherapies. Thus, the use of anti-inflammatory therapy may secondarily reduce surfactant inactivation and contribute to enhanced therapy effectiveness.

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P5.16

Changes of pro-inflammatory and apoptotic markers in an experimental model of acute lung injury

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Model of acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) induced by saline lavage is characterized by lung edema, inflammation and dysfunction of pulmonary surfactant. Aim of this study was to detect a supposed relationship between ALI/ARDS-induced inflammation and apoptosis in the lungs.

In one group of New Zealand rabbits, lungs were repetitively lavaged with saline (30 ml/kg) to induce ALI/ARDS. Then, animals were oxygen-ventilated for additional 5 hours. Other group of rabbits served as healthy non-ventilated controls. After sacrificing the animals, total and differential numbers of leukocytes in blood, and total and differential numbers of cells, and viability of cells in bronchoalveolar lavage fluid (BAL) were measured. Lung edema was expressed as wet/dry weight ratio and apoptosis of lung cells was estimated by TUNEL method. Concentrations of IL-1ß, IL-6, IL-8, TNF-α, caspase-3, endogenous secretory receptor for advanced glycation end-products (esRAGE), and sphingosine-1-phosphate receptor 3 (S1PR3) in the plasma and tissue homogenates were determined by ELISA.

In ALI/ARDS group, increased total number of cells, mainly neutrophils, in the BAL fluid was observed. Their activation resulted in increased lung edema formation and apoptosis of cells in the lung tissue and BAL fluid. In ALI/ARDS group, significantly higher concentrations of IL-1ß and IL-8 were found in plasma and higher levels of IL-1ß and esRAGE were detected also in the lung tissue homogenates compared to healthy controls. Increases in other pro-inflammatory markers were, however, not significant. Concluding, inflammation in ALI/ARDS is associated with apoptosis of the lung cells.

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P5.17

Gene polymorphisms of surfactant protein B are associated with respiratory distress in neonates

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Pulmonary surfactant is a surface active substance that reduces the surface tension at the air-liquid interface and thus prevents the collapse of the airspaces at the end-expiration. It consists mainly of phospholipids and four specific proteins SP-A, B, C and D which are essential for the proper surfactant function. Genetic disorders of genes encoding SP-B, SP-C and transport protein ABCA3 are rare but if present they are associated with respiratory distress in the neonatal period or later in life. We present the case reports of three premature neonates with adequate postnatal adaptation. Within a few days after birth they developed tachypnea and required oxygen. All clinical and laboratory tests were negative. Blood sample was analysed for possible genetic disorders of pulmonary surfactant. The most common sites for the occurrence of mutations of surfactant proteins genes were analysed by direct DNA sequencing - exon 3 for SFTPC gene, exon 9 for ABCA3 gene and all exons encoding the gene SFTPB. The direct causal mutation was not detected. In all three cases in SFTBP gene heterozygous functional SNPs - rs1130866, and in one case homozygous rs2077079 were found.

Conclusion: Detected functional polymorphisms are related to the development of otherwise unexplained tachypnea and respiratory problems in neonates. Molecular diagnostics allows accurate detection of genetic disorders of surfactant proteins responsible for the development of neonatal respiratory distress and offers the possibility to determine the correct patient prognosis and risk for future pregnancies.

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Key words: surfactant proteins, preterm newborn, molecular analyses, DNA sequenation

P6

Gastrointestinal Physiology

P6.1

The possible role of Apelin on formation and healing mechanisms of ischemia reperfusion (I/R) induced mucosal lesions in rats

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The aim of the study was to determine the possible role of apelin on formation and healing mechanisms of ischemia reperfusion (I/R) induced mucosal lesions. For this purpose, 1.Control group, 2.Sham group, 3.I/R groups (the animals were sacrificed at five different time points following the I/R. 3a. immediately, 3b. 24 hrs later, 3c. 72 hrs later, 3d. 120 hrs later, 3e. 240 hrs later I/R.) 4. F13A (150µg/kg/day) + I/R group, 5. I/R + F13A groups (the animals were exposed to the I/R and taken apelin antagonist then were sacrificed at four different time points following the I/R. 5a. 24 hrs later, 5b. 72 hrs later, 5c. 120 hrs later, 5d. 240 hrs later.). Gastric mucosal blood flow, lesion index, MPO, 4-HNE-MDA, NOx, PGE2, TNF-α, HO activity apelin, VEGF, HO-1 protein expressions and immunoreactivities of Nrf2, HIF-1α and VEGF were measured. Mucosal lesion formation was observed in animals, which are exposed to I/R. It was observed that F13A application before I/R increased mucosal injury. The application of F13A following I/R caused a delay in healing process. Mucosal blood flow decreased in I/R groups, and reached into control levels in healing groups but lower blood flow was observed in all F13A applicated groups. Increased gastric MPO actvity due to I/R was greater in 24 hours later I/R and started to decrease in 72 hours later I/R. High MPO activity was determined in healing process of F13A applicated I/R groups. I/R application caused to increase in 4HNE-MDA levels but 4-HNE-MDA levels reached into control leves in healing groups. It was observed that decreasing PGE2 levels due to I/R, reached into control levels in healing groups but this increase delayed in F13A applicated healing groups. Higher TNF-α levels were determined in F13A applicated healing groups compared to non F13A applicated groups. I/R application caused to increase in Apelin protein expression. VEGF protein expression did not changed due to I/R but an increase was detected at 120th and 240th hours following I/R. F13A depressed the increase of VEGF expression in healing groups.
In conclusion Apelin has a preventing effect on mucosal lession formation and accelerating effect on healing process.

P6.2

Effect of chronic systemic Nesfatin-1 treatment in intestinal ischemia/reperfusion

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Introduction: Intestinal ischemia-reperfusion (I/R) injury causes local production of reactive oxygen species, which is known to play an important role in gut epithelial damage. It is highly associated with high morbidity and mortality rates. Nesfatin-1 is a peptide secreted by peripheral tissues and nervous system, involved in the regulation of homeostasis. It is also described as an anti ischemic agent. We aimed to evaluate the effects of nesfatin-1 on intestinal I/R injury in rats.
Material and Method: In this study, 28 two-months-old, Wistar albino male rats were used. The rats were randomly divided into 4 experimental groups (in each group n=7): Sham rats were underwent laparotomy (L); rats were underwent occlusion of superior mesenteric artery for 30 min followed by 2h reperfusion (I/R); rats were treated with nesfatin-1 intraperitoneally (i.p.) (0,25 nmol/gr) for 10 consecutive days and underwent laparotomy (N+L); rats were treated with nesfatin-1 intraperitoneally (i.p.) (0,25 nmol/gr) for 10 consecutive days and underwent occlusion of superior mesenteric artery for 30 min followed by 2h reperfusion (N+I/R). Serum levels of TAS-TOS were determined by colorimetric measurement method. Plasma concentrations of endotelin-1 and eNOS were analyzed by rat ELISA assay kits.
Results: Plasma level of endotelin-1 in N+L and N+I/R groups were significantly higher compared to L and I/R groups and plasma level of eNOS in N+L and N+I/R groups were significantly lower compared to L and I/R group, respectively. The decrease of serum TAS level in N+I/R group was statistically significant compared to L group. Serum level of total oxidant status (TOS), although not statistically significant, was found to be lower in N+I/R group compared to I/R group. Increase of OSI level of N+L was statistically significant compared to L group.
Discussion: Nesfatin-1 can compensate withered microvascular response at the acute phase of I/R injury by inhibiting eNOS and activating endotelin-1. This can also lead to the inhibition of oxidant parameters independently than increase in antioxidant capacity. This effect of nesfatin-1 seems to be more specific for I/R conditions.

P6.3

Effect of chronic systemic ozone treatment on endogen level of Nesfatin-1 in intestinal ischemia-reperfusion created rat

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Introduction: Tissue damage due to ischemia is increased after reperfusion. One reason for reperfusion injury is induced microvascular dysfunction, which is related with disrupted endothelium-dependent dilation in arterioles and enhanced fluid filtration in capillaries, and this causes plasma protein

extravasation in vennules. Ozone therapy accepted as a simple and harmless method that provides a new tool to protect organs from I/R injury. Nesfatin-1 is an anorexic peptide and its protective effect against I/R injury has been implicated too. We aimed to identify chronic systemic medical ozone application on endogenous plasma level of nesfatin-1 in intestinal I/R created rats.

Material and Method: In this study, 28 two-months-old, Wistar albino male rats were used and randomly divided into 4 experimental groups (in each group n=7): Rats were underwent laparotomy (L); rats underwent occlusion of superior mesenteric artery for 30 min followed by 2h reperfusion (I/R); rats were treated ozone/oxygen mixture intraperitoneally (i.p.) (1,1 mg/kg) for 10 consecutive days and underwent laparotomy (O3+L); rats were treated ozone/oxygen as in O3+L and underwent I/R (O3+I/R). Plasma concentration of nesfatin-1 was analyzed by rat ELISA assay kits.

Results: We have observed statistically significant difference for serum level of nesfatin-1 between all groups; L (5,9343± 0,71697 pg/ml), I/R (9,6957±2,03122 pg/ml), O3+L (9,8471 ± 1,11653 pg/ml) and O3+I/R (34,3743 ± 5,54082 pg/ml) groups. Plasma level of nesfatin-1 in O3+L groups was significantly higher compared to L group. Plasma level of nesfatin-1 in O3+I/R groups was significantly higher compared to I/R group.

Discussion: Chronic systemic ozone application increases endogen nesfatin-1 levels in both laparotomy and I/R groups. Ozone may induce systemic protective processes through nesfatin-1 induction. These results can be one the indication for the crosstalk between the protective effects of ozone and nesfatin-1 in I/R injury. We believe that further analyses are necessary to clarify effects of ozone on other systemic responses during different conditions to be sure for mechanism of its action.

P6.4

The dynamic of non-invasive predictive markers for incipient experimental liver fibrosis

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Liver fibrosis is a reversible condition which can be observed in most of the chronic liver injuries. There is a close relation between the severity of the injuries, hepatocytes necrosis and the observed inflammation and fibrosis. In order to assess the fibrosis, usually invasive methods are used. For this reason, there is a search for non-invasive methods which allow a quick and accurate diagnosis and prediction of the progression of the fibrosis.

The aim of the study was to identify some correlations between invasive markers of the liver fibrosis and blood serum, non-invasive markers which might have a predictive value. Forty male Wistar rats have been used. The liver fibrosis was induced using CCl4. All the animals received 1.2 ml/kg CCl4 25% in sunflower oil (intraperitoneal injection), twice a week. Randomly, 10 rats were sacrificed at the beginning of the experiment, then 10 rats after 2, 4 and 6 weeks. Blood samples and liver tissue samples were taken. Malondialdehyde, carbonyl proteins, reduced glutathione and hyaluronic acid levels were assessed both from the blood samples and from the liver tissue homogenate. The liver function was assed using AST and ALT blood levels. The liver samples underwent histopathology examination. The lesions were scored according to Knodell Histological Activity Index. The dynamics of the measured markers was analyzed using MedCalc 12.0 software. Oxidative stress markers significantly increased first in plasma (2 weeks) then in liver samples (4 weeks) and they maintained their elevated values during the entire experiment. Hyaluronic acid followed the same pattern. Necroinflammatory activity grade increased after 2 weeks; incipient fibrosis was observed after 4 weeks. We found a strong significant correlation between the plasma levels of the malondialdehyde, hepatic enzymes, hyaluronic acid and Knodell Activity Index. In conclusion, oxidative stress markers and plasma hyaluronic acid level might be used as non-invasive markers for the incipient liver fibrosis.

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P6.5

Involvement of interleukin-24 in the pathogenesis of inflammatory bowel disease

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Background and aims: The exact pathomechanism of inflammatory bowel disease (IBD) is not fully understood. Recently, therapies targeting cytokines of the interleukin (IL)-10 family came into focus. IL-24, a new member of IL-10 family was suggested to be involved in immune regulation and tissue regeneration; however its role in IBD is not clarified. The aim of our present study was to examine the presence of IL-24 and its receptor in the colonic mucosa of children with IBD and to investigate their possible role in the pathomechanism of IBD.

Methods: The mRNA expression and localization of IL-24 and its receptor were determined in the colonic biopsy samples from children with newly diagnosed IBD (n=29) and controls (n=20) by real-time RT-PCR and immunofluorescence staining, respectively. Expression of collagen-I and -III was measured in the colon of IL-24-treated C57Bl/6J mice. Amount of platelet-derived growth factor (PDGF-BB) and tumor growth factor (TGF-ß) were determined by flow cytometry in IL-24-treated HT-29 colonic epithelial cells. Expression of collagen-I and -III was measured by real-time

RT-PCR in IL-24, PDGF-BB or TGF-ß-treated CCD18Co colonic fibroblasts.
Results: The mRNA expression of IL-24 was elevated in the colonic mucosa of children with IBD compared to controls ($p < 0.05$) and strong IL-24 and receptor immunopositivity were detected in their colonic epithelial and fibroblast cells. IL-24 treatment increased the expression of TGF-ß, PDGF-BB and collagen-I and -III in the colon of mice and the level of PDGF-BB and TGF-β in HT-29 cells. IL-24, PDGF-BB or TGF-β treatment increased the collagen-I and -III expression of CCD18Co cells.
Conclusion: IL-24 itself and also the IL-24-induced expression of PDGF and TGF-ß may alter the expression of collagens in vitro and in vivo as well. These data suggest that elevated level of IL-24 may act on the fibrotic processes in the colon of children with IBD. However further studies are needed we suggest that IL-24 may be a potential therapeutic target in the future.

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P6.6
Local poly(ADP-ribose)polymerase activation in children with Crohn's disease

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The pathomechanism of Crohn's disease (CD) is not fully understood, however several models have suggested the role of inflammatory factors and oxidative-nitrative stress. The possible role of consequential poly(ADP-ribose)polymerase (PARP) activation was also addressed, although there are conflicting data in the literature about the nature of the PARP activity changes in inflammatory bowel disease. As PARP activation in CD was not examined in a human setting yet, our aim is to examine the level of local PARP activation in children suffering from CD. Three types of intestinal biopsies were analyzed: duodenal biopsy with CD (CD: n=22), with macroscopically and microscopically intact (CDintact: n=12; CDintact micr: n=8) and inflamed (CDinflamed.: n=10; CDinflamed micr: n=14) intestinal mucosa, and healthy controls (C: n=9). Paraffin embedded sections of biopsies were immunostained with anti-PAR antibody to determine PARP activity, there were scored 1–10 (sections) by a blinded experimenter.
There was significant difference in the Pediatric Crohn's Disease Activity Index (PCDAI), but not in the CRP level, thrombocyte count between the two diseased groups, however there was significant difference in these markers between the healthy controls and patients as expected.
The PARP activity of the affected duodenum regions were significantly higher than in the controls, independently of the macroscopic image (C: 4.1±1.8 vs. CDintact: 13.2±1.7, CDinflamed: 15.9±1.4, $p \leq 0.001$). On the other hand there is a significant difference in PARP activity if we consider the microscopic image (CDintact micr: 11.5±2.0 vs. CDinflamed micr: 16.1±1.1, $p \leq 0.05$). PARP activation was correlated with PCDAI (R=0.46) and with the neutrophil (R=0.48) and lymphocyte counts (R=-0.43). After excluding the control group, these correlations disappeared. Significant PARP activation can be observed in the inflamed and intact duodenum area of children affected by CD. Local PARP activity doesn't depend on the clinical activity, but the level of duodenal involvement.
Further studies are required to explore the function of PARP activation regarding possible usage as diagnostic marker or therapeutic target.

P6.7
The Transient Receptor Potential Vanilloid 1,4 (TRPV1, TRPV4) and Ankyrin 1 (TRPA1) receptor mRNAs are expressed in the human gastric mucosa

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The importance of sensory-immune interactions in gastritis have recently been emphasized with special focus on Transient Receptor Potential ion channels, such as Vanilloid 1, 4 and Ankyrin 1 (TRPV1, TRPV4, TRPA1) localized predominantly on capsaicin-sensitive peptidergic sensory nerves. They are activated by a variety of exogenous spices and endogenous inflammatory mediators and play an important role in thermo and pain sensation, as well as neurogenic inflammation. They have also been described on several non-neural cells, but their functional significance is unknown. Therefore, we investigated the mRNA expressions of these TRP receptors related to their local, non-neuronal synthesis in the human gastric mucosa and their alterations in chronic gastritis.
Gastric mucosal biopsies were collected during upper gastrointestinal endoscopy. Non-inflamed control and chronic gastritis samples with (IM+) or without intestinal metaplasia (IM-) were selected after histopathological evaluation. The isolated RNA samples were reversed transcribed and quantitative polymerase chain reactions were performed. TRPV1, TRPV4, and TRPA1 specific primers were used to amplify genes of interest, while beta-2-microglobulin was used to amplify the reference gene.
Messenger RNAs of TRPV1, TRPV4 and TRPA1 were similarly detectable in all control and gastritis samples. It should be mentioned that average Ct values of the reactions suggest a lower expression for TRPV4 and TRPA1 mRNA compared to that of TRPV1. Relative expressions of TRPV1 were by 0.2- and 0.45 folds less abundant in IM+ and IM- gastritis samples, respectively, than in control samples. In IM- gastric samples TRPA1 and TRPV4 transcription levels did not show alterations. Interestingly, in IM+ samples a 1.46-fold increase of TRPA1, whereas a 0.68-fold decrease of TRPV4 mRNA

expressions were detectable compared to control samples, but these alterations were not statistically significant. Further investigations are in process to analyze the expression of these receptors in reactive and chronic active gastritis, as well as to determine their localization with immunohistochemistry and functional relevance using animal models.

P6.8

Hydrogen sulfide confers protection in TNBS induced colitis in rat: role of heme oxygenase

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Hydrogen sulfide (H2S) is an endogenous mediator that exhibits several antiinflammatory activities and contributes to protection. We investigated the beneficial effects of H2S donor and whether heme-oxigenases (HO) are involved in the H2S-induced colonal cytoprotection against 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis in rats.
Male Wistar rats were treated with TNBS (10 mg) to induce colitis. H2S donor (Lawesson's reagent) were used at different concentrations (60; 30; 15; 7.5; 3.75; 1.87; 0.93 μM; dissolved in carboxymethylcellulose) twice daily per os (from 0 day to 3. day). 72 h after TNBS treatment colon samples were collected to measure extent of inflammation, myeloperoxidase (MPO), and HO activities and TNF-α content.
In a separate experiment, HO activity was inhibited by tin protoporphyrin (SnPP, 30 micromol/kg/day, s.c) at the day of TNBS challenge (10 mg) co-treatment with H2S donor (2 x 1.87 μM, per os). Twice-daily treatment with H2S donors significantly decreased the extent of colonal inflammation in a dose dependent manner compared to vehicle-treatment. The most effective concentration was 2 x 1.87 μM at the extent of inflammation (27.4 ± 1.5 vs. 46.2 ± 4.3; %). Per os administration of H2S donor reduced significantly TNBS-provoked MPO activity (19.07 ± 3.6 vs. 42. 98 ± 10; mU/mg/protein), TNF-α levels (89.95 ± 9.43 vs. 531.67 ± 32; pg/mg protein) while increasing colonic HO enzyme activity (0.98 ± 0.05 vs. 0.82 ± 0.6 nmol bilirubin/h/mg protein).
The protective effect of H2S was abolished by cotreatment with an inhibitor of HO activity (TNBS:35.6 ± 2.4; TNBS+H2S: 21.6 ± 5.6; TNBS+H2S+SnPP: 28.8 ± 2.6 % extent of lesion).
Our findings suggest that H2S confers protection dose dependently, probably by modulation of anti-inflammatory parameters and HO enzyme activity. Our results support the proposal that induction of HO activity by H2S provides a protective mechanism in this model.

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P6.9

Antizyme (AZ) regulates intestinal cell growth independently of polyamines

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Since antizyme (AZ) is known to inhibit cell proliferation and to increase apoptosis, the question arises as to whether these effects occur independently of polyamines. Intestinal epithelial cells (IEC-6) were grown in control medium and medium containing 5mM difluoromethylornithine (DFMO) to inhibit ODC, DFMO + 5μM spermidine (SPD), DFMO+ 5μM spermine (SPM), or DFMO+ 10 μM putrescine (PUT) for 4 days and various parameters of growth were measured along with AZ levels. Cell counts were significantly decreased and mean doubling times were significantly increased by DFMO. Putrescine restored growth in the presence of DFMO. However, both SPD and SPM when added with DFMO caused a much greater inhibition of growth than did DFMO alone, and both of these polyamines caused a dramatic increase in AZ. The addition of SPD or SPM to media containing DFMO + PUT significantly inhibited growth and caused a significant increase in AZ. IEC-6 cells transfected with AZ-1 SiRNA grew more than twice as rapidly as either control cells or those incubated with DFMO, indicating that removal of AZ increases growth in cells in which polyamine synthesis is inhibited as well as in control cells. In a separate experiment the addition of SPD increased AZ levels and inhibited growth of cells incubated with DFMO by 50%. The addition of 10 mM ASN prevented the increase in AZ and restored growth to control levels.
These results show that cell growth in the presence or absence of ODC activity and in the presence or absence of polyamines depends only on the levels of AZ. Therefore, the effects of AZ on cell growth are independent of polyamines.

P6.10

Effects of dietary fatty acids on gut microbiota and IRAP activity

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Nowadays a growing body of evidence indicates that the gut microbiota exerts important effects on the physiology of mammals. The microbiota signals its host through metabolites that enter into the bloodstream via colonic absorption. Nonetheless, the mechanisms by which gut microbiota produce outcomes that expand beyond the gastrointestinal tract and become systemic are largely elusive.
It has been reported that dietary fatty acids determinate the type of gut microbiota influencing insulin sensitive tissues such as liver muscle or adipose tissue, promoting in certain cases insulin resistance. Insulin-regulated aminopeptidase (IRAP) is present in the major insulin target cells such as hepatocytes, muscle cells and adipocytes. Impaired IRAP has

been suggested to play a role in the development of insulin resistance.
Previous results in our laboratory indicate a regulatory effect of dietary fat on gut microbiota, with a significant decrease of Gen. Enterococcus in a diet enriched with virgin olive oil with respect to butter. Olive oil diet also improves several metabolic parameters such as blood pressure and plasmatic lipid profile. The object of this study was to evaluate the effect of this diet on IRAP activity in liver in comparison to a standard diet and to a diet enriched in saturated fatty acids and cholesterol (butter diet). Our results indicated a significant decrease of IRAP activity in liver of animals fed butter diet. These values correlated with the increment of Gen. Enterococcus in this group.
This may suggest a role for IRAP activity in the beneficial effects of virgin olive oil in comparison to other fats.

P6.11

Stress-induced modulation of ileal motility in Capsici fructus-fed female rats

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Introduction: Stress induces various physiological and hormonal disturbances concerning feeding, including digestive motility. On the other hand, capsaicin, which is main component of capsici fructus (CAP) and stimulates TRPV1 receptors, is known to reverse stress-induced negative influences in the digestive tract. Here, we investigated the effects of CAP intake on the ileal motility in female rats.
Methods: Female rats (Wistar, SPF, BW: 158-219 g) were divided into Control (normal diet) and CAP (0.5% Capsici fructus pulveratus, Japanese Pharmacopoeia, mixed in standard rat chow) groups. 3G gravity stress (every day for 10 min) was loaded by centrifugal apparatus for 1, 3, 15 and 30 days in both groups. Just after the stress loading at each day, a 1 cm-long section of the ileum was isolated under barbiturate anesthesia and fixed to a Magnus-type chamber filled with Tyrode solution. Digestive movements were recorded for 60 s under each of three conditions: spontaneous, Acetylcholine (Ach, 10-4,10-5 g/ml) activation, and Adrenaline (Adr, 10-8 g/ml) inhibition.
Results: Both spontaneous phasic and tonic motility patterns were observed in the ileum. After Ach application, we observed a tonic response which was well antagonized by Adr. There were no significant differences in Ach-induced and Adr-antagonized movements between Control and CAP groups. However, spontaneous tonic response in CAP group was clearly decreased after Adr application after 3 days stress loading.
Discussion: The present study showed that feeding of CAP had significant effects on the Adr-induced antagonization of ileal movements. Ach sensitivities were not changed by capsaicin intake, however, it is suggested that the sensitivity of adrenergic receptors in the ileum may be changed by daily capsaicin intake through the activation of TRPV1 receptors.

P6.12

How to use PET/MRI to observe metabolic and cellular effects of portal vein ligation in healthy rat liver

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Aim: PET/MRI could improve diagnostic accuracy of conventionally used methods to avoid portal vein ligation (PVL)-induced tumour progression. The aim of this preliminary study was to image the effect of PVL on glucose metabolism, using PET/MRI imaging in healthy rat liver.
Methods and materials: Male Wistar rats (n=30) underwent PVL. Dynamic 2-deoxy-2- (18F)fluoro-D-glucose (FDG) PET/MRI imaging (60 min, 30 frames, 3D-OSEM Mediso TeraTomo reconstruction, 0.3 mm voxels, radioactivity injected: 6±1.5MBq iv.), Mediso nanoScan PET/MRI) and morphological/histological examinations were performed before (Day 0) and 1, 2, 3, and 7 days after PVL. Dynamic PET data were collected and the standardized uptake values (SUV) for ligated and non-ligated liver lobes were calculated in relation to cardiac left ventricle (SUVVOI/SUVCLV) and mean liver SUV (SUVVOI/SUVLiver). Four minute-integrated static PET frames were also reconstructed for visualization.
Results: PVL induced atrophy of ligated lobes, while non-ligated liver tissue showed compensatory hypertrophy. Altered FDG kinetics were measured in both ligated and non-ligated liver lobes. SUVVOI/SUVCLV significantly increased in both groups of lobes, with a maximal value at the 2nd postoperative day and returned near to the baseline 7 days after the ligation. After PVL, ligated liver lobes showed significantly higher tracer uptake compared to the non-ligated lobes (significantly higher SUVVOI/SUVLiver values were observed at postoperative day 1, 2 and 3). The homogenous tracer biodistribution observed before PVL reappeared by 7th postoperative day.

P6.13

A novel laparoscopic device for quantifying gastric slow wave activity

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BACKGROUND: Gastric slow waves assist in regulating the propagation patterns of peristaltic waves. Dysrhythmic slow wave activity has been implicated in a number of functional motility disorders including gastroparesis and functional dyspepsia. An improved understanding of slow wave activity is important for the development of new treatments to overcome the symptoms associated with these disorders.
The most accurate method for capturing slow wave activity is to record directly from the serosa of the target organ; the majority of high resolution recording devices however, are designed to be deployed during open surgical procedures.

With many surgeries now performed with minimal trauma, development of a laparoscopic device capable of capturing slow waves is essential to progress this area of research.
METHODS: We present the design for a novel laparoscopic device for recording slow-wave activity, together with its validation. The device consists of a rigid shaft (diameter 2.2 mm), and expandable and retractable spiral head (diameter 30 mm at full extension) with a flexible nitnol wire core and a Pebax® coating. The Pebax® coating was exposed in 32 places, approximately 2 mm apart to reveal 32 recording channels. The flexibility of the head allows it to pass through a 5 mm surgical trochar, and all materials used allow for sterilisation to the level of other surgical equipment. Validation was achieved by comparing recordings from the laparoscopic device with those collected from a standard flexible contact electrode in an open-abdomen porcine model.
RESULTS: Activation maps were consistent and showed normal organised aboral slow-wave propagation. Slow-wave velocities were similar between the laparoscopic device and the PCB (mean 3.2±3.9 mm/s vs 5.2±2.0 mm/s).
CONCLUSIONS: This laparoscopic device is capable of achieving high-quality serosal slow-wave recordings. It can be utilised for the investigation of gastric slow wave activity, which offers the potential for development of new treatments for gastric bioelectrical disorders.

P6.14
Contribution of Capsaicin-sensitive sensory nerves and nitric oxide to the protective action of Orexin-A against ischemia/reperfusion-induced gastric mucosal injury in rats

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This study investigates the protective mechanisms of Orexin-A (OXA) against ischemia/reperfusion (I/R) induced gastric injury. Therefore, Sham, I/R+Saline, I/R+OXA, I/R+L-NAME, I/R+L-NAME+OXA, I/R+Capsaicin, I/R+Capsaicin+OXA, I/R+Vagotomy, I/R+Vagotomy+OXA groups were set up. A total of 30 min ischemia/3 hours reperfusion was applied to the I/R groups with parallel infusion of saline (0.01 ml/kg/min) or OXA (500 pmol/kg/min). For each group, gastric blood flow, lesion index, myeloperoxidase (MPO) activity, CGRP, nitrite/nitrate (NOx) levels, iNOS and eNOS protein expressions were measured. Gastric mucosal injury caused by I/R was significantly reduced in OXA infused rats while the blood flow significantly increased. Lesion index and blood flow did not change in I/R groups with L-NAME, Capsaicin or vagotomy alone. However, L-NAME+OXA, Capsaicin+OXA and Vagotomy+OXA groups had increased lesion index and decreased mucosal blood flow. Protective effect of OXA diminished in all three groups and approached to I/R group level. I/R also caused an increase in MPO enzyme activity, which was decreased by OXA infusion. MPO activities in I/R groups with L-NAME, Capsaicin and vagotomy did not change. However, when these agents were applied with OXA infusion, decline in MPO activity that was observed following sole OXA infusion was diminished. CGRP levels decreased in I/R+Saline group compared to the sham but significantly increased after OXA infusion. CGRP level did not change in L-NAME, Capsaicin and vagotomy groups. However it was decreased in L-NAME+OXA, Capsaicin+OXA and vagotomy+OXA groups. NOx levels were high in I/R+Saline group and decreased in L-NAME group, but no change was observed in OXA group. NOx levels of L-NAME+OXA and Capsaicin+OXA groups were significantly lower than OXA group. I/R increased the iNOS protein expression and it decreased significantly in OXA and L-NAME groups. eNOS protein expression decreased with I/R while did not change in either OXA or L-NAME groups. In conclusion, it has been shown that sensory afferent neurons and NOS-NO system mediate the protective mechanisms of OXA against I/R related gastric mucosal injuries.

P6.15
The role of the ICC myenteric plexus network in the anisotropic propagation of intestinal slow wave activity

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Introduction: Slow waves (SW) are initiated and actively propagated by networks of interstitial cells of Cajal residing in the myenteric plexus (ICC-MP) between circular and longitudinal smooth muscle (SM) layers. This activity passively conducts to the neighboring SM layers driving the muscle contraction. Experimental studies have observed a faster circumferential SW propagation compared to longitudinal propagation with circumferential to longitudinal anisotropy ratio (AR) of 1.3. However, spike activity (responsible for contraction) propagates faster longitudinally than circumferentially with AR 0.55. In this study, electrical propagation over an ICC network is simulated to investigate the role of ICC-MP in the anisotropic propagation of SW in the intestine.
Materials and Methods: Confocal images of the ICC-MY networks were obtained from the intestine of normal mice. The images were 0.531 x 0.531mm in size sampled at 1024 x 1024 pixels resulting in a spatial resolution of 0.519µm. A novel finite element mesh was constructed using Delaunay triangulation over isocontours of the segmented images. Each node in the resultant triangular mesh represented either an ICC cell or non-ICC cell, based on the regional attributes obtained from the segmented image. A bidomain continuum modeling framework was used to model the reaction-diffusion properties describing the electrical activity through the network. A circular region of radius 0.04 mm in the centre of the network was initially activated and the subsequent pattern of propagation was analysed for eccentricity.
Results and Discussion: The simulated spread of electrical activity was found to be anisotropic with AR = 0.8 ± 0.08(SD,n=10) i.e., dominant along the longitudinal direction. This observation could explain the preferential spread of spikes longitudinally. It suggests that the rapid circumferential

conduction of SW in the SM layer is not primarily driven by the underlying ICC-MP network but may arise from the circular SM layer where the intramuscular ICC supports the circumferential spread. This may significantly impact the understanding of the role of different tissue layers in maintaining normal peristalsis.

P6.16

Effects of Silymarin on the initiation and progression of liver fibrosis in CCl4-induced experimental model

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Liver fibrosis is a common response that occurs during the evolution of chronic liver diseases and the activation of hepatic stellate cells (HSC) is the key mechanism involved in the hepatic injury. Silymarin (Si) is an herbal product with hepatoprotective potential. We aimed to investigate the effects of two different doses of Silymarin on a CCl4-induced model of liver fibrosis. Fibrosis injury was assessed after 4 weeks of CCl4 administration (early fibrosis) and after 2 months of CCl4 administration (established fibrosis). For each of the 2 assessed periods, fifty Wistar rats were randomly divided into 5 groups (n=10). For the early fibrosis, CCl4 in sunflower oil, was administered by gavage, twice a week for one month (CCl4 group), while Si was added from the third week of experiment in CCl4 + Si 50 group (CCl4 twice a week, Silymarin 50 mg/b.w. in CMC five times a week) and CCl4 + Si 200 group (similar to the previous group, with Si 200 mg/b.w.). For the established fibrosis, CCl4 in sunflower oil (CCl4 group), was administered by gavage, twice a week for two months, and then there was a 2 weeks period of toxic discontinuation. In the Si groups, Si was added after the toxic discontinuation in 2 different doses, Silymarin 50 mg/b.w or Si 200 mg/b.w. For each period, there was a control group (sunflower oil twice a week) and a vehicle group (carboxymethylcellulose five times a week) corresponding to each experimental model.
At each explored interval we assessed hepato-cytolysis (aminotransferases and LDH), oxidative stress, fibrosis (histological score, hyaluronic acid), TGF-β1, markers of HSC activation (α – SMA expression by western blot) and activation of Kupffer cells by immunohistochemistry. Our data showed that Silymarin, in both experimental models, and both doses, has the capacity of reducing oxidative stress, hepato-cytolysis, fibrosis, activation of Kupffer cells and the expression of α – SMA and TGF-β1, with better results for Si 200 mg/b.w. Thus, Silymarin administered in chronic liver injury is a potent inhibitor of the fibrogenetic mechanisms both during initiation and progression of liver fibrosis.

P6.17

Possible activation of immunity by chronic peripheral Ozone and Nesfatin-1 application in ischemia-reperfusion

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Introduction: Ischemia reperfusion (I/R) is a biphasic phenomenon which can damage the graft. Ischemia initiates the injury and reperfusion worsens the ischemic injury by inflammatory responses. Cortisol is released by the hypothalamic-pituitary-adrenal axis in response to inflammation. In animal models, the disrupted release of cortisol in response to inflammation has a great of importance. Nesfatin-1 is a peptide which is involved in the regulation of homeostasis. Ozone (O3) and oxygen (O2) mixture is used for ozone therapy that provides benefit in ischemic diseases, peritonitis and infected wounds. We aimed to identify the effect of chronic peripheral medical ozone and nesfatin-1 application on plasma level of cortisol in intestinal I/R created rats.
Material and Method: In this study, 42 two-months-old, Wistar albino male rats were used and randomly divided into 6 groups (in each group n=7): rats were underwent laparotomy (L); rats underwent occlusion of superior mesenteric artery for 30 min followed by 2h reperfusion (I/R); rats were treated ozone/oxygen mixture intraperitoneally (i.p.) (1,1 mg/kg) for 10 consecutive days and underwent laparotomy (O3+L); rats were treated ozone/oxygen mixture same as O3+L group and created I/R (O3+I/R); rats were treated with nesfatin-1 intraperitoneally (i.p.) (0,25 nmol/gr) for 10 consecutive days and underwent laparotomy (N+L); rats were treated with nesfatin-1 same as N+L group and created I/R (N+I/R). Plasma level of cortizol was analyzed by rat ELISA assay kits.
Results: Plasma level of cortisol in I/R and O3+I/R groups were significantly higher compared to L group. Plasma level of cortisol, although not statistically significant, was found to be lower for animal O3+I/R groupcompared to I/R group. Decreased plasma level of cortisol in N+I/R group was statistically significant compared to I/R group.
Discussion: Chronic peripheral medical ozone and nesfatin-1 application can inhibit anti-inflammatory response in early phase of intestinal I/R and support immune reactions by reducing plasma level of cortisol. This effect of ozone and nesfatin-1 may also increase the rejection of grafts during transplantation period.

P6.18

Transparent, true 3D qualitative and quantitative microscopic investigation of orofacial histological structures

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Objective: Our methodical innovation consists of 3 separate developments: 1) The triethanolamine based optical clearing solutions, which are able to homogenize the refractive indexes of tissue components, thus increasing their transparency. 2) The assembling of a true 3D investigating system, including the so called „stereoconverter", an optical device, which is able to increase the magnification of the conventional light microscope by 5-20 fold without decreasing its depth of field (DOF). This results in a relative increase of DOF at the given magnification. 3) The development of a true 3D measuring and modeling computer software based on stereophotogrammetric methods, for the analysis of spatial structures and functions.
Methods: Several orofacial structures (gingiva, masticatory muscles, lip, tongue, submandibular salivary gland, etc.) of rats were investigated both post mortem and in vivo using the system above. Beside soft tissues, we also commenced the development of hard tissue (tooth, bone) specific clearing solutions in vitro.
Results: With the aid of our clearing materials, we were able to make the structures of deeper tissue layers examinable, e.g. to study their microcirculatory system. Due to the increase of magnification and the relative enlargement of DOF, quite thick tissue sections or blocks can be imaged. By application of our true 3D measuring and modeling computer system, the microscopic structures can be covered by a spatial point cloud and spline surfaces. Mathematical analysis of these surfaces can be performed and shaded photorealistic three-dimensional models and animations of them can be achieved.
Conclusion: The true 3D way of thinking instead of planar approach can result in paradigmatic change also in the field of microscopy and may lead to the development of a new scientific branch i.e. the "transparent, true three-dimensional qualitative and quantitative microscopy". The identification and modeling of the delicate spatial structures of soft and hard tissues might not only provide substantial, new, qualitative and quantitative knowledge, but also might influence their therapy.

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P6.19

The protective effect of microemulsion of sour cherry (prunus cerasus) kernel extract on carbon tetrachloride -induced hepatotoxicity in mice

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Background: Microemulsion (ME) or nano-sized microemulsion has been developed in recent years to produce an orally delivery system. The aim of this study was to find out the protective effect of microemulsion of sour cherry (Prunus cerasus) kernel extract on carbon tetrachloride - induced hepatotoxicity in mice.
Methods: Adult albino Swiss male mice (25-30 g, 8-10 week old) were divided into seven groups; each group consisted of ten mice. Sour cherry kernel extract microemulsion was orally administered to mice at doses of 2.5%, 5%, 10% and 1000mg/kg of normal extract for ten days. Two hours after the last administration, a single dose of carbon tetrachloride (CCl4, 1 ml/kg in olive oil) was injected intrapritoneal to induce acute hepatotoxicity. Animals were sacrificed 24 h after the injection of CCl4. Their blood was collected serum was prepared for determination of enzyme activities or biochemical measurements. Liver was removed and kept in 10% formalin solution for histopathological examination.
Results: The results obtained in this study exhibited elevation in the serum enzymes activity and also various pathological changes were observed. These findings were significant in groups treated with 2.5% ME, 5% ME and 1000 mg/kg Microemulsion (ME) of sour cherry kernel extract ($P < 0.05$).
Conclusion: Under the present experimental conditions, 5% microemulsion of extract and 1000 mg/kg normal extract exhibited nearly similar hepatoprotective effect against CCl4 toxicity.

Keywords: Hepatoprotective, Microemulsion, Carbon tetrachloride, Sour cherry kernel

P7

Renal Physiology

P7.1

Influence of crowding stress on properties of renal Na,K-ATPase in young male normotensive and spontaneously hypertensive rats

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Objective: This study is oriented to the influence of social stress produced by crowding on properties of the renal Na,K-ATPase, a key system in maintaining the homeostasis of Na+ ions in the organism.
Method: Five weeks old Wistar males (mcW) and spontaneously hypertensive male rats (mcSHR) were exposed to crowding for 2 weeks (msW, msSHR). Properties of renal Na,K-ATPase were estimated by enzyme kinetic measurements in wide concentration range of substrate ATP and cofactor Na+.
Results and Conclusions: Spontaneous hypertension and stress independently did not alter the number of active Na,K-ATPase molecules in renal tissue of rats as indicated by unaltered Vmax values. When the organism of rats was loaded by stress and hypertension simultaneously the number of active Na,K-ATPase molecules decreased as documented by lowered Vmax value. Hypertension by itself did not alter the binding properties for ATP or sodium as indicated by stable values of Km and KNa in unstressed rats. Stress by itself decreased the ATP-affinity of the enzyme as indicated by increased value of Km, while Na-binding properties were improved as indicated by decreased value of KNa. Surprisingly, SHR subjected to stress showed improved ATP-binding as revealed from decreased value of Km and return of the Na-affinity to the level like in unstressed control Wistar rats.

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P7.2

Metformin prevents renal ischemia-reperfusion injury: A biochemical and histopathological evaluation of experimental model

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Background: Recent studies have shown that metformin, an oral antidiabetic agent, also posses anti-inflammatory and antioxidant effects. The aim of our study was to determine does pretreatment with metformin (in doses of 3mg/kg or 10 mg/kg) could attenuate acute kidney failure caused by renal ischemia-reperfusion (I/R) injury in anaesthetized rats.
Materials and methods: Male adult Wistar rats (n=57, b.w. 250-300 g) are anesthezied with intraperitoneal bolus injection of sodium thiopentone (120 mg/kg) and placed on their backs on thermostatically controlled heating mat to provide constant body temperature 37.5 ± 1C. Trachea, carotid artery, jugular vein and urinary bladder are separated from surrounding tissue and inserted with appropriate tubes or cannulas. Rats were randomized into six experimental groups (N=6-10 per group) - I: Sham + saline, II: Sham + metformin 3mg/kg, III: Sham + metformin 10 mg/kg, IV: I/R + metformin 3 mg/kg, 30 min before ischemia V: I/R + metformin 10 mg/kg, 30 min before ischemia VI : I/R + saline. Animals randomized into I/R injury groups were subjected to bilateral clamping of renal pedicles for 45 min followed by reperfusion for 4h. In addition, cardiovascular parameters are monitored continuosly, and anesthesia is maintained by supplementary injections of thiopentone sodium (10 mg/kg, i.v.), as required. Both mean arterial pressure and heart rate were monitored continuously. Selected parameters of glomerular and tubular function, as well as histological score, were obtained from the appropriate serum, urine or tissue samples at the end of reperfusion period.
Results: Acute pretreatment with metformin (in doses of 3mg/kg and 10 mg/kg) significantly attenuated I/R-induced increase in specific parameters of glomerular function (serum urea and creatinine concentrations) and tubular function (fractional excretion of Na+). Also, metformin significantly reduced the total and tubular necrosis histological score.
Conclusion: Our study shows that a single dose of metformin could afford significant protection of the injured rat kidney. Its use in the future should be considered to reduce in prevention of development of acute kidney failure

P7.3

Less inflammation and oxidative damage is responsible for the resistance of Rowett rats against focal segmental glomerulosclerosis

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Genetic background has a strong influence on the progression of chronic kidney disease. We found recently that Rowett, black hooded (BH) rats were resistant to renal fibrosis induced by subtotal nephrectomy and salt and protein loading. To study the role of sustained inflammation and oxidative damage in the development of renal fibrosis, we compared renal fibrosis in the resistant BH to sensitive Charles-Dawley (CD) rats in the doxorubicin (DXR)-induced focal segmental glomerulosclerosis model.
In CD and BH rats treated with 5 mg/kg DXR (tail vein) renal function was followed by urinary protein and neutrophil gelatinase associated lipocalin (NGAL) excretion. Renal fibrosis was assessed by PAS sirius red and fibronectin staining 8 weeks post treatment. Messenger-RNA of the slit diaphragm protein (nephrin), pro-fibrotic factors (TGF-β, CTGF, collagen (COL1A1)) and of inflammatory (p47phox, p91phox and MCP1) markers were measured by real-time PCR. Oxidative damage was assessed by 4-hydroxynonenal (HNE) and nitrotyrosine (NT) staining and quantified by western blot. Survival was assessed in separate cohorts.

DXR dose-dependently decreased body weight gain in parallel with a progressive loss of renal function. BH rats survived longer than CD rats. Deterioration of renal function accompanied by glomerulosclerosis, tubulointerstitial fibrosis and matrix deposition was more severe in CD than BH rats. Nephrin loss, TGF-β, CTGF, COL1A1 expression demonstrated better maintained slit diaphragm structure and milder fibrosis. MCP1, p47phox p91phox expression confirmed milder inflammation, and NT and HNE staining demonstrated less oxidative stress in DXR-treated BH than CD rats.
Mediators of fibrosis, inflammation and oxidative stress were suppressed in DXR nephropathy in BH rats underlining the importance of these pathomechanisms in the progression of renal fibrosis.

P7.4

Knockout of the Tau T gene predisposes C57 BL/6 mice to a ST2-induced diabetic nephropathy

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Diabetic nephropathy (DN) is the leading cause of end stage renal disease (ESRD) in man. Although efforts have been made, an ideal animal model that can reproduce the characteristics of human DN has not been developed. In this study, we hypothesize that the absence of the taurine transporter gene (TauT, a kidney protective gene) is one of the critical risk factors for DN development in diabetes mellitus (DM). This hypothesis was tested in vivo in TauT heterozygous deletion (TauT+/-), and TauT homozygous knockout (TauT-/-) in C57BL/6 background mice. We provide evidence that alteration of the TauT gene has a substantial effect on the susceptibility to diabetic kidney disease development in both TauT+/- and TauT-/- mouse models of diabetes. These animals specifically developed characteristic renal histological changes that included glomerulosclerosis, nodular lesions, arteriosclerosis, arteriolar dilation, and tubulointerstitial fibrosis. Immunohistochemical staining of molecular markers of smooth muscle actin (SMA), CD34, Ki67, and collagen IV confirmed these observations. These results have demonstrated that both homozygous and heterozygous TauT gene deletion predispose C57BL/6 mice to develop end-stage diabetic kidney disease, which closely replicates the pathological features of diabetic nephropathy in human diabetic patients and can serve as a model for therapeutic strategies.

P8

Physiology of the Immune System

P8.1

Preventive effects of resveratrol against Schistosoma mansoni-induced liver fibrosis in mice

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Background: In Schistosomiasis, hepatocyte injury and Kupffer's cell activation can result in reactive oxygen species generation, pro-inflammatory and profibrogenic mediators release. This can result in stellate cells activation and consequently, liver fibrosis. Resveratrol, a natural polyphenol, has been shown to possess antioxidant and anti-inflammatory properties. However, studies into its protective effects against Schistosoma mansoni-induced liver fibrosis are limited.
Aims: The present study was designed to examine the preventive effects of resveratrol on Schistosoma mansoni-induced liver fibrosis in mice.
Methods: Sixty male albino mice were divided into four groups of 15 mice as follows: normal resveratrol-untreated, normal resveratrol-treated, Schistosoma mansoni-infected resveratrol-untreated and schistosoma mansoni-infected resveratrol-treated. At the end of the experimental period, blood samples were collected to measure serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and TNFα. Liver tissue was collected for malondialdehyde (MDA) measurement, histopathological examination and fibronectin gene expression analysis.
Results: AST, ALT and TNF-α, and MDA levels were significantly increased in the infected resveratrol-untreated group compared to normal resveratrol-untreated group (all, $P < 0.05$). However, their levels were significantly decreased in the infected resveratrol-treated group compared to infected resveratrol-untreated group (all, $P < 0.05$). In addition, fibronectin gene expression was highly up-regulated in the infected resveratrol-untreated group compared to normal resveratrol-untreated group ($P < 0.05$). Administration of resveratrol significantly down-regulated fibronectin in the infected resveratrol-treated group compared to infected resveratrol-untreated group ($P < 0.05$).
Conclusion: Results of the study indicate that resveratrol can prevent S. mansoni-induced liver fibrosis via mechanisms involving its anti-oxidant, anti-inflammatory and anti-fibrotic properties.

P8.2

Characterization of extracellular vesicles produced during spontaneous death of neutrophilic granulocytes

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Introduction: Neutrophils (PMN) produce antibacterial microvesicles after complex biological stimulation. The antibacterial capacity is reduced if the activation is partial. In order to assess the importance of cell activation in production of antibacterial microvesicles, in this study we characterize the extracellular vesicles (EV) produced during spontaneous death of the neutrophils, and test their antibacterial effect.

Methods: PMNs were prepared from the blood of healthy volunteers. After storing the cells in dulbecco's modified eagle's medium in cell culture incubator for 3 days extracellular vesicles were separated by two step centrifugation and filtration. The separated vesicles were counted and analyzed with EV optimized flow cytometry. The characteristics of EV were analyzed with dynamic light scattering, fluorescent and electron microscopy. The antibacterial effect of the EV was tested in bacterium survival assay.
Results: During the 3 days storing the number of apoptotic EV was increased parallel with PMN count decrease. The flow cytometric appearance (forward and side scatter) of apoptotic EV was similar to antibacterial microvesicles and both populations were positive for annexin V and CD11b, a neutrophil cell surface marker. Dynamic light scattering showed overlapping vesicles populations with microvesicles and exosomes. However unlike microvesicles produced after PMN activation, the apoptotic EV were ineffective in bacterium survival assay.
Conclusion: The apoptotic EV of neutrophils have a right side out vesicular nature, which is similar in size and appearance to the antibacterial microvesicles, but apoptotic vesicles do not have antibacterial effect. These results highlight the difference between distinct vesicle populations and support the specific formation of antibacterial EV.

P8.3

Detection of different gene expression in human residual Epithelial cells of anterior lens capsule after manual and femtosecond laser performed capsulorhexis

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Introduction: Cataracts occur commonly and incidencies increase with age. Nowadays, two surgical methods are available in Hungary, the conventional manually performed and femtosecond laser-assisted cataract surgery. We hypothesized that the laser-assisted method causes less stress to the epithelial cells; therefore the regeneration of epithelial layers is faster than in conventional manual surgery. To investigate the above theory cell culture and molecular studies were performed.
Methods: Samples were obtained from patients who were diagnosed with cataract (n=12) and were operated on either using the traditional or the laser-assisted method. The removed anterior lens capsules were maintained up to 14 days in tissue culture to obtain information about the residual epithelial cells. Ultrastructural investigation was performed using transmission electronmicroscope. Changes of gene expression levels were analyzed with qRT-PCR targeting genes regulating apoptotic, proliferative and inflammatory processes (p53, Bcl2, CyclinD1 and Cytokeratin-7, TNFalpha, IL-6, IL-8).
Results: Several ultrasturctural changes in both surgery samples were observed. To study the molecular background of the structural changes, we examined four genes related to apoptosis, the cell cycle and differentiation. Our results showed that the Bcl-2 expression levels in both samples were reduced which makes epithelial cells more sensitive towards apoptotic signals. In contrast to manual surgery samples the laser-assisted samples showed more elevated levels of Cyclin-D1, indicating more active proliferation. Investigation of prominent inflammatory cytokine expression TNFalpha, IL-6 and IL-8 revealed a marked increase in IL8 mRNA levels in the manual samples.
Conclusion: In the present work we compared two surgical methods in terms of epithelial cell survival and inflammatory cytokine profile. Based on the electronmicroscopic images and the molecular analysis, the manually performed samples showed a more active apoptotic and inflammatory profile, supported by decreased Bcl-2 and increased IL-8 expression levels. Additionally, the laser-assisted samples showed higher division rate and lower apoptotic.

P8.4

Evidence for the involvement of galanin receptor 3 in an inflammatory arthritis model of the mouse

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BACKGROUND: Neurogenic inflammatory components mediated by peptidergic sensory nerves have a crucial impact on the symptoms of experimental arthritis, regarding both pain perception and immune cell recruitment. Galanin is a regulatory sensory neuropeptide, that attenuates neurogenic inflammation, but its targets and mechanisms have not been unravelled. Since there are minimal in vivo data on the role the Gal3 receptor (Gal3R) in inflammation, we analyzed its involvement in a mouse model of rheumatoid arthritis.
METHODS: Polyarthritis was induced by K/BxN arthritogenic serum in male Gal3R gene-deficient and wildtype mice. The mechanonociceptive threshold was determined by esthesiometry, grasping ability by the wire-grid grip test, paw volume by plethysmometry and clinical scoring. Myeloperoxidase (MPO) activity was measured by luminol-based in vivo bioluminescence imaging. The ankle joints were harvested for histological evaluation at the end of the study.
RESULTS: Gal3R gene-deficient mice demonstrated significantly increased and earlier ankle edema and clinical disease severity than wildtypes. Their grasping impairment was also more severe, whereas their mechanical hyperalgesia did not differ in the early, but became greater in the late phase. Neutrophil-derived MPO-activity in the ankle joints was similar in the two groups during the acute phase, but it was significantly lower in the knockouts by day 5.
CONCLUSION: Gal3R activation results in several anti-inflammatory functions in arthritis: it inhibits early edema

formation, grasping disability and mechanical hyperalgesia in the late phase. Meanwhile, Gal3R-mediated late immune cell activation indicates a potent immunoregulatory role, which needs further investigations. Selective Gal3R agonists or monoclonal antibodies could provide a novel approach for anti-inflammatory drug therapy.

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P8.5

Detailed characterization of the antibacterial effect of human neutrophilic granulocyte derived extracellular vesicles

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Introduction: The professional bacteria eliminator cell, the neutrophilic granulocytes (PMN) produce extracellular vesicles (EV) to many different stimuli. One specific type of these EV, (the aEV) show antibacterial effect. This effect is rather bacteriostatic than bactericide, depends on direct bacteria-EV connection and on formation of large aggregates, but it is independent from the opsonization of bacteria. Other EV (pEV) are also able to attach to bacteria, but they are unable to form large aggregates with them, and also lack of antibacterial effect. A third EV population, the sEV lack both attaching to bacteria and antibacterial effect. Our aim was to investigate, which factors are responsible for attachment and/or for antibacterial effect.

Methods: PMN were prepared from venous blood of healthy volunteers. MV were isolated with filtration and with 2 step centrifugation protocol after appropriate activation of PMN. Antibacterial effect was measured with bacteria surviving test. Quantitative and qualitative properties of bacteria-EV interaction were examined with confocal fluorescent microscopic techniques. Specific inhibitors were used to examine the importance of identified compounds.

Results: Specific accumulation of the 2 chains of Integrin β2 (CD11b and CD18), the myeloperoxidase (MPO) and phosphatidyl serine (PS) were identified in aEV aggregations, compared to sEV. Conformation of CD18 was "active" in aEV, but "inactive" in sEV. pEV showed similar CD18 presence and conformation as aEV. However, the amount of MPO and PS in pEV-bacteria interactions was lower, compared to aEV. Specific blocking of CD18 function inhibited connection of aEV and pEV to bacteria, masking of PS with Annexin V prevented formation of aggregates with aEV. Blocking enzymatic function of MPO, or titrating the MPO-substrate hydrogene peroxide concentration had no effect on aEV antibacterial action.

Conclusions: Amount and conformation of CD18 are both determinates of the connection of EV to bacteria, while PS rather plays a role in formation of large aggregates. To our great surprise, enzymatic function of MPO is indifferent in antibacterial effect of EV.

P8.6

Direct inhibition of complement c5a has long-term anti-inflammatory effects after partial aortic occlusion

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Background: The mesenteric circulation is highly sensitive to systemic hemodynamic derangements thus vasoactive treatments targeting circulatory malfunction of the splanchnic area could be of significant therapeutic relevance. Circumstantial evidence suggests that complement activation plays decisive role in this process. Our objective was to investigate the effects of complement C5a inhibitor treatment (AcPepA, Nagoya City University) on inflammatory mediator changes and intestinal microcirculation in clinically-relevant time frame in a rat model of mesenteric hypoperfusion.

Methods: In groups 1 and 2 (n=6, each) the mean arterial pressure of the splanchnic area was kept between 40-45 mmHg for 60 min by partial aortic occlusion (PAO), group 3 (n=6) served as control. Group 2 was treated with AcPepA 15 min before reperfusion (4 mg/kg iv) while the vehicle for AcPepA was administered to groups 1 and 3. After 24 hrs the animals were re-anesthetized and small intestinal microcirculation was investigated with orthogonal polarization spectral imaging technique. Blood samples were taken for determination of tumor necrosis factor-α (TNF-α), endothelin-1 (ET-1) and high mobility group box protein-1 (HMGB-1) levels.

Results: 24 hrs after PAO the plasma TNF-α (M=86.0; p25=28.8 p75=172.5 vs M=407.6; p25=236.9 p75=572.4 pg/ml), ET-1 (M=5.78; p25=5.11 p75=6.09 vs M=8.92; p25=8.34 p75=9.87 fmol/ml) and HMGB-1 (M=0.66; p25=0.24 p75=0.86 vs M=2.85; p25=2.68 p75=3.23 mU/mg protein) levels were significantly higher as compared to the control group, while the intramural red blood cell velocity was significantly decreased. The AcPepA treatment moderated the elevation in TNF-α (M=112.2; p25=83.3 p75=155.8 pg/ml), ET-1 (M=5.28; p25=3.84 p75=5.80 fmol/ml) and HMGB-1 levels (M=0.89; p25=0.17 p75=1.96 mU/mg protein) and improved intramural microcirculation.

Conclusion: Direct inhibition of C5a can effectively influence the potentially harmful, local and global long-term effects of acute mesenteric hypoperfusion associated to central circulatory failure.

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P8.7

Release of Diphtheria Toxin Fragment A (FA) from early endosomes into the cytosol

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Diphtheria toxin (DTx) is a well-characterized representative of bacterial protein toxins. It is synthesized and released by toxinogenic Corynebacterium diphtheria strains as a single polypeptide chain of 58kDa. After a mild treatment with trypsin and reduction of its disulfide bonds, toxin is separated into two fragments: Fragment A (FA) of 21 kDa has enzymatic activity (ADP-ribosyltransferase) at the end of the N-terminal end and Fragment B of 37 kDa that is providing connection between the cell and holotoxin. FA in the cell leads to protein synthesis inhibition, DNA fragmentation, actin degradation and apoptosis. Actin supports endosome vesicles to early endosomes in the cell. The acidic milieu in endosomes causes conformational changes and denaturation of the toxin. The transmembrane domain becomes disclosed and mediates through interaction with the endosomal membrane release of FA into the cytoplasm. The recent study report that FA can interact with actin in both in vitro and in vivo conditions. In this present study, in order to determine the effects of cytochalasin D (CD), ammonium chloride (NH4Cl) and primaquine on the release of FA into the cytosol, DTx sensible HUVECs (Human Umbilical Vein Endothelial Cells) were treated with DTx. Cell lysates and isolated early endosomes were prepared with ultracentrifuge. FA release from early endosomes was tested with the ADP-ribosylation assay and Western blot (WB).
The results from our experiments conclude that FA release was increased with the interaction of actin, eEF2 and Hsp 90. In contrast to this finding, in the presence of CD, ammonium chloride and primaquine, inhibition of FA release was observed.

P8.8

The effects of treatment with Simvastatin on liver ghrelin, HIF-1 alpha and trace elements in endotoxemic rats

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Aim: Lipopolysaccharide (LPS) cause to increased cytokine levels in experimental animal models. It is known that simvastatin is an antiinflammatory, antioxidant, antiapoptotic effects is used inhibitor of HMG-CoA reductase enzyme. In literature, Ghrelin levels were decreased and its supplementation protected against tissue damage by preventing proinflammatory cytokines levels in septic animals. There has been shown that copper with bacteriostatic function has been increased enzyme activity and Ghrelin releasing by food intake. Liver plays important roles the clearing infective products, cytokines. LPS caused to secretion of HIF-1 α from macrophages by increasing cytokines. In septic organism, trace elements were to increased transcriptional activity of HIF-1 α in response to oxidative stress conditions. We aimed to investigate the effects of prior treatment of simvastatin on ghrelin, HIF-1 α, selenium, Manganese, copper, and calcium levels on hepatic tissue in rats treated with LPS.
Material / Method: Rats were divided into four groups: control, LPS (20 mg/kg, i.p.), Simvastatin (20 mg/kg, p.o.), and LPS+Simvastatin group. The liver sections examined immunohistochemically react with TNF-α, IL-10, HIF-1α and ghrelin antibodies. Selenium, Manganese, copper, and calcium elements were measured in liver tissue samples.
Results: We found the levels of serum and tissue TNF-α and IL-10 were higher in the experimental groups than controls ($p < 0.05$). In all experimental groups, HIF-1α immunoreactivity was increased compared to controls' except Simvastatin group ($p < 0.05$). In the Simvastatin group, Ghrelin levels were increased in comparison with the other groups ($p < 0.01$). Ghrelin levels were greatly decreased in LPS ($p < 0.05$). Although, Selenium, Copper, Manganese levels were decreased in all experimental groups tissue calcium was found increased in LPS and simvastatin+LPS groups ($p < 0.05$).
Conclusion: We observed that the degree of hepatocellular degeneration was partially reduced depending on the dosage and duration of prior simvastatin treatment in septic group, probably due to alterations of Ghrelin and HIF-1α levels.

P8.9

Contribution of CD40L/Mac-1 interaction to visceral adipose tissue inflammation in mouse model of high fat diet-induced obesity and obesity-related nephropathy

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Obesity epidemic is associated with kidney damage. Hallmark of high fat diet-induced obesity (DIO) is inflammatory cell infiltration in obese adipose tissue and altered endocrine and inflammatory function of the visceral adipose tissue (VAT). We demonstrated previously that the leukocyte integrin Mac-1 binds the co-stimulatory immune molecule CD40 ligand (CD40L) to mediate inflammatory cell recruitment. Given contradictory results in the literature regarding lipid accumulation, inflammation and fibrosis of the kidney in obesity-related nephropathy in mice we aimed to study the possible role of the CD40L/Mac-1 interaction in obesity-associated inflammation of VAT and in the kidney.
Materials and Methods: Male, wild-type, C57Bl/6J mice were fed ad libitum with a high-fat diet (HFD, 45KJ% fat) or standard chow (LFD) for 20 weeks. In HFD animals, the

CD40L/Mac-1 interaction was inhibited by daily i.p. injections of the specific peptide inhibitor cM7. Control groups were treated with the control peptide scrambled-cM7 (scM7) or saline. Epididymal adipose tissue was analysed by flow cytometry, immunohistochemistry, and pro-inflammatory gene-expression was detected by qPCR. In kidney tissue pro-fibrotic (TGF-β, fibronectin (FN1)), tubular damage (NGAL, miR-21) and inflammatory (MCP-1, IL-1β, TLR4) gene-expression were analysed by qPCR.
Results: Inhibition of the CD40L/Mac-1 interaction by cM7 inhibited CD8+ T cell accumulation in VAT during DIO in mice. Infiltration of CD8+CD44+CD62L- effector memory T cells was markedly reduced (cM7 vs. scM7 37±9.6%; p=0.05). The expression of injury markers (NGAL; miR-21) and pro-fibrotic genes (TGFβ; FN1) were not altered in the kidney of the HFD-group compared to the LFD-group (n≥ 9/group) at 20 weeks, which disclose advanced kidney injury. Inflammatory marker expression (MCP-1, IL-1β, TLR4) PCR studies in kidney mRNA isolate are ongoing.
Conclusions: Our results point to the pro-inflammatory role of the CD40L/Mac-1 interaction in VAT inflammation in DIO. The lack of increased pro-fibrotic gene expression in the kidney suggests that this model of DIO may represent an early state of obesity related nephropathy.

P8.10
Effects of 6-hydroxydopamine on fractal complexity of lymphocyte chromatin organization

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Objective: Oxidopamine (6-hydroxydopamine) is a known neurotoxin, commonly used for selective targeting of dopaminergic neurons in the brain in order to create animal models for Parkinson's disease. Recently it has been demonstrated that this substance can induce programmed cell death (apoptosis) in lymphocytes in in vitro conditions. The objective of this study was to determine whether 6-hydroxydopamine affects chromatin organization during early lymphocyte apoptosis.
Method: Human peripheral blood samples were treated with 6-hydroxydopamine at concentration 100μM. Before and after the treatment, blood smears were fixated in methanol and stained using DNA-specific Feulgen method for chromatin visualization. A total of 30 lymphocite chromatin structures were visualized using DEM 200 digital instrument (Oplenic Optronics, Hangzhou, CN) mounted on Olympus CX21FS1 binocular microscope (1000x magnification), and compared to the untreated controls. Fractal analysis was performed using FracLac program code algorithm for ImageJ (NIH, USA) software. For each chromatin structure, the average values of fractal dimension and lacunarity were determined.
Results: The obtained results indicate that treatment with 6-hydroxydopamine induced statistically highly significant reduction of chromatin fractal dimension ($p < 0.01$). On the other hand, chromatin lacunarity (indicator of structural gapiness) was significantly increased ($p < 0.01$).
Conclusion: This is the first study to demonstrate that 6-hydroxydopamine impacts lymphocyte chromatin structure in terms of reducing organizational complexity. The results are in line with the findings of other authors showing that the cell nuclear complexity decreases during the early stages of apoptosis.

P8.11
Nuclear envelope circularity is related to chromatin textural variance in Feulgen-stained medullar thymocytes: application in apoptosis research

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Objective: Recently, many studies have been focused on chromatin textural patterns as possibly important indicators of genetic changes during various physiological and pathological processes such as programmed cell death (apoptosis). In this work we present the data suggesting that some of the parameters of chromatin texture may be correlated with standard morphometric determinants of nuclear size and shape.
Method: We analyzed a total of 100 lymphocyte nuclear structures in thymus medulla, previously visualized in 5 mice thymus sections stained using DNA-specific Feulgen method. Textural features were quantified using Grey level co-occurrence matrix (GLCM) technique. For each chromatin structure, the average value of GLCM entropy, angular second moment, variance and inverse difference moment were calculated. Nuclear circularity as the indicator of envelope roundness, was determined based on the envelope perimeter and nuclear area.
Results: Statistically significant negative correlation was observed between envelope circularity and GLCM variance ($R = -0.20$, $p < 0.05$). No such correlation was present between the circularity and other textural features. The results indicate that in the chromatin digital micrographs, as the roundness of thymocyte nucleus increases, the variance between chromatin resolution units in the texture decreases, and vice versa.
Conclusion: This is one of the first studies to describe such relationship between chromatin textural organization and nuclear envelope shape. The results have potential application in apoptosis research where both nuclear GLCM features and circularity are potentially important indicators of cell apoptotic status.

P8.12

Correlation between fractal and grey level co-occurrence matrix parameters in nuclear structure of toluidine blue - Stained thymus cortical lymphocytes

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Objective: Fractal and Grey level co-occurrence matrix (GLCM) analysis are two well-known mathematical methods that were shown to be useful for quantification of structural and functional complexity of nuclear chromatin during programmed cell death (apoptosis) and other physiological/pathological processes. However, many issues regarding the potential relationship between major parameters of these two methods during chromatin structural analysis remain unknown. In this work we present results indicating the strong correlation between fractal dimension as the main fractal analysis parameter, and some of the GLCM features.
Method: A total of 150 chromatin structures of mice thymus cortical lymphocytes stained with nucleic acid-specific toluidine blue dye, was analyzed using the FracLac plugin and MATLAB-based code algorithms for ImageJ software (NIH, USA). Prior to analysis, the structures were visualized with DEM 200 digital instrument (Oplenic Optronics, Hangzhou, CN) mounted on Olympus CX21FS1 binocular microscope (1000x magnification). For each chromatin structure, values of fractal dimension, GLCM angular second moment (level of textural uniformity), and GLCM entropy (level of textural disorder) were calculated.
Results: There was a strong positive correlation between fractal dimension and angular second moment values ($p < 0.01$). Also, we detected statistically highly significant correlation between fractal dimension and entropy (parameter of textural disorder).
Conclusion: These findings indicate that as the chromatin structural complexity increases, structural disorder decreases and vice versa. This is the first study to demonstrate such relationship and suggests that level of chromatin fractal complexity can be a good predictor of changes in chromatin textural patterns. The findings have potential implications both in conventional microscopy and cell physiology where fractal and GLCM analysis are today frequently applied.

P8.13

Tadalafil (PDE5 inhibitor) suppreeses inflammation after ovalbumin sensitization in guinea pigs

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PDE5 inhibition is widely used in the therapy of erectile dysfunction, pulmonary hypertension as well as other cardiovascular diseases. However, the expression of PDE5 was confirmed in several immune cells, suggesting its potential role in allergic inflammation.
The aim of this study was to evaluate the effect of one-week administration of selective PDE5 inhibitor tadalafil in experimentally induced allergic inflammation in guinea pigs and to compare it with the effects of selective PDE4 inhibition by roflumilast.
24 male adult guinea pigs, divided into 4 groups, have been used in the study. Control group has been left without sensitization. The latter 3 groups have been sensitized with ovalbumin over two weeks and thereafter treated intraperitoneally for 7 days with tadalafil at the daily dose of 1.0 mg/kg b.w., with roflumilast (PDE4 inhibitor) at the same dose, or with vehiculum, respectively.
Sensitization with ovalbumin has led to significant increase in in vivo and in vitro airway reactivity. Tadalafil reduced both specific airway resistance measured in whole-body double-chamber plethysmograph after nebulisation of histamine, and in vitro airway reactivity to cumulative doses of acetylcholine in tracheal and lung tissue strips using organ bath method. These changes have been associated with suppression of haematological markers of inflammation and apoptosis.
Selective PDE5 inhibition seems to play a significant role in allergic airway inflammation. However, its anti-inflammatory potential needs further testing.

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P8.14

Role of hypoxia inducible factor in cytokine secretion responses to cadmium of rat alveolar macrophages in normoxic and hypoxic conditions

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The lung is considered to be one of the main target organs of cadmium (Cd) toxicity, which is present in both air pollution and cigarette smoke and causes pulmonary inflammation. Changes of the inflammatory cytokines in hypoxia-induced lung injury or toxic conditions are not reported yet. It is shown that lung macrophages exposes to different levels of O2 concentrations and, release different mediators. Hypoxia inducible factor (HIF-1 α) is one of most important molecules playing a significant role in the control of hypoxic processes that promotes cellular survival, migration, proliferation. In the present study, we aimed to evaluate effect of different dosages of Cd (1-100 μM) to TNF-α, IL-4, IL-5, IL-10 in rat alveolar macrophage cell line NR8383 in normoxic and hypoxic conditions. NR8383 cells were silenced using HIF-1 α siRNA sequence primers and overexpressed. Quantitative RT-PCR assay was performed to quantify the mRNA expression changes of HIF-1α. Inflammatory cytokine secretion levels were found increased in hypoxic conditions and HIF-1 α overexpressed cells. Cd causes cytotoxicity and chages

inflammatory responses as a dose and time dependent manner. In conclusion, our results suggest that inflammation and hypoxia may contribute in Cd-induced lung damage in lower dosages, HIF-1 α is one of the important mediators of immune function in lung. Cd exposure might be one of the causes of the immune supressor results of long term cigarette smoking and HIF-1 α level changes might be one of the regulator of these responses.

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P8.15

In the long run hyperbaric oxygen therapy attenuates pro-inflammatory processes in streptozotocin induced diabetes in rats

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The possible negative effects of hyperbaric oxygen therapy (HBOT) on cardiovascular system and oxidative balance in diabetes raise questions. Previously we showed that weeks after HBOT cardiovascular functions of type 1 diabetic rats were improved or unaltered. In the present study our aim was to examine the effect of HBOT on systemic oxidative stress and cytokine production in the same setting.

Diabetes was induced in Wistar rats (15) with a single dose of 70 mg/kg streptozotocin. 7 diabetic and 8 controls underwent one-hour long hyperbaric oxygen treatment (HBOT: 2.5 bar) 12 times. 6 controls and 8 diabetic rats remained untreated. Two weeks after the HBOT series, heparinized blood plasma samples were collected for malonyl-dialdehyde assay. Altered cytokines were identified by Rat Cytokine Antibody Array (R&D). According to the results the following cytokines were selected for ELISA measurement: Cytokine-induced neutrophil chemoattractant 1 (CINC-1), lipopolysaccharide induced CXC chemokine (LIX/CXCL-5), tissue inhibitor of metalloproteinase 1 (TIMP-1).

Without HBOT plasma MDA levels were significantly higher in diabetic rats compared to controls, this difference was abolished after HBOT. The plasma levels of pro-inflammatory cytokines were increased in untreated diabetes (CINC-1: 69.10 ng/L [63.85, 74.63] vs. 137.10 ng/L [66.71, 343.80], $p \leq 0.01$; LIX: 282.00 ng/L [141.90, 382.00] vs. 430.80 ng/L [327.00, 604.40], $p \leq 0.05$), however due to HBOT this difference was ceased (CINC-1: 66.96 ng/L [61.30, 83.50] vs. 96.90 ng/L [70.73, 147.50], NS.; LIX: 245.30 ng/L [172.60, 340.30] vs. 346.80 ng/L [276.90, 774.60], NS.). TIMP-1 was not altered by diabetes in the lack of HBOT, on the other hand after HBOT the plasma level of TIMP-1 was significantly higher in diabetic animals (20.76 μg/L [18.43, 25.62] vs. 32.31 μg/L [21.65, 38.43], $p \leq 0.01$).

According to our results, two weeks after HBOT, the oxidative stress and cytokine alterations induced by experimental diabetes were transformed in a favorable direction. Generally these findings suggest that HBOT is safe to use in diabetic patients, and may also have beneficial effect on their chronic subclinical inflammation.

P8.16

Muscle fatigue index and lactate level in sedentary young and elderly women

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Muscle fatigue may be defined as an exercised-induced percentage reduction in the capacity of the neuromuscular system to generate force, work, or power. In humans, fatigue is defined as the decrease in the maximum force or power compared to an individual baseline. This operationalization is particularly important because it allows the comparison of muscle fatigue between diverse populations, such as the young and the elderly. Published data indicate that women have greater resistance to fatigue related to aging process. Several factors have been taken into account to explain these observed differences between the genders that range from differences in muscle mass to hormonal influences. The appearance of muscle fatigue may be due to neurological or metabolic processes.

The aim of this study was to compare and assess the correlation between the knee muscle fatigue index and lactate production among sedentary young and elderly women.

This study was a cross-sectional investigation which 32 young (women; 20–40 years-old who were sedentary) and 32 elderly individuals (women; ≥ 65 years-old who were sedentary) were included. Isokinetic dynamometer was used to evaluate muscle fatigue. Capillary blood was obtained from digital area using heparinized micro tubes, immediately transferred to polyethylene tubes containing 1% sodium fluoride and then stored frozen. The blood lactate level was determined in the samples by using the electro-enzymatic method.

The results showed that muscle fatigue index of the knee extensor of the dominant side was higher in the young individuals ($p=0.02$). In the three measurement periods of the lactate levels, a significant difference was seen between the groups ($p < 0.05$), but no direct correlation with muscle fatigue index was found (p=ns). Decrease in glycolytic metabolism in the elderly supports these results. In conclusion, no correlation was found between muscle fatigue index and lactate levels which indicates the presence of multifactorial etiologic mechanisms for muscle fatigue.

P8.17

Staphylococcus enterotoxin B and thymic stromal lymphopoietin treatment of keratinocytes as a model for atopic dermatitis

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Atopic dermatitis (AD) is a chronic inflammatory skin disease commonly associated with structural abnormalities of the epidermis and chronic immune activation. Although AD was first thought to be chiefly caused by mutations of the filaggrin gene (FLG) and the concomitant defect in the cutaneous barrier function, recent research has shown that it may develop even in the absence of such a genetic abnormality. Indeed it is now clear that the pathogenesis of AD is also dependent on the abnormal colonization of the epidermis by microbial organisms (most notably Staphylococcus aureus) and the production of proinflammatory mediators by keratinocytes (e.g. thymic stromal lymphopoietin [TSLP]). Research into effective therapies is hindered by a lack of easily reproducible yet effective model systems.

To effectively mimic the AD-like environment found in the lesional skin of AD patients we aimed at treating two keratinocyte cell lines and primary normal human epidermal keratinocytes (NHEKs) with mediators known to present in such lesions, namely Staphylococcus enterotoxin B (SEB) and TSLP, modeling pathological microbial colonization and keratinocyte-induced inflammation respectively.

The effectiveness of SEB-TSLP treatment was determined by measuring the production of pro-inflammatory mediators using quantiative real-time PCR (QPCR). On keratinocyte cell lines the mRNA expression of interleukin (IL) 1α, IL6, IL8, chemokine (C-C motif) ligand 17 (CCL17), tumor necrosis factor-α (TNFα) and nerve growth factor (NGF) were all significantly increased, pointing to the initiation of pro-inflammatory processes on these cells. On NHEKs the treatment had similar effects, although the expression of NGF did show significant change. Interestingly the level of two differentiation markers known to participate in effective barrier formation, FLG and loricrin were decreased upon SEB-TSLP application.

These data show that our model could effectively simulate changes characteristic of AD-lesions.

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P8.18

CARD9 mediates autoantibody-induced autoimmune diseas by linking the syk tyrosine kinase to chemokine production

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Background: CARD9 is a CARMA-like intracellular protein that is highly expressed in innate immune cells. In contrast to its well-defined role in fungal recognition pathways in dendritic cells, its function in non-infectious autoimmune inflammation has not been described yet, despite the fact that a genome-wide association screen raised a possible association between CARD9 gene polymorphisms and a human autoimmune arthritis. Here, we investigated the role of CARD9 in autoantibody-mediated diseases by a transgenic method.

Methods: We used the neutrophil-, macrophage-, Fc receptor and Syk-mediated K/BxN serum transfer arthritis and the anti-collagen type VII antibody-mediated epidermolysis bullosa acquisita (EBA) models in the presence or absence of CARD9. Bone marrow neutrophils (or cultured bone marrow-derived macrophages) from wild type, Syk- or CARD9-deficient mice were stimulated through their Fcγ receptors, followed by the analysis of the superoxide release (as a short-term response) and chemokine release (as a long-term response).

Results: The absence of CARD9 resulted in a partial, but significant decrease in arthritis severity and CARD9−/− mice showed a moderate skin inflammation in the EBA model compared to the wild type animals. Neutrophil (and monocyte) accumulation at the site of inflammation was strongly affected, while CARD9-deficient neutrophils (and monocytes) had normal migratory capacities to the joints in wild type/CARD9−/− mixed bone marrow chimeras. Surprisingly, the synovial levels of the chemokines CXCL1 and CXCL2 were dramatically reduced in the absence of CARD9 upon arthritis induction. While Syk−/− neutrophils (and macrophages) failed to produce superoxide or release chemokines compared to wild type cells when stimulated through their Fcγ receptors among in vitro conditions, CARD9−/− neutrophils showed normal short-term, but strongly reduced long-term responses.

Conclusions: CARD9 plays an important role in the development and progression of autoimmune arthritis and autoimmune blistering skin disease in mice, likely by linking the Syk tyrosine kinase to chemokine production in innate immune cells.

P9

Endocrinology and Metabolism

P9.1

The potential contribution of alarin to the regulation of energy balance in rats

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Introduction: In the background of obesity, regulatory alterations in energy balance affecting peptide systems may also be assumed. Regulation of energy balance does not only involve maintenance of body weight but also that of metabolic rate and core temperature. The contribution of alarin, a new member of the orexigenic galanin peptide family to the regulation of energy metabolism has been recently suggested. Our aim was to analyze the thermoregulatory and food intake related effects of alarin in rats.
Methods: Adult male Wistar rats received full-length alarin, its truncated form (Ala6-25Cys) and scrambled alarin in various intracerebroventricular doses at cool or slightly subthermoneutral ambient temperatures. In semi-restrained animals resting oxygen consumption (VO2, indicating heat production) heat loss (assessed by tail skin temperature) and core temperature (Tc) were recorded in an indirect calorimeter system (Oxymax). In freely moving animals the spontaneous and fasting-induced food intake were automatically recorded in a FeedScale system.
Results: Upon alarin injection, even at cooler ambient temperatures a slow increase in VO2 and continuous tail skin vasoconstriction induced a rise in Tc. This effect was not dose-dependent, however, the administration of Ala6-25Cys prevented this action. Fasting-induced food intake was significantly reduced by alarin.
Conclusion: Alarin appears to elicit a hypermetabolic, hyperthermic thermoregulatory response and an anorexigenic effect in rats. Such responses characterize catabolic rather than anabolic mediators. Ala6-25Cys seems to act as an antagonist to the thermoregulatory effects. Further investigations are needed to clarify the complex role of alarin in energy homeostasis.

P9.2

Endocrine distruptor effect of Bisphenol A on the developing cerebellum, through estrogen and thyroid hormone receptor expression level changes

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In animal physiology a wide variety of the hormone-regulated events are influenced by endocrine disruptors (EDs). The potential mechanisms of ED actions are numerous but most of them are not discovered yet. After the ED is ingestedthe resulting symptoms are extremely versatile and they depend on the chemical structure of the molecule, and the hormone signaling pathway of the hormone which is affected by the ED. In our work we demonstrated the effect of bisphenol A (BPA) on the estrogen- and thyroid hormone receptor (ER, TR) expression (in a primary cerebellar cell culture, on transciptional and on translational levels also). Results are compared to non-treated controls and to samples obtained from age-matched in situ cerebella, in cultures that contain glial cells, and in cultures without glial cells. As results we saw that ER and TR expression levels depend on the individual, as well as combined presence of estrogen and thyroid hormones. The glial cells can mediate the hormonal ER-TR expression regulation in the neurons. BPA has strong, characteristic effects on the receptor expression levels. According to the results, both estrogen and thyroid hormones are required for the cerebellar development and the ED BPA may influence this process in a manner more complex than previously thought.

P9.3

Magnesium status and insulin resistance in subjects at risk for type 2 diabetes

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INTRODUCTION: Diabetes type 2, probably polygenic disease, is a major public health problem through its metabolic consequences and long-term vascular. The magnesium deficiency is frequently associated with diabetes mellitus. This depletion ubiquitous cation in the body, alters glucose metabolism and insulin sensitivity in type 2 diabetic patients, is also implicated in the occurrence of complications and the risk of diabetes occurred targeting insulin resistance states, subjects with impaired glucose tolerance, related to 1st degree diabetics subjects and women with a history of gestational diabetes should receive a follow-up search of a magnesium deficiency, expression of a lack of supply and / or increased urinary loss. The cause of magnesium deficiency in diabetic patients is not fully known. Recently, ion-selective electrodes have become available to determine the ionized Mg in the plasma, which is a better indicator of the state of Mg relative to the total concentration of serum Mg.
MATERIALS AND METHODS: It was evaluated the status of magnesium and the HOMA insulin resistance (homeostatic

model assessment) in: known and treated diabetic subjects (controls) = 102, subjects with decreased glucose tolerance = 103 and subjects first degree relatives of type 2 diabetic patients = 95. The blood samples were collected into plain, on EDTA tubes (K3 EDTA) and heparin tubes (lithium heparin), fasting morning. The urine is collected in a plastic container (non-metallic) = Pot urine. All assays were performed on the same day, with the exception of insulin. For this hormone, serum aliquots were stored in a freezer until assayed. Software Statistical Package of Social Sciences (SPSS 17.0) was used. To assess the statistical significance of the parameters, we used the ANOVA test.

RESULTS: The results show overall and significantly (P <0 01) in the different groups studied, fasting blood glucose and postprandial, of HOMA insulin resistance, correlated with lowered plasma levels of magnesium .

CONCLUSION: Our results suggest the interdependence of glucose metabolism with magnesium status.

Keywords: Type 2 diabetes - magnesium- HOMA.

P9.4

Evaluation of spatial learning and memory, level of serum cholesterol and tryglyceride in caloric restriction applied adolescent female rats

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Caloric restriction is to reduce calorie intake while maintaining the essential nutrients and it is different from severe hunger. It prolongs the survival of several species, reduces neuronal damage and blood cholesterol levels. It is thought that there is an association between dyslipidemia and cognitive functions. Cholesterol plays an important role in synaptic plasticity, learning and memory. We investigated the short term and long term effects of caloric restriction in adolescence on serum triglyceride, cholesterol levels and spatial memory. 4 groups of adolescent female sprague dawley rats were used. The first group of animals were taken to caloric restriction for 4 weeks beginnig from the postnatal day 28 and their spatial memory performance were tested in Morris Water Maze task after the restriction.

The same procedure was applied to the second group but they were given 3-weeks standard diet after these 4 weeks restriction. Their performance in Morris Water Maze task were tested after 3-weeks standard diet. Other groups were the controls of these groups, they were fed with standart diet during whole experiment. Serum triglyceride and cholesterol levels of the caloric restricted groups decreased significantly. Caloric restricted rats in adolescence had a significantly better performance in Morris Water Maze task in their adult period than their corresponding control.

These results suggest us that caloric restriction in adolescence may show its improving effects on spatial memory by reducing serum triglyceride and cholesterol levels.

P9.5

Academic stress effects food choice in health school students

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Academic stress effected feeding behaviours in college students (3). Uncontrollable stress increase the hyperphagia and consumption of delicious food (too fatty and sugary). Previous studies show that, long-term high cortisol levels increased high-calorie sweet food intake in the ongoing stress (1). Stress induced eating behaviours and probably neurobiological adaptations as glucose metabolism, insulin sensitivity and other appetite related hormones, controlled the by HPA axis.

We aim to define In this study, stress-related eating behaviours and and food choices investigated in 32 healthy college students(18-22 years old), Nutrition and Dietetic and Nursing students in Kirklareli University Health School. In exam week, blood pressure, body mass index(BMI) and waist to hip ratio measured and a questionnaire was used to measure the sugar intake from sweet foods and Perceived Stress Scale(PSS) and student stress inventory(SSI) scale was used to measure stress levels. The students in 2nd year show higher stress level than 3rd year (p <0,05). Snake foods consumption increased by exam week and sugar intake also increased. The students in 2nd year preferred more sweet foods and sweet drinks. Blood pressure and BMI has no significant effect on food choice or stress level. The students of health school is expected to be preferred better eating habits and better food choice, in a manner similar to other studies, they chose unhealthy food.

As a result academic stress have effects on food choice in both the second and third grade students. This study could be used to understand of the metabolic and psychological disorders which especially increasing among college students.

P9.6

Effects of Quercetin on depression-like behavior

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Objective: Diabetes and depression are frequently concurring health problems that both have increasing prevalence worldwide. There are therapeutic approaches against diabetic depression, which depends on antidepressants, but poor treatment adherence, mostly raised by side effects, among diabetics embodies a serious obstacle. In the present study, we aimed to investigate the effects of quercetin, a naturally occurring flavonoid, on depression-like behavior in rats.

Methods: Adult 45 male Wistar albino rats were randomly divided into diabetic (n=24) and non-diabetic (n=21) groups that each group was consisted of 3 sub-groups (control, quercetin 50 mg/kg, and quercetin 100mg/kg). Single dose of streptozotocin (60 mg/kg, i.p.) was administered to diabetic

group which was fasted overnight. Tail blood was obtained 72 hours following streptozotocin injection, and animals that had blood glucose higher than 250 mg/dL were considered as diabetic. All streptozotocin injected animals were diabetic by day 3 post-injection. Control groups were treated with 0.5% carboxymethyl cellulose (i.p.), and quercetin groups were treated with 50 mg/kg or 100 mg/kg quercetin (i.p.) which dissolved in 0.5% carboxymethyl cellulose for 21 days. The animals were objected to forced swim test for 5 minutes after a 15 minute acclimatization the day before.

Results: Diabetes induced a 63.7% increase of immobility duration as compared to non-diabetic control ($p=0.001$). Application of 50 mg/kg quercetin decreased depressive behavior in both non-diabetic and diabetic groups (respectively, $p=0.007$ and $p=0.033$). Higher dose of quercetin (100 mg/kg) was ineffectual in ameliorating immobility in both diabetic and non-diabetic groups ($p >0.05$).

Conclusion: Quercetin is a naturally occurring flavonoid that is abundant in the plant products, and our results show that 50 mg/kg quercetin supplementation can be beneficial in ameliorating depressive behavior in diabetics. It's well known that excessive intake of antioxidants can make them act as prooxidants and so, higher dose of quercetin (100 mg/kg) did not exhibit such antidepressant-like effect probably due to pro-oxidation.

P9.7

Effect of different doses of Quercetin supplementation on element levels of brain tissue in diabetic rats

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Objective: Quercetin is a potent antioxidant which has ample of natural sources. Although human diet is rich of quercetin, there is limited data in medical literature about interaction of quercetin with bodily elements in diabetics and non-diabetics. We aimed to investigate effects of 50 and 100 mg/kg/day quercetin supplementation on element levels of the brain in diabetic rats.

Methods: Adult 45 male Wistar strain albino rats were divided into six groups: Group I (Sham control): % 0.5 sodium carboxymethyl cellulose (CMC) was given intraperitoneally for 21 days (n=7). Group II (Control Qu50): 50 mg/kg quercetin was dissolved in 0.5% CMC and administered for 21 days (n=7). Group III (Control Qu100): 100 mg/kg quercetin was dissolved in 0.5% CMC and administered for 21 days (n=7). Group IV (Diabetic Control): 0.5% CMC was given intraperitoneally for 21 days (n=8). Group V (Diabetic Qu50): Quercetin was dissolved in % 0.5 CMC and supplemented 50 mg/kg/day for 21 days (n=8). Group VI (Diabetic ik Qu100): Quercetin was dissolved in % 0.5 CMC and supplemented 100 mg/kg/day for 21 days (n=8). In groups I – III, supplementation was begun 72 hours after single application of citrate tampon (i.p.), whilst in groups IV – VI, that after 60 mg/kg, i.p. streptozotocin injection for diabetes induction. At the end of the supplementation, all animals were anesthetized with combination of high dose ketamine + xylazine (respectively, 90mg/kg and 10 mg/kg) and sacrificed following deep anesthesia. Brain tissue samples were taken and stored in -70 oC until analyzing. Zinc, copper, iron, selenium, magnesium, manganese, calcium, cobalt, molybdenum, boron, cadmium, nickel, lead, and phosphor levels in brain tissue by atomic emission and element levels were expressed as µg/dL.

Results: In the brain tissue, there was no statistically significance between experimental groups for analyzed parameters ($p >0.05$).

Conclusion: The findings of the present study show that neither 21-day diabetes nor two different doses of quercetin (50 and 100 mg/kg) affected element metabolism of the brain.

P9.8

Exercise and milk-protein supplements: Effects on skeletal muscle sirtuins in rats with elevated risk factors for metabolic disorders

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Epidemiological studies indicate that exercise and consumption of milk proteins associate with a lower risk of metabolic disorders and cardiovascular diseases. These improvements are partially caused by improved aerobic capacity of skeletal muscles. Sirtuins (SIRT1-7) are important regulators of energy metabolism e.g. by inducing mitochondrial biogenesis and by regulating enzyme activities in metabolic pathways. We studied the effects of long-term milk protein supplements (whey protein; WP, and milk protein product containing carbohydrates; PD) and exercise (running wheel; RW) for 23 weeks on skeletal muscle sirtuins in low-capacity runner (LCR) rats. LCR rats are selectively bred for low aerobic exercise capacity and have elevated risk factors for metabolic syndrome and cardiovascular diseases. Rats supplemented with whey and free access to running wheels (WP+RW) ran significantly less during the first 5-16 weeks of the intervention compared to RW and PD+RW groups. For total running distances run at 23 weeks, the WP+WR, RW, and PD+RW groups recorded distances of 495, 740, and 932 km, respectively. All groups with the access on running wheels improved similarly for maximal treadmill running capacity compared to non-running groups (sedentary control; SED). For plantaris muscle, the largest changes in sirtuin protein expression were in mitochondrial sirtuins SIRT3-5. Running increased by 250% ($p <0.01$) SIRT3 expression compared to SED rats. Protein supplements per se did not increase SIRT3 expression but when combined with exercise, increased by 400% ($p <0.001$). Increased SIRT4 expression was observed in RW and WP+RW groups ($p <0.001$). While running alone did not increase SIRT5 expression, all other groups increased significantly ($p <0.001$). SIRT6, which inhibits glycolysis, exhibited decreased expression in RW and WP+RW ($p <0.01$ and <0.05, respectively) and increased expression in PD ($p <0.001$). SIRT1 and SIRT7 showed more modest differences.

In conclusion, these results suggest that sirtuin activity coordinates an increased oxidation of lipids for energy use in response to exercise and protein supplementation.

P9.9

Age- and nutritional state-related catabolic effects of a central leptin infusion

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In the course of aging well-defined changes appear in the regulation of energy homeostasis: middle-aged people tend to gain weight, while old people show anorexia with sarcopenia. As these trends are also observed in other mammals, alterations in the activity of catabolic and anabolic peptides may be assumed in the background. Leptin is a catabolic adiposity signal produced in white adipose tissue acting mainly in the hypothalamus. It suppresses food intake and enhances energy expenditure leading to weight loss.
Both aging and obesity have been associated with leptin resistance. In the present study we analyzed how central catabolic leptin effects are influenced by aging and nutritional states. Heart rate (indicating MR) and core temperature of male Wistar rats of different nutritional states [normally fed, calorie-restricted (CR) and high-fat diet-induced obese (HF)] and age-groups (from 3- to 24-months) were registered during an intracerebroventricular 7-day leptin infusion (1 μg/μl/h) in a biotelemetric system. Body weight and food intake were measured daily. For statistical analysis of the data repeated-measures ANOVA was used. Leptin resistance of older animals affected hypermetabolic actions (heart rate and core temperature). Leptin-induced anorexia remained significant in all age-groups. Weight reducing effect of leptin was strong in young, diminished in middle-aged and aging animals and became significant again in the oldest group. In HF rats leptin-induced hypermetabolism of the younger middle-aged group and hypermetabolism plus anorexia of the aging one were suppressed. Calorie-restriction reduced body weight and fat mass to a similar extent in all age-groups. It strongly enhanced leptin-induced hypermetabolism at all ages, and hunger prevented the manifestation of sustained anorexigenic actions of leptin with the exception of the oldest group.
Our results suggest an unexpected increase of responsiveness to anorexigenic leptin actions in old rats.

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P9.10

Adipocytokines and inflammation as a link between obesity and related endothelial dysfunction

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Obesity is characterized by a chronic low grade pro-inflammatory state thatcausespathological alterations of adipocytes development. Adipocytokines fulfill their actions via different signaling pathways and their effects may be beneficial and/or detrimental to endothelium function. For example, adiponectin is the only adipokine that exhibits both anti-inflammatory and anti-atherogenic properties, and is shown to be protective against cardiovascular diseases. In addition, it has the ability to directly stimulate the production of NO in endothelial cells, and also reduces oxidative stress. In contrast to the dramatic increase in plasma levels of several adipokines observed in visceral adiposity, the plasma levels of adiponectin are markedly reduced. Leptin, resistin, TNFa and IL-6 has been directly or indirectly, by increasing endothelin-1 and angiotensin II production, implicated in increasedreactive oxygen species production, contributing to endothelial dysfunction, inflammation and atherosclerosis.Some studies suggest that adipocytokines levels may be independent predictors of endothelial dysfunction in apparently healthy subjects, providing a pathophysiological link between adipose tissue inflammation and early vascular alteration.
Elevated abdominal visceral fat is associated with development of endothelial dysfunction and cardiovascular diseases, independently of total body adiposity. Resistance arteries of obese rats exhibit impaired endothelium-dependent vasodilation, similar to resistance arteries in obese patients that we found, suggesting thatvisceral obesity may be associated with elevated peripheral vascular resistance maintaining hypertension in part of obese patients. Subcutaneous adipose tissue is regarded as less metabolically active than visceral adipose tissue, and these two depots of adipose tissue show functional differences; among others visceral adipose tissue contains a greater number of macrophages and monocytes than subcutaneous. Although visceral adipose tissue has the greatest impact as a cardiovascular risk factor, the metabolic role of endothelial function in visceral and subcutaneous fat has should be more elucidate.

P9.11

The uterine and vascular actions of Estetrol delineate a distinctive profile of Estrogen Receptor α modulation, uncoupling nuclear and membrane activation

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Estetrol (E4) is a natural estrogen with a long half-life produced only by the human fetal liver during pregnancy. The crystal structures of the estrogen receptor α (ERα) ligand binding domain bound to 17β-estradiol (E2) and E4 are very similar, as well as its capacity to activate the two activation functions AF1 and AF2 and to recruit the coactivator SRC3. In vivo administration of high doses of E4 stimulated uterine gene expression, epithelial proliferation, and prevented atheroma, three recognized nuclear ERα actions. However, E4 failed to promote endothelial NO release and acceleration of endothelial healing. Furthermore, E4 antagonized E2 effects on these endothelial processes recognized as being dependent on membrane-initiated steroid signaling (MISS).
We conclude that E4 is a less potent estrogen able to modulate the nuclear/transcriptional activity of ERα, and is not only devoid of ERα MISS in the endothelium, but also able to antagonize these effects. This profile of ERα activation allows to characterize E4 as a selective ER modulator which could have medical applications that should now be considered further.

P9.12

Peripheral CCK-1 receptors in age-related regulatory alterations affecting energy balance

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Introduction: In the background of middle-aged obesity and later appearing anorexia, cachexia, complex age-related alterations in the anabolic (orexigenic, hypometabolic) and catabolic (anorexigenic and hypermetabolic) peptide systems may be assumed. One of the most important anorexigenic agents is cholecystokinin (CCK). CCK produces satiety by the activation of the peripherial CCK1 receptors of the nervus vagus. Our previous studies revealed age-releated alterations in the responsiveness to intraperitoneal CCK injection. CCK administration suppressed food intake in the young and the old normally fed (NF) rats, but failed to reduced it in the middle-aged NF group. Further tests demonstrated that high-fat diet-induce obesity (HF), accelerates the appearance of middle-aged CCK-resistance (already at age 6-months) as well as the return of high sensitivity to CCK in further aging (by age 12-months), while calorie-restriction (CR) prevents the development of middle-aged-resistance. In the present study the role of activity of CCK-1 receptors in age-related regulatory alterations were analyzed by peripheral CCK-1 receptor antagonist administration.
Methods: The effects of 100 μg subcutaneous CCK-1 receptor antagonist devazepide injection on food intake were measured by an automated FeedScale system in NF, CR and HF young adult and middle-aged (3 and 12 months, respectively) male Wistar rats. For statistical analysis of the data repeated-measures ANOVA was used.
Results: Subcutaneous devazepide injection increased food intake in young NF rats in contrast to middle-aged ones. Both CR and HF middle-aged animals showed significant orexigenic responsiveness to CCK-1 receptor antagonist administration.
Conclusion: CCK-1 receptor antagonist-induced orexigenic effects appeared in the same groups of rats in which significant anorexigenic effects of the CCK injection were previously detected. Thus, our studies confirm that age- and nutritional state-related differences in the responsiveness to CCK administration are associated with CCK-1 receptor activation.

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P9.13

The electron transport chain component NDUFB8 is required for glucose-stimulated insulin secretion in MIN6 cells

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Background: Type 2 diabetes evolves from the impaired function of pancreatic beta cells in the face of increased insulin demand. Mitochondria play a central role in glucose metabolism and calcium signaling in these cells, and mitochondrial dysfunction can result in beta cell failure and diabetes. The purpose of the present study was to investigate the role that NDUFB8, belonging to complex I of electron transport chain, plays in beta cell function and whether its inhibition may contribute to type 2 diabetes.
Methods: In order to investigate the role of NDUFB8, its expression was efficiently reduced via an siRNA approach in murine insulinoma MIN6 cell line. We then investigated how NDUFB8 silencing affects mitochondrial function, calcium signaling and insulin secretion, using optical imaging and immunoassay-based approaches.
Results: We showed firstly that NDUFB8 is required for glucose-stimulated insulin secretion, particularly, during the first 15 minutes. This decrease in insulin secretion in NDUFB8-deficient cells was accompanied by significant increase in total insulin content of these cells. Decreased expression of NDUFB8 reduced the rise kinetics of mitochondrial membrane potential, as assessed using tetramethylrhodamine ethyl ester (TMRE), upon stimulation with 7 mM glucose. Furthermore, NDUFB8 inhibition resulted in an elevated and more sustained calcium response to depolarisation by 20 mM KCl.
Conclusions: In conclusion, our results indicate that, in pancreatic beta cells, NDUFB8 is an important player in glucose-stimulated insulin secretion, contributing to changes in mitochondrial membrane potential in response to elevated extracellular glucose levels, and calcium signaling.

P9.14

The effect of obestatin on corticosterone secretion and anxiety behaviour

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Obestatin and ghrelin, both isolated from the GI tract are the products of the same peptide precursor, preproghrelin. Obestatin is a 23 amino-acid peptide, originally described to antagonise the orexigenic effects of ghrelin. Later it has been also shown to influence the endocrine pancreatic function, drinking, memory, sleep as well as anxiety behaviour. The aim of the present study, conducted in male CFLP mice, was to investigate the effect of graded doses of intracerebroventricularly (icv) administered obestatin [(0.5 µg, 1 µg, 1.5 µg)/2 µl aCSF], alone or after CRF receptor blockage with antalarmin (0.1 µg/2 µl aCSF) on behaviour and hypothalamus-pituitary-adrenal (HPA) axis activity.
The behaviour of the animals was observed in a computerized open field (OF) and elevated plus maze (EPM) tests. To assess the HPA activity, plasma corticosterone level was measured 30 min after obestatin administration by fluorescent spectrophotometry. According to our results, obestatin had no effect on general locomotor activity in the OF and EPM. The percentage of open arm time (OAT%) and the percentage of central ambulation distance (central/total ambulation %) however were both decreased by the ascending doses of obestatin, and the latter effect was blunted by the pretreatment with antalarmin. Obestatin also caused a marked elevation of the corticosterone concentration.
These results indicate that obestatin exerts anxiogenic-like effect, which might be mediated, at least partly via the activation of the HPA axis.

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P9.15

Ornithine transcarbamylase deficiency in a young boy with acute liver failure

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Pediatric acute liver failure is defined as hepatic necrosis resulting in loss of liver function within weeks or a few months of the onset of clinical liver disease. It may be caused by a variety of diseases including inborn errors of metabolism. Considering urea cycle disorders, acute liver failure has occasionally been reported as the presenting symptom. In this work, we report the joint occurrence of ornithine transcarbamylase deficiency (OCTD) and describe the metabolic diagnosis pitfalls and challenges of biological follow up.
Our patient is a male, the second child of non-consanguineous Algerian parents. He was born at 40 weeks after an uneventful gestation and delivery, with birth weight 3,600 g, and Apgar scores 9 and 10. He was breastfed until 2 months and then received an adapted cow's milk formula (Biomyl1). At the age of 4 months, the child was hospitalized for increased fatigue with episodic somnolence and coma. His weight 6 kg (50th percentile). Liver was slightly enlarged and truncal hypotonia was noticed. Urgent biochemical evaluation showed lactate 1.4 mmol/L, ammonia 309 µmol/L, albumin 27 g/L, ASAT 648 UI/L, ALT 988 UI/L, γGT 21 UI/L, alkaline phosphatase 307 UI/L. glucose 5.4 mmol/L (4.0–7.8 mmol/L), creatinin 18 µmol/L, urea 1.7 mmol/L, total bilirubin 20 µmol/L, conjuresults revealed elevated transaminases and decreased prothrombin level (32,5%). Physicians have immediately contacted our laboratory center for more investigations. Metabolic work-up revealed citrulline 1,93 g/L, orotic acid excretion of 193,95 mg/g creatinine.
The boy was diagnosed with OCTD. Treatment was started with intravenous infusion of glucose and L-arginine, oral sodium benzoate, and restriction of natural protein intake. Concentrations of ammonia, transaminases and orotic acid excretion normalised within two days, three weeks and one month respectively and the child improved markedly. Our case report shows the importance of urgent and thorough metabolic investigations in young children presenting hyperammonemia with acute liver failure. OCTD should be included in the differential diagnosis.

P9.16

A novel kisspeptin antagonist peptide 234 prevents kisspeptin-induced pubertaladvancement in the female rats

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Kisspeptin has an important role in central regulation of reproductive functions. This neuropeptide stimulates gonadotropin releasing hormone (GnRH) neurons prior to puberty and thus is necessary for activation of hypothalamo-hypophyseal-gonadal (HHG) axis. A recently developed kisspeptin antagonist, peptide 234, has been shown to inhibit kisspeptin-induced release of luteinizing hormone (LH) in rats.
Effects of this novel kisspeptin antagonist on reproductive functions have not been well documented. In the present study, effects of kisspeptin antagonist on kisspeptin-induced puberty onset were studied. The prepubertal Sprague-Dawley female rats were weaned on day 21. The rats were intracerebroventricularly (ICV) cannulated and daily injected with kisspeptin or the antagonist at doses of 50 pmol and 1 nmol, respectively. Puberty onset was monitored by examination of vaginal opening (VO). Body weight was daily determined, and vaginal opening was daily monitored starting from day 26. The animals were decapitated from day 60 when diestrus, which was determined by vaginal smears, was observed. Kisspeptin advanced VO compared to sham rats

(33 versus 38 days, respectively). Puberty onset was delayed in the rats receiving kisspeptin antagonist (41 days). Combination of kisspeptin and peptide 234 resulted in puberty onset similar to sham rats (37 versus 38 days, respectively). Pubertal weight was found to be lower in the kisspeptin injected rats compared to sham group (66.7±3.8 and 86.4±5.4 g, respectively).
In conclusion, this novel kisspeptin antagonist peptide 234 appears to modulate the effects of kisspeptin on puberty onset in female rats.

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P9.17
Decreased insulin sensitivity in multiple sclerosis

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Introduction: Multiple sclerosis (MS) is autoimmune neurological disease characterized by demyelinisation, leading to various neurological symptoms including movement impairment, vision problems, loss of sensitivity and others. Less is known about metabolic alterations during MS, so the aim of our study was to assess glucose metabolism status in MS patients.
Methods: We examined 19 patients with MS and 19 healthy controls matched for sex, age and body mass index (BMI) (9 males/10 females, age 30.4±7.1 and 28.7±6.7 years; BMI 23.7±4.5 and 24.4±5.3 kg/m2 respectively). MS patients were newly diagnosed; first occurrence of MS symptoms was treated by short term metylprednisolone therapy. Examinations were performed 2-3 months after that and patients were currently in remission without any therapy. We used standard oral glucose tolerance test (oGTT), blood was drawn in 15 minute intervals for 2 hours. Glucose, insulin and GLP-1 and lipid parameters were measured. Insulin sensitivity indices (ISI) were calculated.
Results: Fasting plasma glucose was similar in both MS patients and controls (5.2±0.3 vs. 5.0±0.4 mmol/l, p=0.05) with similar levels of insulin (5.6±5.2 vs. 3.9±2.6 mIU/l, p=0.216), resulting in comparable index of insulin resistance IR-HOMA (1.33±1.28 vs. 0.90±0.62, p=0.197). During oGTT glucose levels tended to be higher in MS group, but not significant (p=0.076). However we found clearly increased levels of insulin in MS group (p=0.022) during oGTT. Insulin sensitivity was significantly lower in MS group compared to control group [ISI(Matsuda) 6.95±3.44 vs. 10.60±4.81, p=0.011 and ISI(Cederholm) 49.9±15.3 vs. 61.3±16.3, p=0.032]. Levels of GLP-1 were comparable at the baseline and during oGTT in both groups. We did not find any difference in total, HDL and LDL cholesterol as well as in triglycerides levels between groups.
Conclusions: We found decreased insulin sensitivity in patients with MS compensated by hyperinsulinemia. This could predispose MS patients for future Type 2 diabetes mellitus development.

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P9.18
The effects of hyperbaric oxygen therapy (HBOT) on blood viscosity and erythrocyte aggregation in diabetic patients

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There are only a few studies about hyperbaric oxygen's effect on hemorheological parameters and their results showed an increase in blood viscosity and RBC aggregation both in vivo and in vitro. Some many other studies showed abnormal hemorheological parameters in diabetics and so, this would suggest more complications after HBO therapy however; reality is not consistent with this suggestion. Therefore, in this study, the effects of hyperbaric oxygen therapy on blood viscosity and erythrocyte aggregation have been investigated.
After the approval of local ethical committee, 11 diabetic ulcer patients aged between 42 and 82 were taken to our study. 100% oxygen was applied at 2.4 ATA for two hours in three cycles of 25 minutes of oxygen- 5 minutes air break. Treatments were carried on five days a week. Blood that was collected before the initial HBO therapy was accepted to be control. Samples were also collected after the initial therapy and twentieth one to be evaluated. Corrected whole blood viscosity was measured using a cone/plate viscometer with a hematocrit of 45%. RBC aggregation was measured using a Myrenne aggregometer in both autologous plasma and dextran70 solution.
Our results showed that there were no significant changes in corrected blood viscosity between the samples collected before and after the first and twentieth HBO treatments. Also RBC aggregation in both autologous plasma and dextran70 solution after the first and twentieth HBO treatments were not significantly different than the control samples.
These results were in contrast with the previous experimental studies. The reason of these contradictory results may be caused by experimental method and HBO application differences and/or different reactions of humans and animals. Still, this topic needs further studies to clear such an important effect.

P9.19

Effects of cholesterol, FSH and LH on steroidogenic activity in cat granulosa cell culture

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The aim of this study was to examine the effects of 22R-hydroxycholesterol (22R-HC), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) on estradiol and progesterone production by cat granulosa cells.

Eighteen female adult animals borrowed from cat fanciers were included in the study after the approval of the local ethics committee. Following surgical operation, the ovaries were transferred to the laboratory under sterile conditions. Afterwards, the granulosa cells from follicles were collected and cultured for up to 5 days in 24 well plates coated with 5% fetal calf serum (FCS) in Dulbecco's Modified Eagle's Medium (DMEM)/HAM F-12: supplemented with 10-7 M androstenedione, 1% ITS premix and 0.1% bovine serum albumin (BSA), in the presence or absence of 22R-HC (10 μg/ml), FSH or LH (10, 100 ng/ml each) on first and third day.

Treatment of cells with 22R-HC resulted in an increase ($p < 0.05$) in progesterone and estradiol production on day 3 and day 5 of the culture. When 22R-HC was used at a concentration of 10 μg/ml on day 3 and 5, it resulted in a 9.1 and 13.5 fold increase in basal progesterone production, and 3.7 and 4.7 fold increase in basal estradiol production, respectively. Incubation of cells with both concentrations of FSH (10 ng/ml and 100 ng/ml) resulted in significant stimulations of progesterone ($p < 0.001$) on days 3 and 5 whilst incubation had no effect on estradiol production. None of the doses of LH had any effect on estradiol production on day 3, nor on progesterone production on days 3 and 5 by granulosa cells.

In conclusion, it is the first time a successful protocol has been established for granulosa cells isolated from queens. Basal estradiol synthesis is increased as incubation time advanced in all groups unlike basal progesterone production. The cells secrete substantial amounts of estradiol inresponse to cholesterol treatment at least 5 days of incubation. FSH but not LH has significant effect on progesterone production during incubation of cat granulosa cells. This cell culture system might be useful for investigating a variety of aspects of granulosa cells function in female cats.

P9.20

Exposure of pregnant rats to angiotensin 2 leads to an increase in blood pressure in their adult male offspring

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Renin-angiotensin-aldosterone system (RAAS) has an essential role in the homeostatic control of arterial blood pressure (BP), in tissue perfusion and control of extracellular fluid volume. Some pathological conditions such as preeclampsia or gestational diabetes mellitus may lead to increased activity of certain pathways the RAAS during pregnancy and modulate the development of key organs in regulating blood pressure. The aim of our experiment was to evaluate the effect of increased angiotensin 2 (Ang2), as a key hormone of RAAS, during pregnancy of female Wistar rats on BP of their offspring and sensitivity to salt intake in adulthood. Experimental females (n = 5) were on 6th day of gestation implanted with osmotic minipumps releasing Ang2 (2 mg×kg-1×h-1) and control females (n = 4) were sham-operated at the same time. For analysis, we used their offspring, 14 males affected by prenatal Ang2 (A) and 12 control (C) males. We measured BP using tail cuff plathysmography method and determined plasma renin activity (PRA), aldosterone, triiodothyronine (T3) and thyroxine (T4) levels by radioimmunoassay in their plasma. Fixated kidneys were frozen and used for morphological and immunohistochemical analyses. Prenatal exposure to Ang2 led to increase in BP in adult male rats, with no effect of increased salt (2% NaCl) intake. Relative heart weight did not change between the groups, but we observed a tendency to increase in the relative weight of the kidneys in C rats fed with 2% NaCl. Aldosterone levels did not change between A and C males, however we observed in both groups a decline as an effect of higher concentrations of salt in their diet. In PRA, we observed a decline after applying salt only in A rats.
Our results demonstrate an organizational impact of a prenatal exposure to Ang2 on increase in BP and suggest the role of early ontogeny for the setup of BP regulation.

P9.21

Asiatic acid improves vascular functions in mesenteric vascular beds isolated from high-carbohydrate, high-fat diets-induced metabolic syndrome rats

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Objective: We investigated the effect of asiatic acid (AA) on vascular responses to sympathetic nerve stimulation (SNS), or exogenous vasoactive agents in mesenteric vascular beds (MVBs) isolated from metabolic syndrome (MS) rats.
Methods: Male Sprague-Dawley rats were fed with high-carbohydrate, high-fat diets (HCHF) diets plus 15% fructose in drinking water for 12 weeks to induce MS and orally administered with vehicle or AA (20 mg/kg BW/day) for the last 3 weeks. Then, blood pressure (BP), heart rate (HR) and metabolic indices were evaluated. MVBs were isolated and perfused with physiological salt solution. Pretreatment capsaicin (0.1 μM) was performed to abolish sensory neurgenic vasorelaxation. Contractile responses to SNS (electrical field stimulation, EFS (5-40 Hz, 90V, 1 ms for 30s, at 5-min intervals)), and exogenous noradrenaline (NA) (0.15 nmol-15 nmol) were examined. Moreover, vasorelaxation responses to acetylcholine (Ach) (10 pmol-1μmol) and sodium nitroprusside

(SNP) (10 pmol-1μmol) were examined under methoxamine-raised tone conditions.
Results: Rats fed with HCHF diets exhibited signs of MS including, high BP, dyslipidemia, and increased fasting blood glucose and plasma insulin. MS rats treated with AA significantly improved BP, HR and metabolic alterations ($p < 0.05$). Contractile responses to EFS in MS group were enhanced comparing to the response in control rats (at 30 Hz, 85.38 ± 13.43 mmHg vs. 31.75 ± 9.09 mmHg, $p < 0.001$) while, there was no significant difference of contractile responses to EFS in preparations of MS rats treated with AA (at 30 Hz, 48.30 ± 10.30 mmHg) and control group ($p < 0.01$). The response to exogenous NA did not differ across all groups of rat. The blunted response to Ach in MS was significantly improved after AA treatment ($p < 0.01$). There was no significant difference in the response to SNP across all groups of rats.
Conclusions: AA decreases blood pressure in MS rats. This might be related with an inhibitory effect on sympathetic neurogenic vasoconstrictor responses which is likely to involve the pre-junctional site. Furthermore, AA also improves endothelium-dependent vasorelaxation in MVBs of MS rats

P9.22
Examination of macrophage migration inhibitory factor(MIF) and pituitary adenylate cyclase-activating polypeptide(PACAP) in human breast milk samples

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Breast milk contains several bioactive compounds that play important roles in the development of the nervous system and in gaining immuncompetence. PACAP is a neuropeptide with important functions in reproductive and developmental processes. Recently, we have shown that PACAP38 is present in high levels in breast milk and we have described changes of PACAP levels during lactation in the first 17 months. Earlier we have also examined the presence of MIF, a proinflammatory cytokine, in human milk samples, but previous prospective studies have only focused on the water phase of breast milk in the first 3 months of nursing. In the present experiment we aimed to examine the changes of MIF level both in the water and lipid phase of milk samples during the first 6 months of lactation. It is well-known that the constitution of breast milk is influenced by numerous external agents, for example the gender of the newborn. Therefore, in the second part of our research we analyzed the difference between the PACAP concentration of the milk samples of male and female newborns. We collected 5 ml milk every month during the first 6 months of nursing. First we separated the milk samples to lipid phase and water phase by centrifugation. We used ultrasonication to factor the lipid phase, and with this method we obtained an additional lipid fraction and water fraction. We measured the MIF concentration with ELISA technique from each samples.
The PACAP level of the milk samples were measured by RIA examination. In our experiment we detected the presence of MIF in the lipid phase of human milk for the first time. We measured higher MIF concentrations in the water fraction than in the lipid fraction. We were also the first to show an increasing tendency of the MIF concentration in the lipid layer of human milk during a long-term 6-month follow-up period. Our preliminary examinations did not find significant differences between the PACAP level of milk samples from male and female newborns. Our future aim is to establish the exact influence of MIF and PACAP in the process of lactation with additional clinical and molecular biological experiments.

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P9.23
Correlation of visfatin mRNA expression in subcutaneous adipose tissue with anthropometrical measures and biochemical parameters of youth male

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INTRODUCTION: Visfatin is adipokine regulating metabolic function, insulin sensitivity and insulin secretion. The aim of this study was to assess the correlation of visfatin mRNA level in adipose tissue and protein visfatin level in blood with anthropometrical and biochemical parameters of youth prepubertal male.
METHODS: Case control study was carried out at University Hospital Osijek, Department of pediatric surgery. From youth male from 3 months to 10 years old, subcutaneous and visceral adipose tissue, anthropometrical measures and biochemical parameters were taken. Visfatin protein level was assessed by ELISA (Fenixpeptides, SAD) and mRNA expression by real time PCR (Bio Rad, SAD). Tissue level of visfatin mRNA expression was correlated with protein expression of visfatin in blood and with anthropometrical and biochemical parameters. Spearman correlation is used for analyzing correlations and $p < 0.05$ was considered statistically significant. Experiment was approved by Ethical Committee of University Hospital Osijek.
RESULTS: The results show negative correlation of visfatin mRNA expression in subcutaneous adipose tissue with height ($P=0.009$, $R2=-0.461$), body weight ($P=0.002$, $R2=-0.521$), age ($P=0.008$, $R2=-0.468$), thigh circumference ($P=0.006$, $R2=-0.480$) and creatinine ($P=0.005$, $R2=-0.494$), but not with body mass index, glucose blood level and visfatin protein expression in blood. There was negative correlation of visfatin mRNA expression in visceral adipose tissue with thigh circumference ($P=0.019$, $R2=-0.439$) and abdominal circumference ($P=0.003$, $R2=-0.545$), but not with body mass

index, blood glucose level and protein expression of visfatin in blood.
CONCLUSION: Adipose tissue expression of visfatin mRNA in is not correlated with protein expression of visfatin in blood. Subcutaneous adipose tissue had higher visfatin expression than visceral adipose tissue and negatively correlates with height, body weight, age, thigh circumference and creatinine. Visfatin mRNA expression is higher in adipose tissue of younger prepubertal youths. Visfatin may have bigger metabolic effect at earlier age, and higher expression is connected with different pathologies.

P9.24

Effect of boron on spontaneous and oxytocin induced contraction in rat myometrium

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Boron is a natural semimetallic element and considered to be an essential micronutrient. Some effects of boron compounds were determined such as inhibition of cell proliferation in prostate cancer cells, intracellular calcium signaling and enhancing effects on the action of estradiol on calcium and magnesium homeostasis. There is a little finding about the effect of boron on smooth muscle contractility and reproductive system.
We aimed to investigate the effect of borax, a boron compound, on myometrial contraction in rats in isolated organ bath. Virgin female Wistar rats were daily exposed to vaginal smear. Only diestrous animals were included to experimental procedure. Myometrium strips were removed from rats following decapitation and placed in a jacked tissue bath containing Krebs solution at 37 oC and pH 7.4, constantly bubbled with 95% oxygen and 5% carbon dioxide. The myometrial strips were allowed to equlibrate under 1.5g tension and isometric contractions were measured by force displacement transducer. Control contractions were recorded for 15 min and increasing concentrations of boron were added to the tissue bath cumulatively (0.1, 0.5, 1, 5 and 10 mg/ml). Boron applications were performed on spontaneous (n=6) and oxytocin induced contractions (n=7). The amplitude and frequencies of contractions were evaluated at 15-min interval before and after applications of boron and determined as mean±SEM. Boron completely inhibited both amplitude and frequency in spontaneous contractions at 5mg/ml (p=0.0001). There was no difference in the other concentrations of boron. Similarly, boron reduced both amplitude and frequency in oxytocin induced contractions at 5mg/ml (p=0.001 and 0.0001, respectively). Treatment of boron at 10mg/ml concentration completely abolished the contractions in oxytocin induced group (p=0.0001). In this study, we demonstrate that boron has an inhibitory effect on myometrial contractility in a dose dependent manner in rats. Further detailed investigations are needed to clarify the exact mechanism(s) of inhibition boron on myometrium contractility and the possible role of boron in pregnancy and parturition processes.

P9.25

Effects of application of chlorpyrifos ethyl and rose water on rat pancreas

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Unwanted insects, rodents, plants, algae and other harmful applied for the prevention of false and excessive use of pesticides, the environment, animals and humans may cause adverse effects on.
Rosa damascena Mill. (Rosaceae) in the world, rose water and rose oil is used in the production of various important laughed. Food products made from these varieties of roses and cosmetic industry is heavily consumed.
In this study, an organophosphate pesticide, chlorpyrifos ethyl and rosewater application of the hormone insulin in rats and effect on pancreatic tissue was investigated. 4 groups of rats were formed into 8 groups for this purpose. The study of 32 adult male Wistar albino rats were included in Group I: control (normal feed), II. Group: chlorpyrifos ethyl (CPE) group (0.3 mg / kg / day), III. Group: rose water (100 mg / kg / day) group (RW), and IV. Group: CPA (10 mg / kg / day) + RW (100 mg / kg / day) group including rats were divided into 4 groups. Twenty rats were sacrificed one day after the administration of blood were taken. In blood insulin analysis, the pancreatic tissues malondialdehyde (MDA) and superoxide dismutase (SOD) has been analyzed.
As a result, compared with the control group treated rats CPE insulin levels significantly ($p < 0.001$) increase was determined. RW treated rats compared to the control group could not be identified by a significant change ($p > 0.001$). If RW + CPA group there is a significant increase in the amount of insulin but this increase is less than that of CPA group.
Compared to the control group, RWE pancreatic tissues of rats treated with SOD were significantly higher ($p < 0.001$). CPA pancreatic tissues of rats treated with SOD was significantly lower compared to the control ($p > 0.001$). These data; CPE application, on insulin hormone in rats resulted in a negative result indicates that. This result CPE toxicological effects on the pancreas may be caused.

Key Words: Chlorpyrifos ethyl (CPE), rose water (RW), insulin, pancreatic.

P9.26

Nesfatin-1 levels in response to the patients with different glucose tolerance levels

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The close relationships between nesfatin-1 levels and diabetes have been suggested in previous studies. In this in the study we aim to investigate serum nesfatin-1 levels in 4 different metabolic disorders, including; metabolic syndrome (MS),

Type 2 diabetes mellitus (T2DM), impaired glucose tolerance (IGT) and impaired fasting glucose (IFG).
Twenty control and 80 patients with different metabolic disorders (n=20 for each group) were participated to this study after giving a signed informant consent which was approved by the local ethical committee. Serum nesfatin-1 levels were measured by commercially available ELISA method. Unpaired t-test was used to analyse data between control and each group.
There are significantly different in fasting blood glucose and HbA1c levels between the groups and control ($p < 0.05$). Serum nesfatin-1 level was 1.045±0,12 ng/ml in control group. It was found to be 0.972±0.05 ng/ml in MS group; 0.937±0.06 ng/ml in T2DM group; 1.057±0.08 ng/ml in IGT group and 0.917±0.05 ng/ml in IFG group. These observed differences between control and other groups were not statistically significant ($p > 0.05$).
There is no close agreement between increase or decrease of nesfatin-1 levels and diabetes in literature. In this study, we have observed a decrease in nesfatin-1 levels. However this decrease was not statistically different compared with control level. This could be related with the number of subjects used in this study which is less. In addition, this could be related with subjects condition e.g newly diagnosed or already started to treatment. To understand the role of nesfatin-1 in diabetes further investigation are needed with a quite large number of newly diagnosed patients.

Key Words: Nesfatin-1, diabetes mellitus, metabolic syndrome, HbA1c

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P9.27

Irisin level in response to the patients with metabolic impairments

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Irisin, which is a newly described myokine and adipokine hormone, has important role on thermogenesis related energy expenditure and improvement of metabolism. In addition, existence of an important close links between health status and irisin levels has been suggested. In the present study, we aimed to understand to relationships between irisin levels and impairment of metabolic function in intensive care unit patients especially in patients with cerebrovascular disorders, ischemic heart disease and pulmonary disease.
The study protocol was approved by the local ethics committee, and informed written consent was obtained from each patients' parent at the start of the study. Total of 90 patients in intensive care unit (n=40 for cerebrovascular disease; n=30 myocardial infarcts; n=20 pulmonary disease) and 20 control subjects were participated to this study. Venous blood samples were taken and analysed for serum irisin levels using ELISA method. Unpaired t-test was used to analyse data between control and each patients group.
Irisin level was 1.77±0.19 ng/ml for the control group. Its level was found to be markedly higher in cerebrovascular disease group 3.11±0.27 ng/ml ($p < 0.05$), in myocardial infarction group 2.67±0.33 ng/ml ($p < 0.05$) and in pulmonary disease group 2.75±0.48 ng/ml ($p < 0.05$).
The observation of markedly high irisin level in all patients group could be the clue of its protective effects under the condition of impaired body metabolic systems. The further studies concerning post treatment response in these patients group will provide the better answer to these protective effects.

Key Word: Irisin, cerebrovascular disorders, myocardial infarction, pulmonary disease

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P9.28

Can Apelin-13 be a new actor in control of obesity?

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Apelin and their receptors (APJ) are expressed in the central nervous system, including the hypothalamus, and in numerous other peripheral tissues (muscle, adipose tissue etc.)
There are two different types of adipose tissue in the human body, which have opposing functions, it has been known since long time. White adipose tissue (WAT) is the main tissue of energy storage while brown adipose tissue (BAT) has important role in thermoregulation and energy expenditure. One such family of proteins is the mitochondrial uncoupling proteins (UCPs), which are anion carriers located in the mitochondrial inner membrane. There are five known homologues (UCP1 to 5), of which UCP1 predominantly expressed in adipose tissue while UCP3 generally expressed in muscle. The present study was designed to investigate the effects of chronic central administration of two different doses of apelin-13 on food intake, body weight and energy expenditure.
In this study, 30 Sprague Dawley male rats were used. Animals divided into 3 groups (n=10). Rats in the experimental groups, apelin-13 at 1 and 50 µg/kg doses and rats in control group, the same amount of saline was injected intraperitoneally for 14 days. During this time, daily food consumptions and body weights of animals were recorded. End of the experiment, animals were scarified and intrascapular white and brown adipose tissues, biceps muscle samples were taken. UCP1 and UCP3 mRNA levels were determined from taken tissue samples by using RT-PCR.
Injection of low and high dose of apelin-13 caused to significant reductions both UCP3 mRNA levels in biceps muscle tissue and UCP1 mRNA levels in white and brown adipose tissues ($p < 0.05$). While apelin-13 significantly increased daily food consumption ($p < 0.05$), in the body weights of animals did not seen any significant change.
The results of this study showed that peripheral apelin-13 injection caused to reduction in energy expenditure by

reducing UCP1 levels in brown adipose tissue and UCP3 levels in biceps muscle.

Acknowledgement: This study was supported by Inonu University BAP (Project 2013/207).

Key Words: Apelin, UCP1, UCP3, white adipose tissue, brown adipose tissue

P9.29

Effects of chronic central administration of irisin on food intake, body weight and body temperature in the rats

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Homeostasis of energy is regulated by hypothalamus. Regulation of this homeostasis is multi-factorial, involving many appetite-stimulating and appetite-suppressing peptide hormones. Irisin is newly discovered peptide and it has critical role for regulating energy metabolism. Irisin is synthesized principally in the heart muscle and several peripheral tissues such as salivary glands, kidney and liver. Irisin is defined an anti-obesitic and anti-diabetic hormone regulating adipose tissue metabolism and glucose homeostasis by converting white to brown adipose tissue.
The study was designed to evaluate effects of chronic central administration of irisin on food intake, body weight and body temperature in the rats In this study, 250±6.8 g in weight of 40 male Wistar-Albino rats were used. Rats were evenly separated into four groups (n=10). Osmotic mini-pumps were implanted to lateral ventricule and artificial cerebrospinal fluid (vehicle; sham group), 10 and 100 nM concentrations of irisin were infused for 7 days. Throughout the experimental period, the rats were kept in individual cages, and food consumption, body weight and body temperature of the animals were daily recorded. At the end of the study, chronic infusion of both concentrations of the irisin caused to significantly increases in food consumption and body temperature ($p < 0.001$), but in the body weights of rats were not found any significantly difference.
The study results support that irisin is an anti-obesitic myokine also it is evidence that irisin can play important roles in hypothalamus on regulation of feeding behavior and control of energy metabolism.

Acknowledgement: This study was supported by TUBITAK (Project no: 104S138).

Key Words: Irisin, food intake, body weight, body temperature, obesity

P9.30

Estimation of the relationships between irisin concentration and food intake, body weight and body temperature using polynomial regression models in the rats

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Irisin is a newly-identified myokine and is associated with food intake (FI), body weight (BW) and body temperature % (BT%) reported by our previous study. The polynomial regression (PR) models can be used to estimate the relationship between study and predictor variable. Therefore, the present study aimed to estimate the relationships between irisin concentration (IC) and FI, BW, BT% using PR models.
In the current study, 40 male Wistar-Albino rats equally separated into four groups (n=10) were used, and the mean BW of the rats was 250±6.8 g. Osmotic mini-pumps were implanted to lateral ventricule and artificial cerebrospinal fluid (vehicle; sham group), 10 (physiologic) and 100 (pharmacologic) nM concentrations of irisin were infused for 7 days. Throughout the experimental period, the rats were kept in individual cages, and food consumption, BW and BT of the animals were daily recorded. The relationships between FI-IC, BW-IC and BT%-IC were separately assessed. A second order PR models was the best-fitted regression model to estimate the studied nonlinear relationships.
From the results of PR models, the estimated PR models were $FI = -0.0028(IC)2 + 0.3176(IC) + 18.537$, $BW = -0.0062(IC)2 + 0.6862(IC) + 274.72$ and $BT\% = -0.0035(IC)2 + 0.4052(IC) + 100.59$, respectively. PR models had high coefficients of determination ($R2 = 1.0$ for all models) and were significant ($p < 0.05$ for each model).
The obtained results demonstrated that satisfactory second-order PR models were derived to estimate the relationships between IC and FI, BW, BT%. Therefore, nonlinear regression models such as PR models can be used in predicting such non-linear relationships.

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P9.31

Chronic intracerebroventricular apelin-13 infusion in rats increases daily food intake and body weight by reducing leptin levels

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Leptin secreted by adipocytes acts on the brain to reduce food intake by regulating neuronal activity in the hypothalamus. Apelin is the endogenous ligand of APJ, which belongs to the superfamily of G protein-coupled receptors. Apelin and its receptor has been detected in the arcuate and paraventricular nuclei of the hypothalamus, which are involved in the control

of feeding behaviour and energy expenditure. This study was designed to examine the effect of centrally infused apelin-13 on serum leptin levels, food intake and body weight.
A total of 21 Wistar-Albino male rats were randomly divided into three groups (n=7 per group) as control, low (1 nmol) and high (10 nmol) apelin-13. Rats were intracerebroventriculary (icv) infused vehicle, 1 and 10 nmol apelin via osmotic mini pumps. After 7 days of infusion (icv), animals were decapitated and blood samples were collected. Serum leptin levels were measured by ELISA.
At the end of the study, daily feed consumption and body weight of animals both 1 nmol and 10 nmol of apelin-13 infusion was higher than control. The parameters of food consumption and body weight of rats were found to significantly increased compared to control only high-dose apelin-13 group ($p < 0.05$). Icv infusion of high dose of apelin-13 resulted in significant decreases in serum leptin levels ($p < 0.05$) compared to control group.
The study results suggest that centrally apelin-13 infusion dose-dependently increases food intake and body weight by suppressing leptin hormone.

Key Words: Apelin, leptin, food intake, body weight, chronic infusion.

P9.32

Effects of intracerebroventricular infusion of apelin-13 on the metabolism rate and energy expenditure

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Uncoupling proteins (especially UCP3 in muscle tissue) are known to be closely related to the energy expenditure and metabolism rate. Thyroid hormones (thyroxine; T3 and triiodothyronine; T4) also have UCP-like roles in the energy use and the control of metabolism rate. Apelin is a peptide hormone that it leads to gain weight because of excessive food intake.
This study was designed to investigate the relationships between thyroid hormone, UCP3 and energy expenditure by central administration of apelin-13. In this study, 30 Sprague Dawley male rats were used. The animals were divided randomly into three groups (n=10). While intracerebroventricular apelin-13 (1 and 10 nmol) was infused to the rats in the experimental group for 7 days, the artificial cerebrospinal fluid (vehicle) was infused via osmotic mini pumps implanted in the lateral ventricle to rats in the control groups (10 µl/h/7 days). At the end of experiments, blood and biceps muscle tissue samples of animals were taken. UCP3 mRNA levels of the groups in muscle tissue were determined by RT-PCR method. Serum T3 and T4 levels were measured by using ELISA method.
The infusion of both doses of apelin-13 caused decreases serum T3 and T4 levels in the rats, but it was found that these decreases were statistically significant only in the high dose apelin-13 group ($p < 0.05$). The UCP3 mRNA levels in the muscle tissue were significantly decreased by both doses of apelin-13 ($p < 0.05$).
Our results are evidence that apelin-13 can be cause obesity by reducing the energy expenditure and metabolism rate.
Acknowledgement:

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P9.33

Does apelin-13 affect the development of brown fat?

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It is known that brown adipose tissue is responsible for the conversion of burning excess calories to heat and thermogenic response to cold in the body. Uncoupling protein 1 (UCP1) in Brown Adipose Tissue (BAT) generates heat and serves as a marker of energy use. The increase of UCP1 in white adipose tissue (WAT) is indicative of the formation of BAT by combustion of WAT. Apelin peptide is a hormone originated from adipose tissue, and shows biological activities binding APJ receptor. In vivo studies have shown that apelin is effective on the body weight and feed consumption.
The aim of this study is to determine the effects of the central infusion of apelin-13 on use of energy and development of BAT.
In this study, Spragua Dawley 30 male rats were used. The animals were divided into three random groups (n=30, in total). While intracerebroventricular apelin-13 (1 and 10 nmol) was infused to the rats in the experimental group for 7 days, the equivalent amount of artificial cerebrospinal fluid (vehicle) was infused by osmotic mini pumps implanted in the lateral ventricle to rats in the control groups (10 µl/h/7 days). At the end of infusion time, the animals were euthanized, and interscapular BAT and WAT tissue samples were taken. UCP1 mRNA levels in tissue samples were determined by RT-PCR.
At the end of the study, it was found that reduction of the amount of UCP1 in both white and brown adipose tissues. Apelin-13 infused at 1 and 10 nmol concentrations was significantly different ($p < 0.05$).
The results of this study showed that apelin-13 reduced the levels of UCP1 in white and brown adipose tissues. According to these results, it can be said that, apelin-13 will be able to reduce the energy use and thus cause to gain weight. Decreasing of UCP1 level in WAT indicates that there is a reduction in browning; decreasing of UCP1 level in BAT indicates that there is a reduction in energy usage. Thus, it can be said that apelin-13 can lead to the formation of obesity affecting in the hypothalamic level.

Acknowledgement: This study was supported by Inonu University BAP (Project 2013/180).

Key Words: Apelin, UCP1, white adipose tissue, brown adipose tissue

P9.34

Changes os C-AMP level during oestrus cycle in normotensive and sponataneous hypertensive rats

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The mammalian pineal gland is under adrenergic control; however, the physiological oscillations of gonadal steroids could strongly affect the melatonin synthesis and secretion by acting on the pre- and postsynaptic levels and by modulation of the target cells replay. The aim of this study was to determine the basal levels of cAMP in the pineal gland during the various phases of oestrus cycle in normothensive (NTR), Wistar rats and spontaneously hypertensive (SHR) Okamoto and Aoki rats and to describe the histological finding of the pineal gland tissues. Two hundred female mature rats (100NTR and 100SHR) were investigated. They were divided in 4 groups according to the phases of the oestrus cycle (diestrus, proestrus, estrus and metaestrus). The phase of oestrus cycle has been determined by microscopic analysis of the vaginal smears. The level of cAMP (RIA) in the pineal gland was the parameter of its intracellular activity. The pineal gland tissues were stained on HaEo. In SHR there is a slight shortening of the oestrus cycle. In NTR there was an increase of the cAMP level from proestrus to metaestrus, contrary to the dramatic decrease in SHR. Histological findings of pineal glands showed the presence of many changed pinealocytes with picnotic nucleuses, while the neuroepithelial cells, in the upper parts of the glands, were separated in gland-like islets. There was a normal pineal histology in NTR. This study indicated significant neurohormonal differences between NTR and SHR. The changed adrenal activity in SHR correlated with histological findings in the pineal gland.

P9.35

Increasing selenium concentration in animal tissues by wheat agrofortification

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Objective: Considering whole human population, more than a third is exposed to the lack of essential elements, among which Se is very important. But, Se is not essential for plants, and conventional agricultural and food production practices are failing to provide quantities of Se adequate for human health. The objective of this work was to determine if increased content of Se in feed achieved by wheat agrofortification could increase Se concentrations in animal tissues.
Materials and Methods: Winter wheat cultivar Divana was grown with 2 treatments: 1) control without Se and 2) foliar Se application as Na2SO4 in amount of 10 g/ha Se. Harvested wheat grain was analyzed and used for feed mixture low in Se and feed mixture high in Se content. Sixteen male Sprague Dawley rats were 10 weeks from weaning fed with 2 types of custom made rat chow: a) low Se group (N=8) and b) high Se group (N=8). After decapitation, the 15 animal tissues are sampled on each rat and stored at -80°C. The wheat grain samples (after milling in heavy metal free mill) and animal tissues were digested in microwave oven using nitric acid. Se concentration was determined by inductively coupled plasma (ICP), optical emission spectrometry (OES) technique. Statistical difference of results was tested by ANOVA test at significance $P < 0.05$.
Results: The applied Se did not affect wheat yield, but increased (12 fold) Se grain concentration from 30 µg/kg to 363 µg/kg. The increment of Se concentration was determined in all analyzed rat tissues fed with agrofortified feed, but it wasn't significant in lungs, heart, lymph node, gut and fat tissue. The significant Se increase was measured in samples of kidneys (37%, from 1.08 to 1.48 mg/kg), liver (42%, 0-63 – 0.90), blood (109%, 0.38 – 0.80), spleen (30%, 0.36 – 0.46), thymus (38%, 0.27 – 0.37), skin (32%, 0.16 – 0.21), bone (37%, 0.13 – 0.18), muscle (125%, 0.09 – 0.21), cerebellum (31%, 0.13 – 0.17) and brain (47%, 0.10 – 0.15). In average, Se concentration increased 30%.
Feeding using agrofortified wheat resulted in higher Se in all animal tissues, but from the human nutrition point of view, most important is increase in muscle tissue (125 %).

P9.36

Glutathione-S-Transferase induction effect of red mud contaminated food

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The environmental harm of the 2010 Hungarian red mud catastrophy is inestimable. We can just guess the quality and the quantity of the toxical materials, which became the part of the ecological circle. We have written down earlier, that the contrary to dissolution tests (official measurements), the plants are amass heavy metals in even more quantity than the soil concentration. By consuming the primer producers, theese toxical metarials can get in the organism of animals and humas as well, even in concentrated amount. The Glutathion-S-transferase (GST) enzyme is the part of the non-specific detoxification apparatus. Observed the GST induction of the occurrence due to toxic agents for many different extent in different tissues. We have fed rabbits (Oryctalagus cuniculus) with plants, which have been grown on red mud harmed soil. After a period of ten days we analysed especially the activation changes of the liver and the central nerve system GST. We found significant correlation between the rate of the contamination and the GST activity of the nerve system and liver. It means we can say that the contamination of the soil induced the second member's detoxifying apparatus of the food chain. It overstrained their organism and caused physiological stress.

Our research shows, in a long term, the contamination of the soil caused by red mud affects other members of the food chain as well.

P9.37

Role of the hypothalamic CRF and AVP in mediating the activation of the HPA axis in alcohol-treated and alcohol-deprived rats

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The aim of our study was to determine the role of the hypothalamic corticotropin-releasing factor (CRF) and arginine vasopressin (AVP) in mediating the activation of the hypothalamic-pituitary-adrenal (HPA) axis, reflected by the elevation of the plasma ACTH and corticosterone concentrations in alcohol-treated and alcohol-deprived rats. The participation of the two distinct CRF receptors (CRFR1 and CRFR2) in this process was also investigated. Male Wistar rats were treated with ethanol or saline solution intraperitoneally (ip) for 7 days, 4 times/day. One half of the animals was decapitated in the morning of the 8th day, the other half was sacrificed in the morning of the 9th day, 12 hours and 24 hours after the last ip administration, respectively. The brains were removed and the hypothalami were isolated for determination of CRF and AVP concentrations with enzyme-linked immuno-sorbent assays (ELISA). In parallel, the trunk blood was collected for determination of the plasma ACTH and corticosterone concentrations with ELISA and chemofluorescent assay. Thirty minutes before their decapitation, the alcohol-treated rats were pretreated intracerebroventricularly (icv) with antalarmin, a selective CRFR1 antagonist, or astressin 2B, a selective CRFR2 antagonist. Hypothalamic CRF and AVP concentrations and plasma ACTH and corticosterone concentrations were increased significantly (and differently) at 12 hours and 24 hours after the last ip administration of alcohol. Antalarmin reversed significantly the elevation of the hypothalamic CRF, AVP and plasma corticosterone levels and considerably, but not significantly the elevation of the plasma ACTH levels. Astressin 2B abolished completely the augmentation of the hypothalamic AVP levels. The present study demonstrates that chronic alcohol intake and the consequent acute alcohol withdrawal induce activation of the HPA axis in rats by stimulation of the hypothalamic CRF and/or AVP production. Our results suggest that this activation is mediated excusively by CRFR1 and that CRFR2 may be implicated in other AVP-mediated processes induced by alcoholism.

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P10

Neurophysiology

P10.1

Nociceptive role of hemokinin-I, the newest member of the tachykinin family, in chronic traumatic neuropathy of the mouse

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Introduction: Traumatic neuropathy is a chronic pain condition caused by mechanical injury of peripheric nerves. There is no satisfactory therapy and the pathophisiological mechanisms are not completely understood. Although regulatory roles have earlier been proposed for tachykinins, but our researches did not verify the role of substance P and it's NK-1 receptor in neuropathic pain. The Tac4 gene coded hemokinin-1 has similar immunological and pharmacological properties to substance P. Our goal was to examine the role of hemokinin-1 in a model of neuropathy using gene-deleted mice.
Methods: We induced traumatic mononeuropathy in male C57Bl/6 (wild-type) and hemokinin-1 gene-deficient (Tac4-/-) mice by 1/2-1/3 ligation of the right sciatic nerve. On the 3rd, 5th and 7th postoperative days we examined the mechanonociceptive threshold with aesthesiometry, cold tolerance with paw withdrawal latency from 0°C water and motor coordination by measuring time spent on accelerating RotaRod wheel. On the 7th day we determined the level of nerve growth factor (NGF) with ELISA from the paw homogenizates.
Results: By the 5th day the mechanonociceptive threashold of WT mice decreased by 35% (mechanical hiperalgesia), the cold tolerance by 70% (cold hyperalgesia), the motor function didn't change. Compared to WT mice in Tac4-/- group mechanical and cold hyperalgesia was significantly reduced after surgery. Tac4-/- motor function did not change due to operation, but was worse throughout the entire experiment. The control NGF-level in Tac4-/- mice was lower compared to WT group. After nerve ligation the NGF level didn't change in WT mice, but significantly increased in Tac4-/- mice compared to their own control measurements.
Conclusion: We were the first to prove hemokinin-1's role in the developement of mechanical and cold hyperalgesia in traumatic mononeuropathy, wherein the inhibition of NGF production can be an important factor.

P10.2

Crosstalk between CB1 and TRPV1 receptors in primary sensory neurons

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The capsaicin-responsive transient receptor potential vanilloid type 1 ion channel (TRPV1) and the G protein-coupled cannabinoid type 1 (CB1) receptor exhibit a high degree of co-expression and interaction in primary sensory neurons (PSN). However, the complexity of that interaction, which could be a considerable component of nociceptive processing in PSN, remains largely unknown. We found that the responsiveness of PSN to capsaicin and to the endogenous agonist of both TRPV1 and the CB1 receptor anandamide defines two distinct types of PSN: those which respond to both anandamide and capsaicin (ACR neurons) and those which respond only to capsaicin (COR neurons). Blocking the CB1 receptor reduces capsaicin-evoked whole-cell currents and increase in intracellular calcium concentration only in ACR neurons. Deleting the CB1 receptor reduces both anandamide- and capsaicin-evoked response in ACR neurons, and the proportion of ACR neurons without affecting the overall proportion of capsaicin-sensitive cells (ACR+COR neurons). While all ACR and the great majority of COR neurons express the CB1 receptor mRNA, the spatial distribution of TRPV1 and the CB1 receptor protein also defines two populations of neurons; cells in which the two receptors are closely associated and cells in which the two receptors are segregated. These findings show that the CB1 receptor has a constitutive TRPV1-sensitizing activity only in ACR neurons, and that the CB1 receptor contributes to anandamide-sensitivity of native TRPV1. These findings also suggest that spatial proximity of the CB1 receptor and TRPV1 may be needed for CB1 receptor-mediated TRPV1 sensitisation.

P10.3

Effects of intraamygdaloid microinjections of RFRP-1 on anxiety and positive reinforcement

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RFRP-1 belongs to the RFamide peptide family. RFRP-1 positive nerve cells were detected in the rat hypothalamus and RFRP-1 immunoreactive fibers were identified in the central amygdaloid nucleus (CeA). The CeA, part of the limbic system, plays an important role in learning, memory, regulation of anxiety and emotional behavior. RFRP analogues bind with relatively high affinity to the NPFF1 and NPFF2 receptors (NPFF-R). RFRP-1 has potent activity for NPFF-1 that is expressed in the CeA. In the presents experiments behavior related effects of RFRP-1 were studied in the CeA.

In different behavioral paradigms two doses of RFRP-1 were examined (50 or 100 ng/side, RFRP-1 dissolved in 0.15 M sterile NaCl/0.4 µl, respectively). Control animals received only vehicle. Other animals received 20 ng NPFF-R antagonist RF9 alone, or antagonist 15 min before 50 ng RFRP-1 treatments.

Open-field test (OPF) was used to investigate the effect of 50 ng and 100 ng doses of RFRP-1 on spontaneous motor activity of animals. During 5 min observation period number of crossings and the distance moved were recorded. There were no any alterations in these parameters in the RFRP-1 treated animals compared to controls. Anxiety was evaluated in elevated plus maze (EPM) test. In the EPM test application of 50 ng RFRP-1 significantly increased time spent in the open arms and in the end of the open arms. Pretreatment with 20 ng NPFF-R antagonist RF9 blocked the effect of 50 ng RFRP-1. Antagonist applied in itself did not influence the EPM test. Conditioned place preference (CPP) test was used to examine in the CeA the possible effects of RFRP-1 on positive reinforcement. Microinjection of 50 ng RFRP-1 significantly increased the time rats spent in the treatment quadrant. Prior treatment with antagonist eliminated the effects of RFRP-1.

Our results are the first indicating that RFRP-1 injected into the CeA proved to be anxiolytic. RFRP-1 microinjected into the CeA has positive reinforcing effects in the CPP. These effects are specific because they can be eliminated by NPFF receptor antagonist.

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P10.4

A maternally induced thalamic neuropeptide mediates the effect of suckling to hypothalamic centers of maternal motivation and lactation

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Extensive maternal caring is critically important for the survival of a newborn offspring. Different sites of the hypothalamus are involved in central maternal adaptations: the preoptic area whose lesion abolishes maternal behavior regulates maternal motivation while neurons in the arcuate nucleus are responsible for the regulation of suckling-induced prolactin release. In this study, we describe novel neuronal pathways containing a thalamic neuropeptide that are involved in the regulation of maternal responses.

We demonstrated that suckling activated neurons containing tuberoinfundibular peptide of 39 residues (TIP39) in the posterior intralaminar complex of the thalamus (PIL). Indeed, our retrograde and anterograde tract-tracing studies determined that neurons in the PIL receive ascending information from the spinal cord and project to maternal brain centers including the preoptic area and the arcuate nucleus. For functional analysis of the projections, genetically modified lentivirus expressing an antagonist of the receptor of TIP39, the parathyroid hormone 2 receptor (PTH2R) was injected into the mediobasal hypothalamus of female rats. Infected cells around the injection site permanently express and release the PTH2R antagonist HYWY-TIP39. We observed a lower basal prolactin level and a significant reduction in suckling-induced prolactin levels in the PTH2R antagonist expressing dams. The same virus was injected into the preoptic area and conditioned place preference test was performed in virus injected mothers. PTH2R antagonist expressing mothers showed no preference for pup-associated chamber as did control virus injected dams suggesting reduced maternal motivation following blockade of endogenous TIP39 action.

In conclusion, TIP39 neurons are ideally positioned in the PIL to convey suckling information towards maternal brain centers to regulate both maternal motivation and lactation during the postpartum period.

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P10.5

Role of capsaicin-sensitive nerve terminals in chronic restraint stress induced increase of nociception

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Experimental and clinical data suggest that chronic psychological distress enhances pain sensation and plays an etiological role in some conditions characterized by clinically significant pain (e.g. fibromyalgia, irritable bowel syndrome). Changes in the central pain processing were extensively examined in patients with stress-related pain disorders, but the mechanisms and the role of peripheral sensory nervous system is unclear. Thus, we studied the effect of chronic restraint stress (6 hours/day, 4 weeks) on nociception and behaviour of male CD1 mice. Mechanonociceptive threshold and noxious heat sensitivity were measured with dynamic plantar aesthesiometer and hot plate test, respectively. Cold tolerance was determined by the measurement of paw withdrawal latency from 0°C water. Behavioural effects of chronic stress were examined in open field- (OFT), light-dark box- (LDB) and tail suspension tests (TST). Weight of the thymus and adrenal glands were also assessed. The role of capsaicin sensitive neurons was investigated with desensitization with resiniferatoxin (RTX), an ultrapotent capsaicin analogue. Chronic stress caused a significant mechanical hyperalgesia and cold allodynia, but thermonociceptive threshold did not change. The decrease of thymus and increase of adrenal weight were accompanied by an increase of the time spent in the lit compartment in LDB. Very interestingly, pre-treatment with RTX significantly increased the stress induced mechanical hyperalgesia. Chronic stress did not alter the behaviour of RTX-treated animals. These are the first data suggesting an important role of capsaicine-sensitive neurons in stress induced changes of nociception and behaviour.

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P10.6

How does maternal smoking influence the early neurobehavioral development of rat pups?

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Exposure to tobacco smoke during perinatal life is known to have various deleterious effects. Among others they worsen cognitive functioning, decrease locomotor behavior and present a risk factor for future pshychiatric disorders. The aim of our study was to investigate the influence of maternal smoking during pregnancy on the early physical and neurobehavioral development of newborn rats. Wistar rats were exposed to whole-body smoke exposure for 2x40 minutes daily from the mating until delivery. For the treatment, TE2 manual closed-chamber smoking system and 4 research cigarettes per occasion were used.

The neurobehavioral development of the pups was monitored by a battery of tests until postnatal day 21. Weight was measured daily until postnatal day 21. On the 4rd week of life motor coordination tests were carried out. Some parameters appeared earlier, like eyelid and eartwitch reflexes, forelimb and hindlimb placing, forelimb grasp. On the other hand we observed a delay in the appearance of forelimb and hindlimb reflexes.

These results suggest that maternal smoking during pregnancy has unfavourable effects on the early neurological development of the rat pups.

P10.7

IL-1ß modifies the taste reactivity in the cingulate cortex of the rat

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IL-1ß is an important primary cytokine which has been shown to be involved in the central homeostatic control. As a key part of the limbic system, the cingulate cortex (cctx) plays determinant role in the central regulation of feeding and metabolism. In our extracellular single neuron recording experiments, the existence of IL-1ß and taste sensitive neurons has been proven in this cortical area.

The aim of the present study was to examine whether IL-1ß in the cctx has any effect on the rodent taste perception. To do so, taste reactivity test was performed after bilateral microinjection of this primary cytokine or vehicle solution into the cingulate cortex of male Wistar rats.

In the taste reactivity test, the gustatory stimulation induced characteristic behavioral reactivity patterns were evaluated. The gustatory stimuli corresponded to the five basic tastes in two concentrations: salty (NaCl 0,05 M and 0,5 M), bitter (quinine-HCl 0,03 mM and 3 mM), sweet (sucrose 0,05 M and 0,5 M), umami (Na-glutamate 0,05 M and 0,5M) and sour (HCl 0,03 M and 0,3 M). Responses to the pleasant (both concentrations of sucrose and the lower concentrations of NaCl and MSG) and unpleasant (both concentrations of HCl and QHCl and the higher concentrations of NaCl and MSG) tastes were analyzed separately.

The IL-1ß treated group showed lower ingestive and higher aversive responsiveness to pleasant tastes than rats of the control group. Taste reactivity patterns to the unpleasant tastes did not differ significantly in the two groups.

The findings of our present experiment indicate that these primary cytokine mediated processes in the cingulate cortex have modulatory effect on taste perception mechanisms.

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P10.8

Investigating the retinoprotective effects of PACAP eye-drop in ischemic retinopathy

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Pituitary adenylate cyclase activating polypeptide (PACAP) has neuroprotective effects in different neuronal and retinal injuries. Retinal ischemia can be effectively modelled by permanent bilateral common carotid artery occlusion (BCCAO), which causes chronic hypoperfusion-induced degeneration in the entire rat retina. The retinoprotective effects of PACAP 38 and vasoactive intestinal peptide (VIP) are well-established in ischemic retinopathy. Our research group previously investigated and proved the retinoprotective effect of intravitreal administered PACAP. However, little is known about the effects of PACAP eye-drop, which is an easier form to use in clinical practice. The aim of the present study was to investigate the potential retinoprotective effects of PACAP 1-38 and 1-27 eye-drops in BCCAO-induced ischaemic retinopathy. Wistar rats (3-4 months old) were used in the experiment. After performing BCCAO, the right eyes of the animals were treated with PACAP 1-38 or 1-27 eye-drops (1µg/drop). Each eye-drop contained different vehicles: saline, aqua destillate, thiomersalo, benzalkonium-chloride. The left eyes, serving as control eyes, were treated with the adeqate vehicle. Sham-operated (without BCCAO) rats received the same treatment. Rats were treated by 2x1 drops a day for 5 days. Routine histology was performed 2 weeks after the surgery, cells were counted and the thickness of retinal layers were compared. According to our results the PACAP 1-27 solved in benzalkonium-chloride was the most retinoprotective eye-drop in ischemic reinopathy.

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P10.9

Complex functional attributes of cingulate cortex glucose-monitoring neurons and their metabolic significance

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The cingulate cortex (cctx) has intimate interrelationship with limbic system structures containing glucose-monitoring (GM) neurons known for their involvement in the central regulation of feeding and metabolism. The GM cells were also demonstrated to be influenced by catecholamines already proved to participate in feeding-associated perceptual learning and memory mechanisms. Main goal of the present experiments was to determine complex functional characteristics of GM neurons in the cctx. Our further aim was to examine whether selective destruction of the GM cells in the cctx induce any alteration of the control of carbohydrate metabolism. To do so, 1) extracellular single neuron activity of anesthetized male rats was recorded with tungsten wire multibarreled glass microelectrodes in the cingulate cortex during a) microelectrophoretic application of chemicals, b) intraoral gustatory stimulations, and c) intragastric infusions. In addition, 2) glucose tolerance test (GTT) was performed 20 minutes (acute) and 4 weeks (subacute) after bilateral microinjection of streptozotocin (STZ) or vehicle solution into the cctx. More than 15 % of the neurons changed firing rate in response to microelectrophoretic administration of D-glucose. Appr. twice as many GM neurons, compared to the glucose-insensitive (GIS) ones, changed their activity to dopamine microiontophoresis. The proportion of taste responsive GM units was also significantly higher than that of the GIS cells. In case of intragastric infusions, one fifth of the tested neurons responded to MSG and to the lower concentration of NaCl. The dynamics of the blood glucose curves of the acute GTT, compared to the control group, proved to be different in the STZ treated group. Our findings indicate that the GM neural network plays important role in the maintenance of the homeostatic balance. Damage to these chemosensory neurons int he cctx appears to cause complex feeding and metabolic disturbances.

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P10.10

Induction of amylin in the preoptic area of lactating dams depends on TIP39-containing posterior thalamic neurons

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Amylin, a peptide previously known as a pancreatic hormone, was found to be expressed in the preoptic area of mother rats in our previous microarray study. The increase in mRNA expression was validated by RT-PCR and the appearance of the peptide was detected by immunohistochemistry. Examining the time course of amylin induction, we found that

amylin is not expressed in the brain before and during pregnancy but its significant increase was observed in rats and mice immediately after parturition in the preoptic area, a region whose lesion abolishes maternal behaviors. Within the preoptic area, the distribution of amylin neurons, examined by in situ hybridization histochemistry and immunolabeling, was the same as the neurons showing Fos activation by pup exposure: the medial preoptic nucleus, parts of the medial preoptic area, and the ventral part of the bed nucleus of the stria terminalis. Amylin-positive neurons were activated by pup exposure in dams. Since our previous studies suggested that suckling effect on maternal motivation may be mediated by posterior thalamic neurons expressing tuberoinfundibular peptide of 39 residues (TIP39), we examined the relationship of amylin and TIP39. Fiber terminals containing TIP39 and the parathyroid hormone 2 receptor (PTH2 receptor; the receptor of TIP39) have the same distribution as amylin neurons in the preoptic area. Furthermore, TIP39 terminals closely apposed amylin neurons suggesting their innervation by TIP39 neurons. In addition, the maternal induction of amylin was markedly reduced in mice lacking the PTH2 receptor suggesting a functional relationship between amylin and TIP39.
These results imply that amylin is a novel neuropeptide with maternal functions, and that its maternal induction is driven by posterior thalamic TIP39-containing neurons that have been suggested to convey suckling information.

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P10.11
New semicarbazide-sensitive amine oxidase (SSAO) inhibitor as a dual antagonist of TRPA1 and TRPV1 ion channels

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Background: A newly developed SSAO inhibitor, SZV-1287 was described to inhibit both acute and chronic inflammation. Transient Receptor Potential ion channels, such as TRP Vanilloid 1 and Ankyrin repeat domain 1 (TRPV1 and TRPA1) are expressed on nociceptive primary sensory neurons and regulate inflammation. Since the chemical structure of SZV-1287 is similar to the selective TRPA1 antagonist HC030031, we investigated the effects of SZV-1287 on the TRPV1 (by capsaicin) and TRPA1 (by mustard oil (MO)) receptor activation of trigeminal ganglion neurons in comparison with SZV-1911, the reference SSAO inhibitor with different structure.
Methods: Ratiometric technique of [Ca2+]i measurement with the fluorescent indicator fura-2-AM on cultured trigeminal cells was performed. Calcitonin gene-related peptide (CGRP) release from the peripheral sensory nerve terminals of the isolated rat trachea was measured by radioimmunoassay.
Results: SZV-1287 and SZV-1911 were used in three concentrations (100, 500, 1000 nM, respectively). SZV-1287 decreased the capsaicin- and MO-induced Ca2+-influx in trigeminal neurons, and MO-induced CGRP release from the sensory nerve endings in a concentration dependent manner. SZV-1911 had no effect in either model. Neither compound had any effect on KCl-evoked Ca2+-influx.
Conclusion: These are the first evidence for an antagonistic action of SZV-1287 on TRPV1 and TRPA1 ion channels on the cell bodies of sensory neurons and peripheral sensory nerve terminals. This effect is independent of its SSAO inhibitory action.

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P10.12
Analysis of connexin 26 expression in hearing loss

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Background: Mutations in connexin 26 (Cx26) and Cx30 have been associated frequently with hearing loss and deafness, where a few mutations in connexin (Cx) 26 have been found to contribute to over 50% of the incidence of hearing loss or non-syndromic deafness in different human populations. The aim was to detect the Cx 26 expression levels in patients with hearing defects and highlight the role of gap junctions and hemichannels in K (+) removal and recycling in the ear, as well as possible roles for nutrient passage, in the cochlea.
Methods: In this study we compared groups of patients with hearing loss and tinnitus using objective measurements with distortion otoacustic emission method (DPOAE system) and Tympanometer.
Results: The expression of Cx 26 has been altered in the three groups of patients. Normal Eustachian tube function was detected in all patients from the 3 subgroups. As the middle ear ventilation found to be normal („A" type tympanogram) further objective measurements could be performed, thus these patients were included. In the C-group (cochlear implant candidates) the outer hair cell functions were diminished, as part of the inner ear lesion, leading to complete hearing loss. This Corti-organ malfunction could be substituted by different cochlear implant systems.
In the pathological group from mild to moderate/severe outer hair cell dysfunction could be detected, which could be explained by co-morbidity (i.e. diabetes, hypertension), tinnitus, age or noise exposure in the case history. These results suggest that the physiologically active connexin 26 channels are crucial to maintain the normal hearing and the altered Cx 26 level exhibits distinct cellular pathologies.

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P10.13

Neuroprotective effect of early postnatal environmental enrichment in a rat model of Parkinson's disease

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Environmental enrichment is a popular strategy of neuroprotection. The aim of our study was to investigate the effect of early postnatal environmental enrichment in a rat model of Parkinson's disease in adulthood.

We used adult Wistar rats in the experiment. The animals of the standard group were placed under regular circumstances. For environmental enrichment, we placed rats in larger cages, supplemented with different toys during the first five postnatal weeks. Two moths later the rats were treated with unilateral injections of 2 µl 6-OHDA (5 µg/ 1 µl) into the left substantia nigra, contol animals received 2 µl physiological saline. Behavioral experiments were done preinjury, and 1 & 10 days after the operation. Tyrosine-hydoxylase immunohistolochemistry was performed after the behavioral testing to label dopaminergic cells of the substantia nigra.

We found that physiological saline did not make significant difference between the treated and non-treated side of the brain. The 6-OHDA treatment made significant cell loss in the standard group: more than 40% of dopaminergic cells died, while in rats which were kept in enriched environment the cell loss was significantly lower.

Our experiments provided evidence for protective effect of early postnatal environmental enrichment in adulthood, because rats under regular circumstances showed more severe acute neurological sings and dopaminergic cell loss after 6-OHDA lesion of the substantia nigra compared to animals grew up in environmental enrichment.

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P10.14

Polysulfide compound dimethyl trisulfide is analgesic in heat-injury-induced hyperalgesia in mice

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Several tissues contain protein-bound sulfane sulfur. Sulfane sulfur species are is also known as polysulfides. Recently, polysulfides were shown to initiate S-sulfhydration of proteins leading to potential biological effects. Polysulfides protected cells from oxidative damage. They were reported to activate TRPA1 receptors on astrocytes. TRPA1 receptor is a member of transient receptor potential ankyrin receptor subfamily. TRPA1 is mostly expressed in unmyelinated and thinly myelinated nociceptive primary sensory neurons. Participation of TRPA1 in inflammatory and nerve-injury-evoked mechanical hyperalgesia is well documented. Based on the above data, in the present study we examined the effect of polysulfide compound dimethyl trisulfide (DMTS) in heat-injury-induced mechanical hyperalgesia in mice. Involvement of TRPA1 receptors was tested with gene knockout animals.

Heat injury was induced in one hindpaw of TRPA1 wild type (WT) and knockout (KO) mice by submerging the paw into 51°C water for 15 seconds under diethyl ether anesthesia. Mechanical pain threshold of the hindpaws was determined by dynamic plantar esthesiometry every 10 min for 60 min. Baseline measurments were taken before heat injury. DMTS or vehicle were administered i.p. 30 min before heat challenge of the paws.

Mechanical hyperalgesia caused by mild heat injury was similar in TRPA1 WT and KO animals. DMTS in 500 µmol/kg dose showed analgesic activity in TRPA1 WT mice. The same DMTS dose was less effective in TRPA1 KO animals. At 10 min DMTS had significantly larger analgesic effect in KO animals.

According to our knowledge the present study provides the first in vivo data on analgesic effect of polysulfide compound DMTS and activation of TRPA1 receptors by polysulfides. Analgesic effect of DMTS is mediated via TRPA1 receptors. However, 10 min after heat injury protective effect of DMTS was only present in TRPA1 KO animals suggesting pro-algesic role of TRPA1 receptors at this time point.

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P10.15

Kallikrein 8: A promising novel biomarker in brain tumors

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Kallikreins are a subgroup of serine protease family. One of the members of kallikrein subgroup is the human tissue kallikrein family and it consists of 15 genes which are located on the 19th (19q13.4) chromosome. This family plays a significant role in different physiological processes.

Tissue kallikrein 8 (KLK8) (neuropsin/ovasin), which is a member of the human kallikrein gene family, is expressed in different areas of the human brain. KLK8 plays an important role in the physiology of the central nervous system. Several studies have shown that KLK8 may be related with cancer and its expression profile varies in several malignancies. In this study the aim was to determine mRNA and protein expression of KLK8 in meningioma and glioblastoma brain tumors.

Meningioma (n=15) and glioblastoma (n=15) brain tumor samples were examined for KLK8 mRNA gene expression using reverse transcriptase polymerase chain reaction (RT-PCR). Its protein expression was determined using Western blot. Pearson Chi-Square and Yate's correction tests were used for the statistical analyses and $p < 0.05$ was considered statistically significant.

The results showed that both protein and mRNA expression of KLK8 were relatively higher in meningioma group than those were in glioblastoma group.
It can be concluded that KLK8 mRNA and proteins are expressed more frequently in meningioma group. Further studies should be carried out in order to consider KLK8 as a novel marker in brain tumors.

Key words: KLK8, Meningioma, Glioblastoma

P10.16
Caffeine improves MK-801-induced learning and memory deficits

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Accumulating evidence indicates that the antagonist of adenosine receptors caffeine which have important roles in the brain such as improves memory and learning processes despite conflict results. The N-methyl-D-aspartate (NMDA) receptor, a glutamate receptor, is important for synaptic plasticity and memory function. The MK-801, NMDA receptor antagonist, has shown amnesic properties in animal model. Also, MK-801 induce schizophrenia-like behaviors in animals. The current study was to find out whether intraperitoneally (i.p) administration of caffeine can prevent MK-801-induced learning/memory deficits in rats. The animals were trained and tested different memory phases in passive avoidance task. The performance of rats was evaluated in the retention tests 24 and 72 h after a single acquisition trial. Animals were divided to two main groups. In a subgroup of the first main group, 0.2 mg/kg MK-801 was injected i.p. before the acquisition session and in the second sub group, MK-801 was injected 30 min before the retention session. Analysis of data showed that in both sub groups, MK-801 impaired learning and memory. In the second set of experiments, administration of caffeine (10mg/kg) after the acquisition session (i.e. before MK-801 i.p injection) improved the MK-801 induced memory impairment and administration of same dose caffeine before acquisition session prevented also memory impairment induced with injection of 0.2 mg/kg MK-801, 30 min before retention test. In conclusion, these results show an interaction between caffeine and glutamatergic system.
A novel finding in this study is that low-dose caffeine can prevent amnesia produced by NMDA antagonist in rats when injected in pre and post-training phase and may be contribute for treating cognitive impairments in schizophrenia.

P10.17
Anterior cingulate responses evoked by mechanical nociceptive stimulation in female rats

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Introduction: Limbic structures within the so called emotional system can be affected by various physiological conditions; in particular, the cingulate cortex is strongly involved in emotion related to pain. In female rats, the estrous cycle is key condition that affects emotional behavior. Therefore, in the present study, we investigated changes in cingulate-cortex responses to noxious stimuli across the estrous cycle.
Methods: Wistar albino female rats (bw 130-146 g) were used. Animals were kept in a temperature-controlled room (24 ± IºC) under a 12:12 (light on from 0700 to 1900 hr) light/dark cycle. The rat estrous cycle is usually divided into four phases: proestrous, estrous, metestrous and diestrous. The phases of the rats estrous cycle were determined from vaginal smears with Giemsa-stain using low-power light microscopy. Noxious stimuli were applied on three body sites (nose, back, tail) using a quantitative pinch stimulator. Neuronal activities were recorded from single neurons in the cingulate cortex. The firing frequency of neurons before and after 5s-pinch stimuli was compared.
Results: Stronger cingulate responses were observed in the estrous phase to noxious stimuli applied to the back and to the tail. No significant changes were observed in cingulate responses across the estrous cycle to stimuli applied to the nose.
Discussion: The present results indicate that nociceptive responses in the anterior cingulate can be modulated by the different phases of the estrous cycle. Moreover, the modulation of cingulate responses was different among body sites, suggesting that the estrous hormones may have specific effects throughout the body.

P10.18
The role of intraamygdaloid oxytocin in reinforcing mechanisms

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Neuropeptide oxytocin (OT) is thought to play a role in social and non-social behavior. The central nucleus of amygdala (CeA), part of the limbic system, plays an important role in learning, memory, anxiety and reinforcing mechanisms. CeA was shown to be rich in OT-receptors (OTR). The aim of our study was to examine in the CeA the possible effects of OT

and OTR antagonist on reinforcement in conditioned place preference test.
Male Wistar rats were microinjected bilaterally with 10 ng OT or 100 ng OT (Sigma: O6379, injected in volume of 0.4 µl) or 10 ng OTR antagonist (sigma: L-2440) alone, or OTR antagonist 15 min before 10 ng OT treatment or vehicle solution into the CeA.
Rats receiving 10 ng OT spent significantly longer time in the treatment quadrant during the test session, while 100 ng OT treatment did not influence the place preference. Prior treatment with the non-peptide OTR antagonist blocked the effects of OT. The antagonist in itself did not influence the place preference.
Our results show that in the rat CeA OT has (a dose dependent) positive reinforcing effect via OTR, because selective OTR antagonist can block this action.

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P10.19

A possible way to decrease "crowdedness" through functional asymmetry in the hypothalamus

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Hypothalamus is a key regulator of many physiological processes and homeostatic pathways in the body. The properties through which the hypothalamus is able to orchestrate all these functions make the hypothalamus an anatomically "overcrowded" brain structure. On the other hand, it has been known for higher brain areas, even they seem to be morphologically symmetric, that usually the left and right sides have distinct physiological functions. This phenomenon provides a solution for the "ergonomic" use of brain resources. Although hypothalamus is also a morphologically symmetric brain structure, it has been considered as an unpaired midline structure and the two sides regulate the same biological functions. Here we show evidence for the functional asymmetry of the neuroendocrine hypothalamus by measuring the mitochondrial respiration rates in isolated left and right sides of rat hypothalami.
We demonstrate that hypothalamic mitochondrial oxygen consumption shows an asymmetric lateralization during the estrous cycle. Results imply that the workload in the hypothalamus may be just as side-linked as in the case of the cerebral cortex and many other well-known paired CNS regions.

P10.20

Putting the fission illusion into a new context

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Presenting two short sounds just after a visual stimulus causes in many observers the illusion of two images. This is the fission illusion and it is very popular to study multisensory integration since the auditory stimulus causes a qualitative change in the perception of the visual stimulus. Several physiological correlates of the illusion have been described indicating that it is not the criterion level of the observers (they tendency to respond) that has been changed. It is a common opinion that the three stimuli have to be presented within a time window of 100 ms, a time frame characteristic for the integration time of multisensory neurons. The present study was design to investigate a possible widening of the time window and to find a plausible explanation for the illusion. A psychophysical experiment was designed. A white (1.5°) disk on black background at 8° excentricity was used as visual stimulus (K) for 17 ms. The auditory stimulus (H) was a 3500 Hz sound presented for 10 ms. The time lag between the stimuli was 67 and 133 ms. The conditions were:1K1H,1K2H,2K1H,2K2H,1K,2K,1K2H,2K1H.
We demonstrated that the illusion could be triggered with a time window of 133 ms, while the participants successfully detected the physically present one or two flashes of the visual stimuli. Our data also indicate that there is no substantial fusion (2K1H) when using a wide time window, proving that the participants were not counting merely the auditory stimuli. Our results indicate that the illusion is rooted rather in the function of neuron population than in single neurons. It could make sense to think in two limits: one, which makes fusion of the stimuli possible and a second, which makes induction by the second sound possible (this might be parameter dependent: sound level, contrast). It could be important to understand the differences in processing of the basic stimuli in different populations (people with synesthesia or schizophrenia).

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P10.21

Surgical level of ketamine anesthesia induces EEG microstructure and respiratory pattern disturbances following pedunculopontine tegmental nucleus lesion in rat

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We followed the impact of surgical level of ketamine anesthesia on sleep, sleep/wake state-related EEG microstructure and respiratory pattern following the bilateral pedunculopontine tegmental (PPT) lesion in adult, male, Wistar rats (n=20). Bilateral PPT lesion was done by stereotaxically guided 100 nl ibotenic acid microinfusion under ketamine/diazepam anesthesia. After 14 days of recovery, we recorded sleep for 6h. Surgical level of

anesthesia was induced by ketamine/diazepam (100 mg/kg, i.p.) 24h later, and we recorded EEG and respiratory movements during 60 min, using piezo-sensor. We repeated sleep recordings following 48h and 6 days after the anesthesia administration.
Fourier analysis was applied on 6h recordings, and we differentiated each 10s as Wake, NREM and REM state, calculated the group probability density distributions of all EEG frequency bands relative amplitudes for each state, and during 20 min of the surgical level of anesthesia. Respiratory pattern time-domain analysis was done using Monotone Signal Segments Analysis for breaths detection, differentiation and quantification of the eupnea, bradipnea-apnea and sigh breath-to-breath intervals. Statistical analysis was done using Mann-Whitney U two-tailed test. PPT lesion was identified by NADPH–diaphorase histochemistry.
PPT lesion did not change sleep before, 48h or 6 days after anesthesia administration. Ketamine anesthesia induced REM and NREM EEG microstructure disturbance from 48h-6 days after anesthesia induction. In the PPT lesioned rats the surgical level of anesthesia disturbed EEG microstructure and respiratory pattern: there was enhanced EEG delta and diminished sigma and beta relative amplitude during the increased number of apneustic bradipnea/apnea breaths.

P10.22

Synergism between NMDA receptor antagonists ketamine and magnesium in lowering body temperature in rats

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Objectives: Ketamine and magnesium, both NMDA receptor antagonists, are known for their anesthetic, analgesic and anti-shivering properties. This study is aimed at evaluating the effects of ketamine and magnesium sulphate on body temperature in rats, and to determine the type of interaction between them.
Methods: The body temperature was measured by insertion of a thermometer probe 5 cm into the colon of unrestrained male Wistar rats (200-250 g).
Results: Magnesium sulphate (5 and 60 mg/kg, sc) showed influence neither on baseline, nor on morphine-evoked hyperthermic response. Subanesthetic doses of ketamine (5-30 mg/kg, ip) given alone, produced significant dose-dependent reduction in both baseline colonic temperature and morphine-induced hyperthermia. Analysis of the log dose–response curves for the effects of ketamine and ketamine-magnesium sulphate combination on the baseline body temperature revealed synergistic interaction, and about 5.3 fold reduction in dosage of ketamine when the drugs were applied in fixed ratio (1:1) combinations. In addition, fixed low dose of magnesium sulphate (5 mg/kg, sc) enhanced the temperature lowering effect of ketamine (1.25-10 mg/kg, ip) on baseline body temperature and morphine-induced hyperthermia by factors of about 2.5 and 5.3, respectively.
Conclusion: This study is first to demonstrate the synergistic interaction between magnesium sulphate and ketamine in a whole animal study and its statistical confirmation. It is possible that the synergy between ketamine and magnesium may have clinical relevance.

P10.23

Long term consequences of early postnatal domoic acid administration on spontaneous behavior of Wistar rats

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Consumption of seafood containing toxin domoic acid (DA) causes an alteration of glutamatergic signaling pathways and could lead to various signs of neurotoxicity in animals and humans. Neonatal treatment with domoic acid was suggested as valuable model of schizophrenia and epilepsy. We tested how repeated early postanatal DA injection influences the spontaneous behavior of rats in adulthood. Rats were injected by 30 μg DA/kg from postnatal day (PND) 10 until PND 14. Their behavior was observed in the open field test for one hour (Laboras, Metris) at PND 35 and PND 42. We did not find any difference between DA treated rats and animlas injected by equivalent volume of saline. DA rats exhibited in both test session same vertical and horizontal exploratory activity (tested parameters: locomotion, distance travelled, maximall speed reached during test, grooming and rearing). We conclude that at least in mention experimental design, DA does not influence the spontaneous behavior of rats in early adulthood.

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P10.24

Acupuncture modifies neuronal activities in the nucleus reticularis lateralis in rats

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Introduction: A descending inhibitory mechanism from the periaqueductal gray (PAG) to the spinal cord through the nucleus raphe magnus (NRM) is strongly involved in the endogenous analgesic system activated by acupuncture stimulation. In addition to the PAG to NRM system which descends in the medial pathway of the brain stem, the nucleus reticularis lateralis (NRL) situated in the lateral part of the brain stem is reported to play an important role in modulating centrifugal pain. In the present study, to clarify the role of NRL in acupuncture analgesia, we investigated the response properties of NRL neurons to acupuncture stimulation.
Methods: Fourty-four female Wistar albino rats weighing about 300 g were used in the present study. Single unit

recordings were made from left or right NRL neurons with a two-barrel glass microelectrode filled with 2M-NaCl and 0.1 M naloxone hydrochloride. To test whether changes in NRL activities were induced by the endogenous opioid system or not, naloxone was injected for 30 s microionophotetically 60 s after the cessation of acupuncture stimulation by passing an anodal current. Cathodal acupuncture stimulation was delivered to bilateral Ho-Ku through stainless steel needles. Rectangular constant current pulses of 0.1 ms in duration were delivered at 45 Hz for 15 min.

Results: The majority of NRM-projecting NRL neurons were inhibited by acupuncture stimulation. This effect was antagonized by ionophoretical application of naloxone, indicating that endogenous opioids act directly onto these NRL neurons. By contrast, about half of spinal projecting NRL neurons were excited by acupuncture stimulation.

Discussion: The present study showed that the NRL has two different functional roles in the acupuncture analgesia. One is the disinhibition of NRM neurons; the other is the direct descending inhibition to the spinal cord. The former can modulate descending inhibition through NRM from PAG.

P10.25

Complex functional attributes of glucose-monitoring neurons in medial orbitofrontal cortex and their homeostatic significance

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The orbitofrontal cortex (OBF) plays important role in the central regulation of feeding and metabolism. The glucose-monitoring (GM) neurons are important constituents of the prefrontal-orbitofrontal neural circuitry, and the streptozotocin (STZ) is known to selectively destroy these GM cells. The present study was designed to characterize the endogenous and exogenous chemical sensitivities of these chemosensory cells and their role in the maintenance of glucose homeostasis.

Extracellular single neuron activity was recorded in the medial OBF (mOBF) of male Sprague-Dawley rats by means of tungsten wire multibarreled glass microelectrodes, during microelectrophoretic application of various chemicals, as well as during intraoral gustatory stimulations.

To examine glucose homeostasis control, i.p. glucose tolerance test (GTT) was performed in male Wistar rats 20 min (acute) and 4 weeks (subacute) after STZ or vehicle microinjection into the mOBF.

One fifth of tested neurons changed their activity in response to microelectrophoretic administration of D-glucose. Acetylcholine elicited activity changes in 80% of all neurons, whereas noradrenaline did so in 20%. Appx. 40% of the examined cells changed in firing rate to microelectrophoretic application of dopamine. During gustatory stimulations, each primary taste and orange juice were shown to evoke activity changes in mOBF neurons.

Characteristic alteration of blood glucose levels of STZ treated animals were observed in the acute GTT: the maximum was higher and it was reached later, than in the control group. In the subacute phase, there was no significant difference between the two groups.

Our data demonstrate that GM neurons in the mOBF play important role in the adaptive mechanisms of the central regulation of feeding and metabolism by the integration of relevant signals arising from the endogenous and exogenous environments.

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P10.26

The effect of kisspeptin on cocaine-evoked behavioral changes

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Kisspeptin, a hypothalamic neuropeptide well-recognized for its role in the regulation of the reproductive axis, is a member of the RF-amide family, which have been known to modify opioid activity and has been previously implicated in drug dependence. With regard to the brain distribution data and the receptor profile of kisspeptin, in the present study we investigated the effect of kisspeptin-13, an endogenous derivative, on the behavioral changes induced by acute cocaine treatment and chronic cocaine dependence. The peptide was administered intracerebroventricularly (icv.) in different doses (0.5-2 μg) 30 min before intraperitoneal cocaine treatment (25mg/kg) to adult male CFLP mice. After of which, in a computerised open field system we measured the horizontal and vertical locomotor activities as well as the time spent in the center of the arena indicative of anxious behavior. Observing the behavior of mice in a computerised open field test after combined treatment with kisspeptin and cocaine for five consecutive days assessed the effect of kisspeptin on chronic cocaine dependence. Our results showed that kisspeptin did not alter the effect of acute cocaine administration on locomotor activity; however, it significantly reduced the anxiety-related behavior. Furthermore, kisspeptin significantly decreased the behavioral sensitisation characteristic of the development of cocaine dependence.

In conclusion, our data indicate that central kisspeptin may have a role in the development of cocaine dependence.

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P10.27

Dopamine and serotonin in frog and turtle retina: An immunofluorescent study

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Dopamine and serotonin are monoamines belonging to the group of the low-molecular weigh neurotransmitters. In the central nervous system (CNS) they are widely used being involved in variable functions as motor control, reward and punishment, mood etc. In retina, which may be regarded as a natural biological model of the CNS, the dopamine and serotonin functions are not entirely clear. That is why the aim of the present work was to study their distribution in the retinas of frog Rana ridibunda and freshwater turtle Emys orbicularis, which possess mixed and predominantly cone type of retina respectively.

All procedures with a frog and a turtle were in accordance with the Bulgarian law for scientific experiments. The animals were deeply anesthetized with halothane and decapitated. The eyes were dissected, and the posterior eyecups with retinas were immediately immersed in 4% (w/v) paraformaldehyde in 0.1 M phosphate buffer for 15–30 min. After fixation, the retinas were dissected from the eyecups and cryoprotected in graded sucrose solutions (10%, 20%, and 30% w/v). Cryostat sections were cut at 14 μm and stored at −20 °C. Antibodies directed to the dopamine- and serotonin transporters were applied, using the indirect immunofluorescent method. The results obtained showed that both dopamine and serotonin were well expressed in frog and turtle retinas.

Dopamine transporter antibody caused staining of great number of amacrine cells' perikaria located very close to the border of inner nuclear layer (INL) and inner plexiform layer (IPL). In addition, in the more distal part of INL single perikaria, most probably dopaminergic interplexiform cells, were also stained. Both plexiform layers: the outer (OPL) and inner (IPL), showed dopamine transporter immunoreactivity as well.

Serotonin transporter antibody also caused well expressed staining in both plexiform layers of the retinas. The final endings of the serotoninergic amacrine cells were revealed in the IPL. The OPL staining might be due to the synapses made by the putative serotoninergic interplexiform and/or horizontal cells. Well expressed staining of the glial Müller cells in turtle retina was also evident.

The participation of the dopamine- and serotoninergic neurons in the complex retinal networks of frog and turtle retinas is discussed.

P10.28

Inhibition of transient receptor potential ion channels by endogenous lipid mediators

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Background: Transient Receptor Potential ion channels, such as TRP Vanilloid 1 and Ankyrin repeat domain 1 (TRPV1 and TRPA1) are expressed in nociceptive primary sensory neurons and sensory nerve endings. Resolvins are endogenous lipid mediators. Resolvin D1 (RvD1) is described as a selective inhibitor of TRPA1. RvD2 has been reported as a potent inhibitor of TRPV1 and TRPA1 in dorsal root ganglion cells. Our aim was to analyse the effects of RvD1 and RvD2 on TRPV1 (by capsaicin) and TRPA1 (by mustard oil (MO)) receptor activation on trigeminal sensory neurons and nerve endings.

Methods: Ratiometric technique of [Ca2+]i measurement with the fluorescent indicator fura-2-AM on cultured trigeminal cells was performed. Calcitonin gene-related peptide (CGRP) release from the stimulated peripheral sensory nerve terminals of the isolated rat trachea was measured by radioimmunoassay.

Results: Significant decrease in the percent of capsaicin- and MO-sensitive cells was observed after 10 nM RvD2 incubation on trigeminal neurons. RvD1 (10 nM) inhibited the MO-induced Ca2+-influx but had no effect on capsaicin-evoked TRPV1 activation. Both RvD1 (100 nM) and RvD2 (10 nM) decreased the capsaicin-evoked CGRP release from nerve terminals.

Conclusions: We demonstrated the inhibitory effects of resolvins on sensory neurons and sensory nerve terminals, but further investigations are needed to understand the mechanisms of these effects.

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P10.29

Effect of visual and auditive stimuli in amygdala neurons

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The amygdala has been usually associated with learning and is a key structure of the brain's reward system. However, some authors found that visual stimuli, not directly associated with rewards or punishments, could elicit responses in amygdala neurons. But the amygdala receives inputs not only from visual but also from auditory areas.

OBJECTIVES: We investigated how the amygdala neurons in one awake monkey (Macaca mulatta) might be involved in the evaluation of visual and auditive stimuli associated with reward or with no reward.

METHODS: All procedures were approved by the Institutional Animal Care Committee of the University of Santiago de Compostela in accordance with the European Community Council Directive. The monkey sat, with its head fixed, in a primate chair. The stimulus consisted of a 2 second video clip with sound and showing a front view of a human face (18.5 × 18.5°) presented on a conventional computer screen in front of the animal. The animal learned to perform an operant task in that two types of videos and two types of

sounds were presented randomly. In 'Yes' trials (video of a face nodding, pronouncing or not the word 'Yes') the animal had to press the lever to get the reward (a drop of juice). In 'No' trials (video of a face denying, pronouncing or not the word 'No') the animal could not press the lever and had to keep waiting, no reward was obtained in 'No' trials. These stimuli were presented to the animal using three types of tasks: VIDEO + SOUND, VIDEO, SOUND. We analyzed the data offline by using Matlab scripts. Neuronal responses were assessed by using the ANOVA test ($P < 0.05$)

RESULTS: Preliminary data showed that some amygdala neurons were decreasing their activity just after the video onset in both 'yes' and 'no' trials in the VIDEO+SOUND and VIDEO CONDITIONS, while these neurons did not change their activity in the SOUND condition. This activity was not modulated by reward or motor action.

CONCLUSIONS: The findings suggest that the amygdala may be involved in the processing of pure visual information. Different groups of amygdala neurons processed visual and auditive information.

P10.30
In vivo imaging of brain after cerebral ischaemia using SPECT/CT in mice

M. Semjéni
CROed Ltd

Imaging techniques to assess brain injury in a non-invasive manner are routinely used in patients and in experimental studies. However, it is technically challenging to visualize inflammation, blood brain barrier breakdown and brain injury simultaneously in vivo, which is further limited by image resolution and the small size of a rodent brain. Since systemic inflammation is often associated with the development of neurological disorders such as stroke, novel tools to simultaneously investigate systemic inflammation and brain injury in vivo would be required. It is currently not understood, how early events of blood brain barrier breakdown after stroke are altered by preceding peripheral inflammation or infection.

! To address these questions, we used single-photon emission computed tomography (NanoSPECT/CT Plus) utilizing specific radioligands combined with magnetic resonance (MRI) imaging to investigate central and systemic inflammation after experimental stroke in mice. Blood brain barrier breakdown was visualized by using penetration of 99mTc-DTPA into the brain parenchyma, which showed an overlap with changes in 1T MRI (nanoScan PET/MRI) images after stroke. Stroke-induced inflammatory changes were simultaneously monitored by imaging 125I-BSA in the same mice, which enabled visualization of systemic inflammation induced by bacterial lipopolysaccharide prior to experimental stroke and also peripheral inflammatory changes induced by cerebral ischaemia.

! Our imaging protocols might be useful tools to assess inflammation and brain injury in small rodents in vivo, and to develop techniques for evaluating systemic inflammation and blood brain barrier permeability changes in patients presenting with neurological disorders.

P10.31
Intracellular Fe2+ and 4-hydroxynonenal suppresses a swelling-activated chloride current in microglial cells

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Efflux of chloride through swelling-activated chloride channels upon cell swelling restores the initial cell volume. However, excessive loss of Cl- may induce DNA fragmentation and cell death. Accordingly, negative feedback loops to control Cl- conductance are required to maintain cell integrity. Using whole-cell patch clamp recordings we investigated the impact of Fe2+ and the lipid peroxidation metabolite 4-hydroxynonenal (4-HNE) on the swelling-activated chloride current (ICl, swell) in the microglial cell-line BV-2. Cell swelling was induced by a trans-membrane osmotic gradient. In control cells under symmetrical Cl- solutions dialysis of the cells with hyperosmotic pipette solution led to the activation of an outwardly rectifying Cl- current over time and which was sensitive to the Cl- channel blockers 5-Nitro-2-(3-phenylpropylamino)benzoic acid (NPPB), 4-(2-butyl-6,7- dichlor-2-cyclopentylindan-1-on-5-yl) oxobutyric acid (DCPIB) and flufenamic acid (FFA). Dialysis of the cell with iron lactate significantly suppressed current activation and reduced maximum current amplitudes compared to control cells with an apparent IC50 of 53.7 fM, but did not affect current rectification. Likewise dialysis of BV-2 cells with 4-HNE caused a significant reduction of current amplitudes without affecting the rectification characteristics. In contrast to intracellular administration of Fe2+ or 4-HNE, superfusion of cells with iron lactate led to a significant increase of ICl, swell compared to control cells. We suggest that a Fe2+–lipid peroxidation cascade may prevent cell death by suppression of excessive Cl- efflux.

P10.32
Median raphe can establishes glutamatergic synapses in the mouse forebrain

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The typically serotonergic median raphe nucleus (MR) takes part in the subcortical modulation of cortical information processing and our workgroup showed recently that MR establishes fast and precise, partly glutamatergic excitatory synaptic contacts with a specific subpopulation of GABAergic interneurons in the hippocampus (HC). This feature of the synapses of the MR was not known so far in other brain areas. In these raphe-hippocampal synapses the terminals contain vesicular glutamate transporter type 3 (vGluT3) and we have shown the presence of postsynaptic AMPA-type glutamate receptors before. Because NMDA receptors (NMDARs) play an important role in excitatory transmission and synaptic

plasticity mechanisms, we tested, whether MR synapses can act via NMDARs in its different target areas. Using double immunohistochemistry in the HC, we labeled the vGluT3-positive boutons originating in the MR and we found that at least about 88% of these synapses are NMDAR-positive. Using anterograde tracing and NMDAR immunohistochemical staining, we showed that at least one-third of the synapses established by the MR in the HC, medial septum (MS) and prefrontal cortex (PFC) contain NMDARs, which changes our view about this pathway. Finally, using double retrograde tracing, we found that there are cells in the MR that innervate the HC and PFC or MS and PFC simultaneously.
These observations suggest that MR can act via glutamatergic NMDA receptors not only in the HC, but also in the MS and the PFC and these areas may be synchronously stimulated by the same MR neurons.

P10.33

Hedonic impact of sweet taste on food consumption and activation of reward related neurons in intrauterine undernourished rats

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Intrauterin undernutrition is a risk factor of metabolic and cardiovascular diseases formed in adulthood. We hypothesized that disturbed central non-homeostatic food intake regulation contributes to the phenotype. In order to investigate this, we set up a model for maternal food restriction by keeping the rat dams on reduced protein diet (PR) during the whole period of pregnancy. Hedonic food intake was examined by presenting sweetened condensed milk solution to control and PR offsprings at 10 weeks of age on two following days. Drinking was allowed for 10 min, milk consumption was measured. One hour after the second experiment, animals were sacrificed and the brains were processed to determine neuronal activation by Fos immunohistochemistry. The number of activated cells in the subdivisions of the accumbens nucleus, recognized as a key center of reward, as well as in the central amygdala a known center of novelty for taste, was counted. Parameters of drinking behavior were analysed from video recordings.
Birth weights of PR rats were lower, but they grew more rapidly, thus by the time of the experiments they reached the weights of controls. In both experiments PR rats drunk more condensed milk. Interestingly, controls consumed significantly more milk during the second experiment. Duration of drinking showed a tendency to increase in PR animals, but frequency of licking did not differ. The quantity consumed correlated with the drinking duration in both groups and with the licking frequency in controls only. The number of Fos+ cells in the accumbens nucleus was similar in the groups. However, reward value of taste reflected well as a correlation between the drunken quantity and the number of Fos+ cells in the medial shell region of nucleus accumbens in both groups and in the core region in PR rats. Significantly more cells were activated in the central amygdala in the control group. Data show that hedonic component of food reward plays a dominant role in consumption of PR animals. Reduced sensitivity of the reward center (accumbens nucleus) and the central amygdala responsible for novel taste may contribute together to altered food intake regulation in PR rats.

P10.34

Effects of resveratrol and resveratrol delivered in liposome carrier system on penicillin-induced brain epileptic activity in male rats

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Resveratrol, which is a type of natural phenol produced by plants, has attracted attention over the past decade because of its anti-inflammatory, anti-oxidant, chemopreventive properties. However, there is a limited data about its possible antiepileptic effects. Epilepsy is defined spontaneous and recurrent seizures that caused by uncontrolled and excessive dyscharges and desynchronized neuronal activity. In the present study, we aimed to elucidate possible antiepileptic and antioxidant effects of pure resveratrol and resveratrol delivered with amphipatic liposomal brain delivery system, which has a high blood-brain barrier crossing potential, on penicillin-induced epileptic seizures. Twenty-four Sprague Dawley adult male rats were divided into four groups as follow; epilepsy, liposome, resveratrol (RES) and resveratrol+liposome (RES+LIP). Rats were anaesthetized with urethane and penicillin administered intracortically in order to establish seizures. Thirty minutes after beginning the seizures 20 mg/kg iv resveratrol and resveratrol in liposome injected. Electrocorticography (EcoG) was recorded with electrodes placed on left somatomotor cortex and spike frequency and amplitudes were analyzed. At the end of the experiments brains were collected and fronto-parietal part of the left hemisphere was separated. Malondialdeyde (MDA), Glutathione (GSH) and Glutathione S-transferase (GST) assays were performed to analyze anti-oxidant effects of resveratrol on penicillin induced epilepsy model. According to EcoG results; RES+LIP group decreased frequency significantly for comparing with saline ($p < 0,01$) and RES ($p < 0,01$). Amplitudes decreased in RES+LIP group in comparison to saline group ($p < 0,05$). According to biochemical analysis; RES+LIP group has higher GST levels ($p < 0,05$), decreased MDA level ($p < 0,01$) and increased GSH level in comparison to control group ($p < 0,01$).
In conclusion, the present findings have shown that; resveratrol inserted into liposomes showed more antioxidant and anticonvulsant effects than only resveratrol by improving resveratrol's bioavailibility. Biochemical analysis have demonstrated that RES+LIP is effective as an antioxidant. To sum up; RES+LIP may be more beneficial than RES on penicillin induced epileptic activity.

P10.35

The role of the melanocortin system and neuropeptide Y in the regulation of energy homeostasis in SHR rats

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Introduction: Caloric intake and body weight (BW) of spontaneously hypertensive rats (SHR) developed for the study of essential hypertension are lower than those of age-matched normotensive controls. Their BW does not reach that of controls even on a high-fat diet suggesting a dysregulation of their energy homeostasis. We assumed an enhanced activity of the hypothalamic anorexigenic melanocortins (MC) in the background, that has been shown to contribute to their hypertension, as well. A diminished tone of the dominant orexigenic neuropeptide Y (NPY) was also hypothesized.
Methods: Food intake (FI) of adult male SHR rats and normotensive Wistar rats (NT) was recorded in an automated FeedScale system upon intracerebroventricular injection of NPY (5 μg) or a MC agonist (5 μg alpha-melanocyte-stimulating hormone, α-MSH, spontaneous night-time FI) or central infusion of MC antagonist HS024 (1 μg/h, 7 days). Using immunofluorescent labeling we determined the number of α-MSH producing cells and also performed the quantitation of α-MSH and neuropeptide Y specific signal density (SSD) in the arcuate nucleus of the hypothalamus.
Results: The α-MSH-injection reduced spontaneous night-time FI more efficiently in SHR than in NT rats. HS024 started to increase daily FI and consequently BW in the NT group already from the 1st, in SHR animals only from the 3rd day. In contrast, responsiveness of day-time FI to NPY injection was found to be suppressed in SHR. We did not find difference between SHR and NT rats in terms of α-MSH-producing cell counts and neuropeptide Y SSD in the arcuate nucleus, but the SSD of α-MSH immunosignal was significantly higher in SHR rats than in NT animals.
Conclusion: Our in vivo and also in vitro results suggest higher MC-production and -responsiveness and lower NPY-responsiveness in SHR rats, what may contribute to the explanation of the dysregulation of their BW.

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P10.36

Synapse-specific distribution of neuroligin-2 in the hippocampus

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Neuroligin 2 (NL2) is a synaptic transmembrane protein. NL2 is specifically found in GABAerg synapses in the postsynaptic membrane. The hippocampal interneurons are the most fundamental regulators of network activity in the central nervous system, consequently, structural and functional changes in their synapses give rise to several disorders, such as certain mutations and modifications of this molecule can be connected to anxiety. In our expreiment, we examined two GABAergic interneuron populations in the CA1 region of the hippocampus: the parvalbumin- (PV-) positive and CB1 endocannabinoid receptor positive basket cells.
Our aim was to quantify the NL2 desity in the synapses of these cell populations. With using preembedding immunhistochemistry, we visualized the NL2 molecule with immunogold method, and reconstructed the synapses using electron microscopy and measured the NL2 density in the synapses of these interneurons. According to our results, the neuroligin density in the synapses made by the the parvalbumin positive interneurons is 50% higher than in the synapses of the CB1 positive interneurons. On the other hand, the total amount of this protein in the postsynaptic side is higher in the CB1 positive interneurons, according to their size. The exact function of the cell adhesion molecule NL2 is still unkown, but the higher density of this molecule in the PV positive cells can be essential for its oscillation rhythm generating functions.

P10.37

CRT and LCD monitors in science

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Widespread availability of cheap computers accelerated psychophysical research. The relative cheap CRT and LCD monitors are used to show visual stimuli. Both monitor types have advantages and disadvantages originating from the working principle which have a deep impact on the perception and on the reaction to the visual stimuli. In our work the two monitor types were compared during fast changing temporal conditions (16 ms) in the so called flicker illusion. There is no clear winner between the two techniques, the intended paradigm decides which monitor type to choose.
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P10.38

Pharmacological manipulations of striatal interneurons induce a phenotype of dystonia in the monkey

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Objective. Dystonia is defined as a syndrome of sustained muscular contraction leading to repetitive movements and

abnormal postures. To date, its pathophysiology remains unclear. A line of evidence suggests that the cholinergic (Ach-I) and GABAergic (GABA-I) interneurons of the striatum may play a critical role in its pathophysiology. Indeed, cholinergic antagonists are the only pharmacological drugs effective in the treatment of dystonia and acetylcholine applied on striatal brain slides corrects the abnormal cortico-striatal plasticity observed in transgenic mice exhibiting the mutant form of the torsinA. Furthermore, blocking GABA-I in rodents induce dystonic-like movements. Taken as a whole, this data suggests that increasing intrastriatal acetylcholine and decreasing GABAergic transmission may induce a phenotype of dystonia. Our aim was to test this hypothesis in non-human primates.

Methods. Intra-striatal (AC-2mm, L+12) microinjections of 2-8µl Oxotremorine (Oxo, Ach-agonist), 0.5-4 µl Bicucculline (Bic, GABAA antagonist) or saline (Nacl 9‰) were performed in two macaca fascicularis under neuronavigation control. Animal behavior was studied in a primate chair before and for two hours following injections. It was then blindly assessed on videos for clinical symptoms using an adapted form of the dystonia BFM rating scale.

Results. Abnormal involontary movements (AIMs) associating both tonic dystonic postures and myoclonic jerks were observed in the hemibody contralateral to injections whereas saline injections had no effect. These AIMs affected distal parts of the limbs, the trunk and sometimes the face and led at the highest volume to focal epileptic seizures. Bic injections had a quicker but shorter effect whereas Oxo injections had a delayed but prolonged effect.

Discussion. These preliminary results suggest that modif

P10.39

Disruption of sensorimotor integration in writer's cramp

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Writing is a sequential motor task in which proprioceptive information is critical to adapt hand posture and finger grip during successive movements, a process disturbed in writer's cramp (WC). To study the intra-cortical functional connectivity occurring during sequential sensorimotor integration, we designed two non-writing motor tasks in which right-handed subjects had to perform a four-finger motor sequence, either on the basis of sensory stimuli previously memorized (SM task) or freely generated (SG task). Neuronal activation was compared between 15 WC patients and 15 healthy controls by event-related functional magnetic resonance imaging (spatial dimension) and coherence electroencephalography (temporal dimension). No dystonic features were observed during these tasks whereas reaction times were clearly impaired in WC patients. The bold signal was decreased in WC patients in the left sensorimotor, bilateral parietal cortices and supplementary motor area (SMA). EEG coherence between the sensorimotor cortex, SMA and premotor cortex was diminished and negatively correlated with severity. ANOVA failed to show any group differences during movement execution but ROI analysis revealed impaired activation in the primary motor cortex and SMA. The coherence between the parietal cortex, SMA and premotor cortex was modified and positively correlated with disease duration, suggesting that some compensatory mechanism might occur over time. The impaired functional connectivity between the parietal and premotor cortices might explain the inability of WC patients to correctly control the muscular pattern recruited during movement execution when it requires a subtle analysis of sequential proprioceptive information, as occurs during writing.

P10.40

The voltage-dependent anion-channel (VDAC) is dephosphorylated by beta-amyloid peptide. Involvement in AD mechanisms of toxicity

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Our laboratory has studied the modulation of the activity of the anionic high-conductance channel located in the neuronal membrane (pI-VDAC), specifically in lipid rafts, by phosphorylation-dephosphorylation state after exposure to toxic agents such as β-amyloid peptide. pI-VDAC is a mitochondrial porin related with apoptotic phenomena, and is also found located at the plasma membrane where it modulates the toxicity of β-amyloid peptide (Aβ), one of the parameters of the pathology of Alzheimer's disease.

Objectives: To characterize the potential modulation of VDAC phosphorylation state in response to Aβ To analyze whether VDAC regulation may be involved in Aβ-induced mechanisms of neurotoxicity

Results:

- At the neuronal membrane, pI-VDAC is present in at least three different isoforms, similar to those observed in mouse and human brain tissue. Studies in cell types from different origins (SN56, HT22, septum and hipocampal murine cells and SHSY5-Y from human neuroblastoma cells) have reported that pI-VDAC isoforms correspond to tyrosine phosphorylated modifications.
- Treatment with tyrosine phosphatase inhibitor (Bipy) prior to Aβ preserves channel phosphorylation, thereby indicating that the peptide may induce phosphatase activation to dephosphorylate VDAC.
- Real-time cell proliferation studies were performed to determine HT22 and SN56 cell mortality in response to Aβ. In the time-course experiments, we established dose-response neurotoxicity in the presence of the peptide. Furthermore, pre-treatment with Bipy followed by Aβ exposure resulted in a reduction of cell mortality. Overall, these results suggest that VDAC dephosphorylation may be a strategy developed by Aβ to induce cell death.

Conclusions: We established the relevance of post-transductional regulation of pI-VDAC in neuronal death by Aβ. Exposure to beta-amyloid peptide seems to cause changes in the

phosphorylation pattern of pI-VDAC. Moreover, we found a dose-time modification of VDAC phosphoisoforms leading to Aβ induced toxicity. Also, the presence of the phosphatase inhibitor (Bipy) provokes a reduction of Aβ toxicity most probably through the decrease of VDAC desphosphorylation.

P10.41

The treatment of orofacial pain by using theta burst rTMS stimulation

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The aim of investigation: In our previous experiments 10 Hz and 20 Hz rTMS stimulations were used for the treatment of different types of orofacial pain.
All patienst had drug resistant orofacial unilatelar neurogenic pain with duration greater than one year. It was found out that 20 Hz rTMS stimulation is more effective than 10 Hz stimulation. In this experiment we tested the theta burst stimulation as anothet possible tool for pain treatment.
Methods: 19 patients (12 females, 7 males) with chronic orofacial pain participated in the study. We stimulated motorcortex area corresponding to the hand on painfull side. They underwent quantitative sensory testing before and after the stimulation. The intensity of pain was evaluated in all patients before, during and after rTMS or shame stimulation by means of a visual analogue scale (VAS). Also thermal and tactile sensory thresholds and allodynia as a consequence of orofacial pain were measured.
Results: By using VAS measurement the theta burst stimulation was more effective than the shame stimulation. The effect of theta burst stimulatins was still observed 14 days after the stimulation. During the measurement of thermic sensitivity, theta burst stimulation was not significantly improved. The measuremnt of tactile stimulation was significantly effective after real burst stimulation if compared with shame stimulation.
Conclusion: Repetitive transcranial stimulation (rTMS) is non-invasive neuromodulatory tecnigue that allows safe and painless stimulation of the brain cortex. High frequency rTMS theta burst stimulation was also effective in the chronic orofacial pain and could represent another possibility for the treatment of different types of orofacial pain.

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P10.42

Cannabinoid agonists evoke Ca2+ transients in spinal astrocytes

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Endocannabinoid signaling is one of the most abundant neuromodulatory mechanisms in the brain. In hippocampal astrocytes, activation of cannabinoid-1 receptors (CB1Rs) increase intracellular Ca2+ concentration ([Ca2+]i), and stimulates glutamate release, which activates NMDA receptors in pyramidal neurons. Although CB1Rs are strongly expressed by astrocytes in the spinal dorsal horn (SDH), the role of glial CB1Rs activation in the spinal nociceptive information processing is far from being understood. The release of gliotransmitters triggered by the cannabinoid-induced elevation of [Ca2+]i is unexplored in glial cells of the SDH. As a first step of investigating this mechanism, we wanted to know whether CB1-agonists evoke Ca2+ transients in primary astrocyte cultures prepared from the spinal cord of rats, wild-type and CB1-KO mice.
10 μM anandamide (AEA), 2-arachidonoylglycerol (2-AG), WIN55,212 (WIN) and arachidonyl-2-chloroethylamide (ACEA)-evoked changes in [Ca2+]i were monitored by using the ratiometric fluorescent dye Fura-2. To determine the ratio of responding cells, confocal measurements were performed on Fluo-8-loaded cultures, and series of x-y images were recorded in the presence of WIN and AEA.
All the above drugs were capable of evoking Ca2+ transients on rat astrocytes with 94±25 nM to 428±84 nM amplitudes. In cultures from wild-type mice 9±4% of the cells responded to WIN and 20±7% responded to AEA. In rat cultures the ratio of responding cells was 11±2% to WIN and 7±4% to AEA. Even in CB1-KO astrocytes some cells responded to these drugs (3±1% and 8±4%, respectively) with smaller amplitudes.
On the basis of the [Ca2+]i measurements we can conclude that CB1R-mediated Ca2+ signaling is present on astrocytes isolated from superficial SDH. However the observed Ca2+ transients on CB1-KO cells raises the possibility of the presence another, non-CB1-mediated endocannabinoid pathway on these cells.

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P10.43

The role of segmentation interval in detecting seizure from EEG series by using embedding entropy metrics

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In the present study, three well known embedding entropy approaches so called Approximate Entropy (ApEn), Sample Entropy (SamEn) and Permutation Entropy (PerEn) were applied to five experimental data sets consisting of both healthy surface Electo Encephalo Graphic (EEG) signals and epileptic intracortical measurements to obtain the EEG based characterization of seizure with respect to the degree of EEG complexity. In test, the role of the EEG segmentation interval, i.e. the size of embedding dimension (ed) in quantifying the

degree of EEG complexity was investigated. The results show that the degree of EEG complexity is originated by not only neurophysiologic but also neuropathologic status of the brain. In particular, EEG complexity decreased in seizure. Though, the wideness of ed has crucial role in computing the entropy of EEG series. In detail, the performance of both ApEn and PerEn directly depends on the eds in contrast to SamEn. The lower eds provides the more discriminative results by using ApEn, however, the higher eds can provide the more sensitive EEG characterization by using PerEn. Besides, tolerance level affects the estimation performance for ApEn and SamEn. Both of them can provide the more useful results for smaller tolerance level about 0.1 times standard deviation of EEG series.

Keywords: Entropy, embedding dimension, seizure, EEG

P10.44

Effect of intracerebroventricular irisin injection on the uncoupling protein expression in the rat brain

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Uncoupling proteins (UCPs) belong to a family of mitochondrial carrier proteins that are present in the mitochondria inner membrane. These proteins carry protons directly from the intermembrane space to the matrix in mitochondria. UCPs have many physiological roles such as energy homeostasis, gene regulation, neuroprotection and cancer. Irisin (encoded by Fndc5 gene) is a newly defined myokine that is secreted from the skeletal muscle and the other parts of the body. It has been reported that irisin stimulates browning and UCP1 expression in adipose tissue. However, the effects of irisin in the central nervous system are not known.

This study was designed to determine the effects of irisin on the expression of UCP2, UCP3, UCP4 and UCP5 in different areas of the brain. 30 female Sprague Dawley rats (12 wk old, 200-250 g) were used in the study. The animals were divided into six groups (n=5 for each group). Rats were intracerebroventricularly injected at 10 μM concentration of irisin or vehicle (control group) in the right lateral ventricle. The brain tissues of rats were removed and analyzed by using RT-PCR method after the animals were sacrificed at the end of six different time points (30 min, 2, 8, 16, 24 and 48h). In the result, the single injection of irisin in the lateral ventricle was caused significantly increases in the UCP2, UCP3 and UCP5 mRNA levels in the striatum and cerebellum area of rat brain ($p < 0.05$). In the cortex, it was seen to a significantly increase in UCP2 and UCP5 mRNA levels ($p < 0.05$). UCP3 level in the cortex any significantly change was observed. In general, the UCP4 mRNA level showed a decrease in the areas of the brain.

Our results indicate that the irisin may control certain physiologic functions in the different areas of the brain by various effects of UCPs.

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Key Words: Irisin, uncoupling protein, striatum, cerebellum, cortex, intracerebroventricular injection

P10.45

Complexity and coherence analysis on EEG of patients with obsessive compulsive disorder

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In the present study, multichannel EEG complexity has newly been examined for identification of obsessive-compulsive disorder (OCD). Since, EEG series is non stationary signal in nature, resting state eyes closed 19-channel EEG measurements of 3 min were segmented by using a specified window of 2 sec before applying the complexity and coherence metrics. In tests, the well known statistical entropy computation methods so named Approximate Entropy, Sample Entropy and Permutation Entropy (PermEn) were used to obtain the quantitative EEG complexity values. And, Mutual Information (MI) in addition to Coherence Function were used to measure the hemispheric dependency of each symmetrically placed electrode couples. Individual entropy values of 19-channels and both MI and CF values of 8 electrode couples were classified by using Support Vector Machine Classifiers. The results show that PE provides the good discrimination with accuracy of 97%, however, MI can provide the clearly error free EEG classification. In particular, both EEG complexity and hemispheric dependency decreases in patients with OCD.

Keywords: Entropy, Hemispheric Dependency, EEG classification, Obsessive Compulsive disorder.

P10.46

The role of hemokinin-1 and Substance P in acute pain in mice

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INTRODUCTION: Hemokinin-1 (HK-1) encoded by the Tac4 gene, is present in the nervous and immune systems. It has similar structural and immunological characteristics to those of substance P (SP) derived from the Tac1 gene. Moreover, these peptides show similar affinity to the neurokinin 1 (NK1) receptors. We studied the roles of these tachykinins in acute somatic and visceral nociception, as well as acute neurogenic inflammation using gene-deleted (Tac4-/- , Tac1-/- , Tac1/4-/-

and NK1-/-) mice compared to their C57Bl/6 wildtypes. **METHODS:** Acute somatic nocifensive behaviour was evoked by intraplantar formalin injection. Nocifensive reactions arose in two phases (direct stimulation of sensory nerves between 0–5 min and consequent activation of inflammatory mechanisms between 20–45 min), and were quantitatively evaluated by the duration of paw liftings, lickings and shakings. Visceral nociception was induced by i.p. acetic acid injection to elicit abdominal contractions (writhings), which was counted during the 0–5, 5–20 and 20–30 min time-intervals. Acute neurogenic inflammation was evoked by the ultrapotent Transient Receptor Potential Vanilloid 1 receptor agonist resiniferatoxin (RTX; i.pl.). After control measurements, noxious heat and mechanonociceptive thresholds were measured with increasing temperature hot plate and dynamic plantar aesthesiometer, 5-20 min and 2-24 hours following RTX-administration, respectively.
RESULTS: Formalin-evoked paw liftings and lickings were significantly reduced in Tac1-/- and Tac1/4-/- mice, while acetic acid-induced writhings decreased in all gene-deleted animals compared to their wildtypes. RTX-evoked thermal allodynia was totally diminished in Tac1-/- and Tac1/4-/- mice, and partly reduced in Tac4-/- animals, while mechanical hyperalgesia was significantly decreased in Tac4-/-and NK1-/- mice.
CONCLUSION: SP mediates somatic and visceral nociception and inflammatory thermal allodynia, but NK1 receptors are only involved in these actions in the visceral region. HK-1 plays a predominant role in visceral pain and inflammatory mechanical hyperalgesia, which actions are partly mediated by NK1 receptors.

P10.47

A role for the neurokinin-1 receptor in endotoxin-induced fever in mice

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An involvement of substance P and its receptor, the neurokinin-1 (NK1) receptor in the mediation of lipopolysaccharide (LPS)-induced fever has been shown earlier, but no studies have been performed, in which, the development of LPS-induced fever was compared between NK1 knockout (KO) and wild type (WT) mice. Adult NK1 KO and WT mice of both sexes were used. In a telemetry system, we investigated the circadian changes of deep body temperature (Tb) and locomotor activity in freely-moving NK1 KO and WT mice. In a separate set of experiments, mice were habituated to staying inside conical restrainers, then in loosely restrained NK1 KO and WT mice, their Tb and autonomic thermoeffector responses to intraperitoneal LPS (or saline) infusion were recorded. Freely-moving NK1 KO mice were hyperactive during periods of the night, which was accompanied by increased Tb, whilst there was no difference in either the locomotor activity or deep Tb between KO and WT mice during the light phase of the day. Injection of LPS resulted in a marked fever response in the mice of both genotypes ($p < 0.05$). However, when injected with LPS, the increase of Tb in NK1 KO mice was significantly attenuated compared to controls (38.1 ± 0.2 vs. 38.5 ± 0.2°C; $p < 0.05$). The attenuation of the fever response was caused by a reduced elevation of the heat production (oxygen consumption) in the NK1 KO mice as compared to their WT littermates (173 ± 9 vs. 189 ± 6 ml/kg/min; $p < 0.05$). We conclude that the absence of the NK1R results in increased Tb and locomotor activity during the night with no alterations during the light phase of the day. The fever response of the NK1 KO mice is attenuated, which is, at least in part, caused by their reduced LPS-induced elevation of heat production.

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P10.48

Temporal characteristics of binocular visual information processing, a VEP study

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Previous research has found that the phase of steady state Visual Evoked Potentials (ssVEPs) is linearly correlated to the frequency of stimulation. When ssVEP responses are registered for a set of temporal frequencies, apparent latency (i.e. response time) can be calculated from the steepness of the regression line fit to the phase-frequency data. Our goal was to compare the phase-frequency relationship and the apparent latencies of Dynamic Random Dot Correlogram (DRDC-VEPs) and checkerboard reversal pattern evoked responses (PR-VEPs). Eight adults with intact binocular vision were included in the study. DRDC-VEP responses were evoked by DRDC stimuli at temporal frequencies in the range of 2-50 Hz. Stimuli were presented on the red and green channels of the CRT monitor while observers were wearing red-green goggles for dichoptic viewing. Luminance reversing checkerboard pattern VEPs of similar temporal frequencies were used as controls. Reliability of VEP responses were evaluated by T2circ statistics. Significant Fourier components of the fundamental frequencies were plotted and regression lines were fit to the data. Goodness of fit was indicated by R2 values. Apparent latencies were calculated from the steepness of the regression lines. We found significantly longer apparent latencies in case of DRDC-VEPs compared to PR-VEPs. Moreover, in some cases, including DRDC-VEP data, we found at least two linear fits (i.e., two apparent latencies) during data modeling, giving two apparent latency values for higher and lower temporal frequency ranges. The higher apparent latency values for DRDC-VEPs indicate mechanisms in the early stages of binocular visual pathways that take longer time to complete compared to monocular information processing. Moreover, based on the initial results, it is also

possible that binocular information is processed by more than one, possibly two parallel mechanisms in the early processing stages, each having different temporal characteristics.

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P10.49

Effect of antioxidants in preventing trimethyltin-induced neurodegeneration

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The trimethyltin (TMT) model is one of the frequently used approaches to study Alzheimer-type of neurodegeneration. In the hippocampus, TMT evokes the formation of reactive oxygen species, triggers apoptosis and disrupts functional circuits, thus leading to memory impairment.

OBJECTIVE: We studied the effect of TMT on the width of the CA1 area of the hippocampus, the level and the activity of oxidative stress markers in blood serum, and its effect on spatial memory. The aim was to assess the effect of SMe1EC2 and vitamin C on TMT-induced changes.

METHODS: Male Wistar rats (n=24) were used in our experiments. TMT was administered intraperitoneally by a single dose of 8 mg/kg of body weight. The control group received saline. Both the pyridoindole SMe1EC2 and vitamin C were administrated 3 times orally in the dose of 50 mg/kg, 1 hour before TMT administration, 1 and 24 hours after TMT submission. The spatial memory was tested in Morris water maze for 5 days on days 21-25 after TMT. Thickness of the CA1 field of the hippocampus was assessed on 4 μm thick hippocampal slices stained by haematoxylin and eosin. The activity of N-acetyl-β-D-glucosaminidase (NAGA) and the level of malondialdehyde (MDA) in blood serum were assayed according to standard method.

RESULTS: TMT application resulted in marked neuronal loss in the CA1 field of the hippocampus, increased level of MDA and the activity of NAGA in serum and in worsening of spatial memory. Pyramidal cell layer in CA1 field was significantly thicker in rats pretreated with SMe1EC2, while that with vitamin C remained similar as in the TMT group. Both compounds tested decreased the level of MDA compared to the TMT group, while the increased activity of NAGA remained unchanged. None of the substances tested were able to ameliorate the effect of TMT on spatial memory.

CONCLUSION: Vitamin C and SMe1EC2 were effective in preventing an increase in MDA level, but failed in lowering the activity of NAGA. SMe1EC2 partially prevented CA1 neuronal loss caused by TMT. The testing in water maze revealed that the function of the hippocampus was fatally destroyed, resulting in deterioration of spatial memory.

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P10.50

Protective effect of rasagiline in aminoglycoside ototoxicity

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Sensorineural hearing losses (SNHLs; e.g., ototoxicant- and noise-induced hearing loss or presbycusis) are among the most frequent sensory deficits, but they lack effective drug therapies. The majority of recent therapeutic approaches focused on the trials of antioxidants and reactive oxygen species (ROS) scavengers in SNHLs. The rationale for these studies was the prominent role of disturbed redox homeostasis and the consequent ROS elevation. Although the antioxidant therapies in several animal studies seemed to be promising, clinical trials have failed to fulfil expectations. We investigated the potential of rasagiline, an FDA-approved MAO-B inhibitor type antiparkinsonian drug, as an otoprotectant. We showed a dose-dependent alleviation of the kanamycin-induced threshold shifts measured by auditory brainstem response (ABR) in an ototoxicant aminoglycoside antibiotic-based hearing loss model in mice. This effect proved to be statistically significant at a 6 mg/kg (sc) dose. The most prominent effect appeared at 16 kHz, which is the hearing sensitivity optimum for mice. The neuroprotective, antiapoptotic and antioxidant effects of rasagiline in animal models, all targeting a specific mechanism of aminoglycoside injury, may explain this otoprotection. The dopaminergic neurotransmission enhancer effect of rasagiline might also contribute to the protection. Dopamine (DA), released from lateral olivocochlear (LOC) fibres, was shown to exert a protective action against excitotoxicity, a pathological factor in the aminoglycoside-induced SNHL. We have shown that rasagiline enhanced the electric stimulation-evoked release of DA from an acute mouse cochlea preparation in a dose-dependent manner. Using inhibitors of voltage-gated Na+-, Ca2+ channels and DA transporters, we revealed that rasagiline potentiated the action potential evoked-release of DA by inhibiting the reuptake.

The complex, multifactorial pathomechanism of SNHLs most likely requires drugs acting on multiple targets for effective therapy. Rasagiline, with its multi-target action and favourable adverse effects profile, might be a good candidate for a clinical trial testing the otoprotective indication.

P10.51

Neuronal responses of the rat medial prefrontal cortex during appetitive classical conditioning

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It has been demonstrated that the medial prefrontal cortex (mPFC) is involved in the regulation of appetitive and consummative behaviors.

In the present experiments, extracellular multielectrode recordings of mPFC neurons were performed in freely moving rats during Pavlovian trace conditioning (CS: 2 sec; trace interval: 2 sec; US: 12 sec; intertrial interval: 6 sec). Two different tone CSs were associated with two different rewards (sugar solution or water), respectively. Neuronal activity changes in the mPFC were analyzed: (i) during CSs; (ii) just before the first licks of licking clusters; (iii) during consummation of the different rewards. Approaching movements of the rats to the drinking bottles were measured with an accelerometer mounted to the headstage amplifier.

Early occurring approaches (during the first 2 sec of reward delivery) were demonstrated to appear as CS related approach behavior: (i) significantly higher accelerations were observed during approaches to the drinking bottle containing sugar solution than to the water containing one; (ii) for both rewards significantly higher accelerations were observed during early approaches, then during late occurring approaches (2 to 12 sec of reward delivery).

Population activity of neurons discriminated between trials with different US types (sugar solution or water) during CS tones, CS evoked approach behaviors and consummation. Analysis of single neuron activity during early and late approaches suggests that some responses are related to conditioned approach behavior (higher response during the early phase) while other responses code reward prediction independently from approach behavior.

These results proved that the activity of medial prefrontal cortical neurons represent consummation and prediction of different rewards. Our data also suggest that multiple representations of the US are present in the rat mPFC during Pavlovian conditioning.

P11.1

The effect of aerobic training on performance and hormonal changes among prepubertal female handball players

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The aim of our study was to examine the changes of physiological, muscle force and hormonal parameters of young prepubertal female handball players after an 8 weeks training period in the preparatory season and to correlate them with performance changes. 18 young female handball players (11.50±0.56 yrs) participated in this study. Fasting venous blood samples were collected before and after the training program (estradiol, cortisol, testosterone, GH). Anthropometric parameters were measured, the hand grip strength test and maximal concentric quadriceps and hamstring torque were also performed. A spiroergometric treadmill exercise test was conducted to analyze the changes in physiological parameters (e.g. maximal HR, VO2max, RR variability, RER) during the test. Besides regular handball practice there was a specific endurance training program (20-25 min continuous running, 3 times/week). Paired sample t-test was used to analyze the changes and bivariate correlation to analyze correlation between parameters. Mean morphological age (12.52±0.80 years) of handball players significantly differed from their decimal ages (11.50±0.56 years). Improvement but no significant difference was detected in the relative VO2max after the training (b: 43.32±5.68 ml/kg/min, a: 44.05±5.09ml/kg/min). Better results were also registered in force parameters, mean maximal concentric quadriceps torque was significantly ($p=0.022$) higher after (84.39±15.35Nm) then before (76.38±15.93 Nm). Significantly higher ($p=0.033$) level of cortisol was detected after the training program (b: 340.39±168.06nmol/l, a: 423.74±199.34nmol/l).

As previously reported there was no significantly higher VO2max measured after 8 weeks of strong aerobic training. The improvement in force parameters in prepubertal ages are the consequences of neuromuscular adaptation. It is important to monitor the biological age/maturity parallel with physiological and anthropometric parameters of young athletes for good performance and efficient training programs.

P11

Exercise Physiology

P11.2

Development and complete morphological and functional reversibility of athlete's heart in a rat model

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Background: Long-term exercise training is associated with characteristic structural and functional cardiac adaptation termed athlete's heart. However the effect of discontuniation of the training (detraining) on left ventricular (LV) function is unclear. Our aim was to evaluate the development characteristics of athlete's heart and the reversibility of morphological and functional changes during detraining.
Methods: Rats were divided into trained (n=15) and control (n=17) groups. Trained rats swam 200 min/day for 12 weeks. Detrained rats remained sedentary for 8 weeks after completion of the training protocol. We regularly performed echocardiographic measurements to investigate development and regression of exercise-induced cardiac changes. LV pressure-volume analysis was performed to calculate cardiac functional parameters. LV samples were harvested for histological examination. Myocardial gene expression analysis was performed using qRT-PCR.
Results: Echocardiographic examinations showed rapidly developing LV hypertrophy in the trained group according to wall thickness values. This adaptation regressed after detraining, which was confirmed by post-mortem measured heart weight and histological morphometry. Unchanged myocardial expression of TGF-β and β-MHC and unaltered amount of LV collagen confirmed the physiologic nature of the observed cardiac hypertrophy. Hemodynamic measurements indicated decreased LV end-diastolic volume (LVEDV) along with increased stroke volume (SV), improved systolic function (ejection fraction) and contractility (end-systolic pressure-volume relationship), ameliorated active relaxation and mechanoenergetics (mechanical efficiency, ventriculo-arterial coupling) after long-term exercise training. After the detraining period regression of exercise-induced cardiac functional changes were observed: LVEDV, SV, LV contractility and mechanoenergetic enhancement reverted completely to control values. Training and detraining did not affect myocardial stiffness.
Conclusions: Our results confirm that the morphological and functional adaptation of exercise-induced physiologic LV hypertrophy completely regressed after 8 weeks of detraining.

P11.3

Periodical changes in the characteristics of the athlete's heart

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Introduction: Left ventricular (LV) adaptation to training and it's reversibility following the cessation of physical activity is well known in adults, and suspected to be more prominent in young athletes.
Aim of study: was to describe the changes in the LV morphology and function during the training season.
Subjects and methods: 15 elite adolescent swimmers (8 boys and 7 girls, 6 years of training history, 20 hours weekly training) participated in our 1.5 year long follow-up study. Training adaptation of the LV was measured with 2D-Echocardiography made every 3 months, according to the macro cyclic periods of training.
Results: A definite LV morphological adaptation could be detected in the swimmers: the smallest end-diastolic diameter (LVIDd: 44.9±3.4mm) and the greatest LV muscle mass (LVMM: 228.0±46.1g) were detected at the end of the general endurance preparation period-2 (GEP2), but the LVMM index (LVMMI: 84.9±10.4 g/m) did not change during the follow-up period, such as the E/A Ratio (E/A: 2.22±0.58) that describes the diastolic function.
Conclusion: On the basis of our results, echocardiography examination to analyze the training adaptation is suggested once a year even in young athletes. The timing of the examination primarily depends on what we are interested in; nevertheless, we suggest it at the end of the GEP because the performance achievable in the race period is predicted by these values.

Keywords: athlete's heart; echocardiography; seasonal change; left ventricle; young swimmer

P11.4

The effect of exercise on blood plasma markers of skeletal muscle injuries

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The aim of the study was to investigate the effect of one acute exercise on changes of blood plasma gelsolin, orosomucoid and actin level, orosomucoid /gelsolin rate and the correlation between these parameters and other skeletal muscle microinjury markers (plasma creatine kinase activity (CK), muscle soreness measuring with visual analog scale (VAS), lactate concentration) in athletes and controls.
After anthropometric measurements 18 males (12 athletes and 6 controls, between 20-30years) performed eccentric-concentric quadriceps contractions (15 in 6 series) with the dominant limb on a dynamometer (Multicont II isokinetic device). Blood lactate level and CK were measured pre-exercise, immediately and 1, 6, 24 hours after exercise. Plasma concentration of gelsolin and actin were determined in all time points with Western blot technique and Enhanced Chemiluminescence detection. The intensity of muscle soreness was estimated with VAS before and 1 day after the exercise test.

The two studied groups significantly differed in bodyfat percentage, fat mass, training hours, gelsolin concentrations. We found significant differences between plasma gelsolin level before the test and the lowest value after the test, just like with VAS before and 1 day after the test. CK at 24h post-exercise was higher than at any other time points.
Strong correlation was observed between plasma gelsolin level at 6h, 24h, maximal and minimal value after exercise and the CK level at 6h post-exercise (r6h=0.570; r24h=0.481; rMIN=0.664; rMAX=0.507), similarly to between post-exercise VAS and minimal level of gelsolin and orosomukoid concentration at 24h after the test (r=-0.490; r=-0.551). We noted a significant relationship between the plasma orosomucoid/gelsolin rate (in rest, at immediately, 1, 6h post-exercise) and lactate concentration in rest (rPRE= 0.674; r0=0.633; r1h=0.650; r6h=0.608).
Indirect indicators of exercise induced muscle damage strongly correlated with the studied plasma markers (gelsolin, orosomucoid, orosomucoid/gelsolin rate). Further studies are needed to determine the sensitivity and the reliability of these biomarkers as indicators of skeletal muscle damage.

P11.5

Amino acid levels, enzyme activity, and lipid peroxidation in smokers and non-smokers after a 6-week long β-Alanine rich diet

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The purpose of our study was to characterize the biochemical effects of a β-Alanine rich legal trade sport food supplement. β-Alanine is the decomposition and constructive product of the dipeptide carnosin (beta-alanyl-L-histidine). Its main physiological role is to neutralize hydrogen ions, avoiding acidification therefore maintaining the optimal pH level of muscle cells. Participants were volunteers (n=43) 58% male, 42% female. Since we did not find any significant differences between the results of men and women, our final groups are: smoker users (SU, n=9), smoker non-users (SNU, n=7), non-smoker users (NSU, n=14), non-smoker non-users (NSNU, n=13). The food supplement was supplied by Scitec Nutrition. Half of each group got pills containing β-alanine, taking them consistently (50mg/bwkg) on an everyday basis, while the other half of the groups took placebos (microcrystalline cellulose). Before and after the 6-week long training and supplementing protocol, we had measured some anthropological, biomechanical and fitness parameters. Besides these measurements blood samples of the volunteers were collected, and creatine kinase enzyme activity (CK), lipid peroxidation(TBARS) measured, important amino acid concentrations (Histidine, Beta-alanine, Carnosine, HPLC) of blood plasma were also detected. The CK activity showed significant reduction ($p < 0,017$) in smokers (before 228,3U/ml and after 112,9U/ml) after the supplementation. While in the nonsmoker group the normal physiological range of the enzyme activity was detected in smokers only after the training program (before 112,1U/ml and after 130U/ml). Interestingly the TBARS levels had shown the same trend as the CK. The smokers had much higher initial TBARS level which was reduced after the training period, while in non smokers TBARS levels has shown the opposite. It started from a much lower level than the smoker group's and after the 6-week training it increased.
We found significant difference only in β-Alanine levels after analyzing plasma in the supplement users (before 6,64 μM/L, after 16,32 μM/L). Histidine and the Carnosine levels did not show significant changes.

P11.6

Myocardial consequences of a treatment with prolyl-hydroxylase inhibitors used to improve exercise performance

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Tabilization of the Hypoxia Inducible Factor (HIF) using prolyl-hydroxylase inhibitors (PHI) leads to an EPO synthesis which is suspected to be used as a doping practice. Such treatment is suspected to improve endurance performance by increasing oxygen transport. However, the effects of a PHI treatment on heart morphology and function have never been investigated.
The aim of this study was to evaluate whether potential effects of PHI on cardiac function could contribute to explain its beneficial effect on aerobic performance. We tested the effects of a 1-week treatment with a PHI (DMOG, 150 mg.kg-1, intraperitoneal injection) or a placebo (NaCl) on both sedentary (Sed) and trained rats (trained during 5 weeks before treatment started; 40 min at 25 m.min-1 per day; 5 days/week. Our first result was that PHI increased running performance (+12%, $p < 0,05$) in both Sed and Ex groups. This increased performance was associated with a major increase in total hemoglobin in PHI-treated animals (+13% $p < 0,05$). However, regarding cardiac function and cardiac remodeling no beneficial effect of PHI was observed. Indeed, in hearts of sedentary as well as exercised rats, no significant change in any morphological parameters (LVEDs, LVEDd, AWTd, PWTd and RWT) was found. Moreover, no change in systolic function, likely to explain enhanced exercise performance, was observed in PHI-treated hearts, when evaluated by intraventricular pressure probe (Millar®). Finally it is interesting to note that in sedentary rat hearts an impairment of diastolic function characterized by an altered E/A and dp/dtmin ratios was found when they were challenged with isoproterenol (0,5 mg.kg-1).
These results obtained in sedentary hearts could suggest that a more prolonged treatment with PHI could have deleterious consequences on heart function and point out the danger of such a doping strategy; however, this point remains to be more precisely investigated.

P11.8

Carotid-radial pulse transit time compared to the pulse arrival time to the capillary bed of the finger tip during and after aerobic exercise in young healthy subjects

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Physical activity is known to have beneficial effects on prevention of cardiovascular disease and on microcirculation. The aim of our study was to compare the carotid-radial pulse transit time as a measure of small artery compliance being under strong sympathetic control and the pulse arrival time to the capillary bed of the finger tip as a measure of the state of the microvasculature in young healthy subjects before, during and 20 minutes after aerobic exercise. Following ethical approval 8 healthy young men (20.8±0.4years old) with comparable maximal oxygen uptake (VO2max = 54.85±2.00mL kg-1 min-1) were recruited. We measured ECG, arterial blood pressure using Finapres Ohmeda with a finger cuff on the middle finger of the right hand, laser Doppler skin blood flow on the finger pulp of the pointer finger of the same hand and carotid or radial pulse with a tonometer (Millar SPT 30). Subjects mounted the cycloergometer and their right arm was fixed on an armrest. After 5 minutes sitting at rest they started a graded exercise at 40 W, increased by 50W every 3 minutes until 85% of the maximal heart rate was reached. After ceassation the parameters were measured for subsequent 20 minutes. Carotid-radial pulse transit time (c-rtT) and pulse arrival time to the capillary bed (PATc) were calculated.
Our results revealed that c-rtT exhibited no statistically significant differences before and 20 minutes after exercise (111,3±4,1ms and 109,7 ±3,5ms), but was significantly decreased at highest workload (90,1±0,2ms). On the other hand PATc was increased 20 minutes after exercise compared to resting values (130,3 ±8,1ms and 120,7 ±5,5ms) and decreased at highest workload (104,5±1,6ms). A linear correlation between c-rtT and corresponding RR interval duration during exercise was found but no correlation between PATc and RR. We conclude that during exercise increased sympathetic tone is the main reason for increasing c-rtT, but other mechanisms should contribute to the regulation of the finger tip skin microcirculation, where termoregulation plays a major role. Further experiments are needed to elucidate exact mechanisms.

P11.7

Effectiveness of constant load exercise test on critical power output estimation in sedentary male subjects

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OBJECTIVE: The critical power (CP) is the maximum sustainable power output a subject can maintain for a particular period of time without fatigue. CP estimation from the power output versus time relationship has been derived from various mathematical models. The purpose of this study was to examine effect of the multiple work-bout, 3- and 4-parameter models on estimation of CP.
METHODS: After obtaining a signed informed consent which was approved by the local ethics committee, 20 sedentary male subjects (mean±SE, age: 22±3 yr; weight: 75±9 kg) performed an incremental exercise test using an electromagnetically-braked cycle ergometer to determine anaerobic threshold (AT), respiratory compensation point (RCP) and maximal exercise performance. AT and RCP were estimated from respiratory gas exchange parameters. RCP represent the point where the body's buffering mechanisms failure to compensate metabolic (lactic) acidosis. The data were evaluated breath-by-breath. On separate days, each subject completed 4 randomly ordered constant power output rides to exhaustion to estimate CP: work load corresponded to 25% above the AT (W1), 50% above AT (W2) 75% above AT (W3) and 100% above AT (W4). CP was estimated with linear (power-(1/time)) mathematical model using the data obtained from these tests: model A: W1-W2-W3-W4 (4-tests model); model B: W1-W2-W3; model C: W1-W3-W4; model D: W2-W3-W4. The linear regression analysis and the paired t-test were used to compare values between the dependent groups.
RESULTS: The CP was found to be 139±5 W in model A; and it was significantly different than in model B (129±5 W); in model C (134±5 W) and in model D (146±6 W). There was a significant correlation between the values estimated from linear regression analysis power-(1/time) models (4 test model) and RCP (R=0.986, p <0.0001).
CONCLUSION: These findings suggest that the 4-test model may provide more accurate estimation of CP compared to different 3-test models. In addition, RCP can also be used as an important criterion to estimate CP.

Key Word: Anaerobic Threshold, Respiratory Compensation point, Critical power, Respiratory Gas Exchange

P11.9

Effect of high protein diet and exercise on cardiac Aquaporin 7 expression

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Aquaporin7 is known as an aquaglyceroporin that can be transport of glycerol as well as water. Glycerol is an important substrate for cardiac energy production expecially during exercise in addition to fatty acids and glucose. High protein diet is commonly used by athletes in spite of potential adverse

effects. The aim of study was to investigate the effects of high-protein diet and exercise on cardiac AQP7 expression.
Sprague Dawley rats were divided into 4 groups as control (C), exercise training (E), high-protein diet (HPD) and HPD-exercise training (HPD-E) groups (n =12/group) in this study. HPD groups were fed %45 protein contained diet during 5 weeks. Treadmill exercise was applied during high protein feeding period in exercise groups. Real-time polymerase chain reaction and immunohistochemistry tecniques were used to determine the expression and localization of aquaporin 7 in the heart tissue. Differences in AQP7 expression were expressed as fold change (Pfaffl technic) as compared with C group.
AQP7 expression was found to be increased in E (3,47 fold), HPD (5,58 fold) and HPD-E group (3,86 fold) when compared the C group ($p < 0,001$). In addition, protein expression was also increased of HPD and HPD-E ($p < 0,001$).
HPD and exercise caused increasing effects on cardiac AQP7 expression. Cardiac AQP7 may seem to be important facilitator on glycerol permeability when the rats feed HPD and do exercise.

Key words: Aquaporin7, Gycerol, Heart, High-protein diets, exercise

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P11.10
Effects of recreational physical exercise on metabolic and cardiovascular parameters in type 2 diabetic rat model

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Introduction: Alterations in human behavior, lifestyle and environment have resulted in a dramatic increase in the prevalence and incidence of type 2 diabetes mellitus (T2DM). The disease is often associated with cardiovascular complications which are the leading causes of death. The aim of this study was to examine the influence of recreational physical exercise on the metabolic and cardiovascular parameters in T2DM rat model.
Methods: During a 6-week test period we used Goto-Kakizaki (GK) and control Wistar rats. The GK animal is an inbred model of T2DM with impaired glucose tolerance. GK rats were divided into non-running (GK-C) and running (GK-R) groups which were placed into cages installed with a running wheel. We studied 1. the metabolic parameters, such as body weight, the level of blood glucose in response to oral glucose tolerance test (OGTT) and the concentration of insulin and leptin hormones (ELISA), 2. the heme-oxygenase (HO) and nitric oxide synthase (NOS) activities and 3. the infarct size following 45 min left anterior coronary artery (LAD) occlusion.
Results: In our research we found that 1. the recreational physical exercise decreased the body weight and improved the level of blood glucose and insulin in GK-R. The concentration of plasma leptin was significantly reduced in running rats. 2. The HO and NOS enzyme activities had increased in diabetic GK heart and aorta compared to the control group. Furthermore, the NOS activity was upregulated in GK-R tissues. 3. The physical activity reduced the infarct size compared with GK-C rats.
Conclusion: Recreational physical exercise has been indicated as an "insulin-like" activity because of its anti- inflammatory and antioxidant properties. Our results show that 6 weeks of recreational physical exercise reduces cardiovascular risk by interfering pathophysiological mechanism including oxidative stress, which is the key feature of T2DM.

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P11.11
Clinical, functional and inflammatory factors associated with muscle fatigue and self-perceived fatigue in elderly community-dwelling women

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Fatigue is a common and nonspecific symptom associated with aging, chronic health problems and degenerative factors. Comparison of main associated factors and the physiological and psychological aspects are poorly documented in the elderly.
The aim of this study was to investigate the clinical, functional and inflammatory factors associated with muscle fatigue and self-perceived fatigue in older community-dwelling women. This observational, cross-sectional study was carried out on 135 sedentary community-welling women (71.2 ± 4.57, years), with good functionality. Multidimensional structured questionnaire and functional tests were used to record the clinical and functional characteristics. Plasma concentrations of interleukin 6 (IL-6) and soluble receptor of tumor necrosis factor alpha (sTNFR1) were determined by ELISA. Muscle fatigue was measured by the isokinetic dynamometer and index of muscle fatigue calculated using manufacturer´s software. Statistical analysis was performed by multiple linear regressions and Spearman correlation coefficient. The regression models showed that age, body mass index, physical activity level, functional capacity and peak torque were associated with muscle fatigue ($p < 0.01$). Self-perceived fatigue was associated with the number of comorbidities, depression, physical activity level, functional capacity, peak torque and perceived health ($p < 0.01$). Peak torque was the main factor that contributed to explaining the variations of muscle fatigue. Level of physical activity and health self perceived were the factors explained most of the self perceived fatigue. Spearman coefficient demonstrated correlation between self-perceived fatigue and muscle fatigue of knee extensor right ($p < 0.05$) and left $p < 0.05$). No significant association was found between muscle fatigue and perceived self fatigue with plasma levels of IL-6 ($p=0.99$) and sTNFR1 ($p=0.80$). Psychophysical factors of fatigue are

interacted and the level of physical activity, functional capacity and peak torque were predictive of muscle and perceived self fatigue.
These results can facilitate the understanding of the psychophysical aspect of fatigue in older women.

P11.12

Correlation between inflammatory mediators with muscular handgrip strength in community-dwelling elderly women

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Multiple interrelated factors are involved in the pathogenesis of sarcopenia. Among them, high plasma levels of pro-inflammatory cytokines such as interleukin-6 (IL-6) and soluble tumor necrosis factor alpha receptor (sTNFR1).
The aim of this study was to investigate the correlation between IL-6, sTNFR1 with handgrip strength (HGS) in community-dwelling elderly women. This is a cross-sectional observational study and included community-dwelling elderly women above 65 years-old. Elderly with cognitive impairment (MMSE), inflammatory, cardiovascular or metabolic diseases sharpened, neoplasia, using medications that act on the immune system, neurological sequelae, surgical osteosynthesis in lower limbs and severe visual impairment and hearing were excluded from this investigation. Plasma concentrations of IL-6 and sTNFR1 were determined by ELISA. Handgrip dynamometer (Jamar®) was used to measure HGS. Descriptive statistics were performed to characterize the sample. The correlation between variables was evaluated by Spearman's correlation coefficient, considering a significance level of $\alpha = 0.05$. One-hundred twenty one women (71.07 ± 4.93 years) were evaluated. We found an average of 21.15 ± 4.59 HG Kg/f, median level of IL-6 0.87 pg/ml and a median level of sTNFR1 of 1051.70 pg/ml. The linearity between the variables, decreased HGS was not correlated with increased IL-6 (r=-0.33, p=0.629) and sTNFR1 (r=-0.05, p=0.413).
The plasmatic levels of inflammatory mediators did not correlate adversely with HGS, possibly due to the levels were not able to influence the muscle tissue.

P11.13

Right ventricular adaptation of the athlete's heart

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Introduction: Standard echocardiography has been widely used to assess left ventricular (LV) adaptation to regular physical exercise. Because of some methodological difficulties, such as the crescent, frequently triangular shape, the large number of trabeculation in its wall, right ventricle (RV) has not been frequently investigated. The aim of the present study was to compare the training effects on RV and LV in elite male endurance athletes.
Methods: The LV and RV volume, and both systolic and diastolic function were analyzed by transthoracal echocardiography (Philips HD 15, USA) in 52 male endurance athletes (E) (24.6 ± 5.1 yr) and 25 pair-matched non-trained subjects (N) (26.5 ± 5.4 yr).
Results: E displayed larger body size related long-axis diameters than non-athletes in the two chambers (rel. RVLADd: E: 63.4 ± 6.3 mm/m, N: 60.7 ± 6.6 mm/m and rel. LVLADd: E: 63.8 ± 5.6 mm/m N: 60.7 ± 6.6 mm/m). In the RV short axis diameter (rel. RVSADd) difference was even more marked (E: 37.8 ± 3.1, N: 35.3 ± 2.4 mm/m). In the athletes ratio of peak early to late diastolic filling velocity in the mitral valves (E/A) (E: 2.07 ± 0.51, N: 1.75 ± 0.36), the TDI determined rates in the septal (E: E's/As': 1.89 ± 0. 55, N: 1.62 ± 0. 55) and lateral walls (E: E'l/Al': 2.62 ± 0.72, N: 2.18 ± 0.87) and the systolic myocardial velocity (S'l) (E: 0.111 ± 0.033 cm/s, N: 0.095 ± 0.027 cm/s) were significantly higher than in non-athletes. In the tricuspidal valves no such differences were seen, Tricuspid Annular Plane Systolic Excursion (TAPSE) only was significantly higher in the athletes (E: 29.3 ± 4.9 mm, N: 25.8 ± 3.9 mm).
Conclusion: It seems that endurance athletes' adaptation to regular physical exercise is nearly similar in the two ventricles. A slightly stronger morphological and poorer functional adaptation can be postulated in the RV than in the LV, the cause of which can be that during physical load the resistance in pulmonary circulation is much higher than in systemic circulation, and therefore, the pressure and the workload of the RV is more extensive. It is possible that the higher morphologic adaptation offers a sufficient increase in the function, and further functional adaptation is not necessary.

Keywords: transthoracal echocardiography, right ventricle, endurance athletes

P12

From Cell Signalling to Bioenergetics and Cell Damage

P12.1

The combined therapy with melatonine and hypothermia prevents apoptosis and improves oxidative stress in a neonatal rat model of hypoxic-ischemic encephalopathy

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Introduction: Perinatal hypoxic events are major causes for neonatal mortality and neurological morbidity resulting in central nervous system injury through hypoxia and ischemia. Hypoxia and ischemia produce massive brain damage following a typical pattern which is defined by selective vulnerability of the brain regions and oxidative stress involvement.
Aims: The main objective of this study was to test the possible protective effect of melatonine and hypothermia in hypoxic-ischemic encephalopathy at newborn rats. The changes in terms of histology and apoptosis were determined in brain so as to assess the local damages induced by hypoxic ischemia and oxidative stress parameters were evaluated as well.
Materials and methods: The experiment was performed on 24 newborn Wistar rats pretreated with melatonine in a dose of 20 mg/kg/day for seven days. At the end of this period the animals were exposed to hypobaric hypoxia (9% O2 for 90 minutes) and ischemia (by clamping the right carotid artery). In order to test the effect of combined therapy of melatonine with hypothermia, several animals were exposed after hypoxic-ischemic injury to whole body hypothermia (with 4OC) for 3 h.
Results: In global hypoxic-ischemic encephalopathy melatonine, at a dose of 20 mg/kg/day as premedication offers neuroprotection by reducing the number of cells expressing apoptosis in CA1, CA2, CA3 and Dentate Gyrus of the hippocampus, thalamus and cerebral cortex under the conditions of conjugation with post-injury hypothermia. Protein carbonyl level was decreased, and antioxidant enzyme defence increased.
Conclusion: The results of this study prove that melatonine offers neuroprotection in hypoxic ischemic brain injuries, but the protection is conditioned in most of the brain regions by conjugation of the protective therapy with post-injury hypothermia treatment.

Keywords: hypoxia, apoptosis, melatonine, hypothermia, hypoxic-ischemic encephalopathy

P12.2

Effect of intestinal cold preservation in PACAP-38 containing solution

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Small-bowel is one of the most sensitive organ to ischemia-reperfusion injury during transplantation. Pituitary adenylate cyclase-activating polypeptide (PACAP) has cytoprotective effect in ischemic injuries of various tissues.
The aim of study was to measure oxidative stress markers, histological damages and changes of PACAP-38 immunoreactivities and cytokine levels in grafts stored in PACAP-38 containing preservation solution. Small-bowel autotransplantation was performed on male Wistar rats (n=35). Grafts were stored in cold University of Wisconsin (UW) solution for 1 hr (GI), 3 hs (GII), and for 6 hs (GIII); and in PACAP-38 containing UW solution for 1 hr (GIV), 3 hs (GV), and for 6 hs (GVI). After preservation reperfusion lasted 3 hs in each group. Tissue biopsies were collected after laparotomy and at the end of the reperfusion periods. Tissue oxidative stress parameters were measured from homogenates. Intestinal PACAP-38 immunoreactivities were measured by radioimmunoassay. To measure cytokine array from tissue homogenates we used rat cytokine array. Tissue lipid peroxidation was elevated in a time-dependent manner in GI-GIII compared to sham operated and control results ($p < 0.05$). Meanwhile, the activity of the endogenous antioxidant system decreased significantly after 3 and 6 hs preservation (GSH: 808.7±5.2; 720.4±8.7 vs. 910.4±µmol/g; SOD: 125.1±1.4; 103.3±1.9 vs. 212.11±5.8 IU/g). Histological results showed destruction of the mucous, submucous layers, and crypts in GI-GIII compared to GIV-VI tissues. Levels of PACAP-38 immunoreactivity decreased after 1 hr and 3 hs preservation. It was significant following 6 hs cold storage ($p < 0.05$). Cytokine array revealed that expression of the sICAM-1, L-selectin and TIMP-1 were increased in GIII, while strong reduction was in these cytokines activation in GVI. PACAP-38 adding to the conventional UW preservation solution decreased tissue oxidative injury and structural damages. PACAP-38 immunoreactivities increased by the administration of exogenous PACAP-38 to the solution. PACAP-38 could mitigate tissue cold ischemic injury-induced changes in cytokine expression.

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P12.3

The effect of hyperbaric therapy on the levels of oxidative stress

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Hyperbaric oxygenation(HBO2)is inhalation of 100%O2 under a pressure greater than 1atmosphere absolute(ATA).Various protocols of HBO2 may influence oxidative status(OXS)of body and its'ability to detoxify the reactive intermediates or repair the resulting damage. This review summarizes our recent studies investigating if is OXS dependent on post HBO2 time when is measured, and if different HBO2 protocols result in different levels of oxidative stress. Measurements of Ferric Reducing Ability of Plasma(FRAP)and Thiobarbituric Acid Reactive Substances(TBARS)were done after 100% oxygenation in hyperbaric chamber at a pressure of 2 bars, for 1.5h or 2h, immediately after, 24h after single HBO2 exposure and after 4

consecutive days HBO2 in healthy male Sprague-Dawley(SD)rats and compared to baseline levels.
Results showed significant increase in TBARS values, without significant changes of FRAP immediately after acute HBO2 and in rats 24h after exposure. The extension of HBO2 protocol for 4days of therapy showed that there were no significant changes in FRAP and TBARS levels compared to control. Second experiments evaluated the effects of 4 days consecutive HBO2 on oxidative stress in SD male diabetic rats. There was no difference in antioxidative capacity between diabetic and diabetic-HBO2 treated rats, oxidative stress(TBARS)was significantly higher in these groups compared to healthy rats. TBARS was not affected by HBO2 treatment. Results suggest that repeated HBO2 exposures and the time in-between can be viewed as a state of intermittent pseudo-hypoxia during which organism has an opportunity to adjust antioxidative protective systems between exposures. To explore if sex can influence level of oxidative stress, healthy male and female rats were subjected to 4 days HBO2 protocol. FRAP or TBARS were not significanlty changed in HBO2 male rats compared to male controls, but TBARS values were significantly higher after HBO2 in female rats compared to its controls, indicating higher level of oxidative stress in control conditions in in male rat, while OXS of female rats was affected by HBO2.In conclusion,impact of HBO2 on OXS depends on HBO2 protocol, sex and health conditions of model used.

P12.4
Pharmacological protections against retinal injuries

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Glutamate excitotoxicity and similarly, ischemia-reperfusion (I/R) injury play a key role in several neurological disorders and pathological states of the eye, including retinal vascular occlusion, glaucoma, and diabetic retinopathy. The hypophysis adenilate cyclase activating polypeptide (PACAP) is an endogenous neuropeptide with highly effective neurotroph and neuroprotective effects, which was proved in many retinal degenerative models to be retinoprotective as well. Melanocyte stimulating hormone alpha (alpha-MSH), a peptide-hormone physiologically occuring in the human organism, has also been shown to be protective against I/R-induced injuries in myocardium, brain, kidney and gastrointestinal tract, but not in retina. With the help of electroretinography, a retinal function measurement, we successfully demonstrated that in the background of the morphological effect of PACAP against MSG-induced retinopathy functional amelioration also exists. This may establish a rationale for possible future use of PACAP as a therapeutic agent in excitotoxic retinal damage. The results of our experiments carried out with α-MSH justify the presumption that α-MSH ameliorates the severity of ischemia/reperfusion-induced retinal damage functionally as well as on tissue and cellular level, and provide evidence on the remarkable but not exclusive participation of HO-1 in the action mechanism of the hormone. Remarkable is the property of α-MSH to be able to restore retinal function after an ischemic period, i.e., post-ischemically applied, which leads to the conclusion that α-MSH can potentially be used in a wide range of I/R-mediated pathological conditions applied either as prevention or as treatment.

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P12.5
Possible anti-tumorigenic usage of angiostatin in oncotherapy

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Introduction and Objective: Angiostatin, a cleavage product of N-terminal kringle domains of plasminogen, is an endogenous angiogenesis inhibitor which decreases tumorous growth. However, the instability of the molecule makes it hard to use in therapy. Our goal was to design a molecule which produces stable, active angiostatin in situ and in vivo. The construction, an angiostatin-protease chimera cleaves angiostatin in the extracellular space after its activation.
Method: PCR amplified protease, angiostatin cDNA and elements needed for bacterial and eukaryotic expression were ligated into an adequate vector in the same reading frame. The vector was used to transfect E. coli, mice and human cells to archieve angiostatin production. Aortic ring assay served for monitoring endothelial proliferation of VEGF-induced rat aortic rings in the presence of transfected cells. BALB/c mice were injected separately with transfected and wild type 4T1 and C26 cancer cells to quantify tumor growth.
Results: The chimera was purified from E. coli. Its autocatalysis was observed using SDS-PAGE: we got the full size chimera if protease inhibitor was used during isolation, otherwise we only detected the cleaved fragments. Endothelial proliferation in hanging drop cultures stopped when transfected cells were propagated at the bottom. The aortic ring assay was negative on transfected cells compared to the controll group. In vivo tumor progression was tested using 20 independent clone lines. Cancerous tissue started growing after 10 and 26 days in mice injected with transfected 4T1 and C26 cells, respectively. We already had tumor-associated death in the controll group while this was absent in the other one. Tumor growth happened at slower rates in mice injected with transfected cell lines. After termination, the quantified primary tumor and lung metastasis sizes were significantly

(p <0,05) smaller in mice inoculated with transfected cells compared to controll mice.
Conclusion: Our construction is able to cleave active angiostatin in situ and in vivo. The anti-tumorigenic effect of the molecule was demonstrated in experiments. Patent application for the chimera is in process.

P12.6
Evaluation of the cytotoxic effect of Diphtheria toxin on human umblical vein endothelial cells

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Diphtheria toxin (DTx) is one of the well-characterised bacterial toxins, both in terms of its structure and mode of action. Fragment A (FA) (21 kDa) catalyzes transfer of ADP-ribose moiety of NAD to eukaryotic elongation factor 2 (eEF2) and inhibits protein synthesis. The cytotoxic effect was not explained as the primary reason of inhibition of protein synthesis and on the other hand, due to inhibition of protein synthesis, cytotoxic effect was not observed. These findings gave rise to possibility of the activation of another pathway. In this context, either apoptotic or cytotoxic effects of DTx was explored in human umblical vein endothelial cells (HUVECs). Cell viability was determined with MTT assay. Nuclease activity and DNA fragmentation was determined with hyperchromasite and diphenylamine techniques respectively. Moreover, ADP-ribosylation and its protein synthesis activity were studied with in vitro poly-phe system. Actin- FA interaction in the cell and degradation of actin filaments was shown either with the co-precipitation after ultracentrifuge or immunofluorescence techniques. Both with colorimetric and western-blot techniques the effect of toxin on apoptotic and anti-apoptotic protein amounts were determined. It was observed that caspase-3 and cytochrome c amounts were induced and in contrast the Mcl-1 amount was reduced. However either Bcl-2 or PARP amounts were stayed constant. In conclusion, due to the inhibition of protein synthesis, cytotoxic effect could be associated with the cell cytoskeleton leading the apoptotic process.

P12.7
Nitric oxide can serve as indicator for severity injury of polytrauma

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Patients with injuries to multiple organs or organ systems are in a serious risk of shock, systemic inflammatory response, multiorgan failure and death. Although there are scoring systems available to assess the extent of polytrauma and guide prognosis, their usefulness is limited by their considerably subjective nature. As the production of nitric oxide (NO) by many cell types is elevated by tissue injury, we hypothesized that serum concentration of NO (and its oxidation products, NOx) represents a suitable marker of polytrauma correlating with prognosis. We measured serum NOx and standard biochemical parameters in 93 patients with various degrees of polytrauma, 15 patients with minor injuries and 20 healthy volunteers. On admission, serum NOx was higher in patients with moderate polytrauma than in both controls and patients with minor injury, and it was even higher in patients with severe polytrauma. Surprisingly, NOx on admission was normal in the group of patients that required cardiopulmonary resuscitation or died within 48 hours after admission. In groups where it was elevated on admission, serum NOx dropped to normal values within 12 hours. Blood lactate levels on admission were elevated in proportion to the severity of subsequent clinical course, with the highest values found in patients that eventually died. In conclusion, elevated serum NOx and blood lactate in patients with polytrauma are markers of serious clinical course, while normal NOx combined with very high lactate may signal fatal prognosis.

P12.8
The effects of Src-family kinase inhibitors on osteoclast development

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Background: Osteoclasts are the unique bone-resorbing cells of hematopoietic origin; their development is regulated primarily by M-CSF, RANKL and integrin-mediated interactions. Our workgroup has previously tested thousands of Src-family kinase inhibitors from the Vichem Ltd on neutrophil granulocytes, which cells show many similarities with osteoclasts regarding signaling pathways. One of the most effective inhibitors was the PD166326, an anti-leukemia drug candidate. Here we tested the effect of PD166326 on osteoclast development.
Materials and methods: Bone marrow cells were isolated from the femurs and tibiae of C57Bl/6 mice on the 0. day and differentiated into osteoclasts in vitro in the presence of recombinant M-CSF and RANKL. Cultures were treated with PD166326 during different stages of osteoclastogenesis. Vehicle control samples received dimethyl sulfoxide (DMSO). Osteoclast differentiation was examined after 4 or 6 days by osteoclast-specific tartrate-resistant acid phosphatase (TRAP)-staining. Survival assay was performed at the 4th day using AnnexinV-PE and 7-AAD apoptosis and necrosis kit. Actin ring formation was observed with Lifeact EGFP transgenic mice.
Results: The number and the size of developing osteoclasts were strongly reduced in case of the early administration of the inhibitor. The IC50 value of the inhibitor was around 5 nM. The treatment of the cultures by 10 nM PD166326 practically blocked osteoclast development. When we administered the inhibitor later, from the 4th day – when the

last main point of osteoclast development happens – only minor reduction was observed. The tendency to apoptosis did not increase in the PD166326 treated cultures. The administration of PD166326 did not cause the degradation of actin rings.

Conclusions: The anti-cancer drug PD166326 inhibited osteoclastogenesis in a very small concentration. We suppose that it has an effect on the early stage of osteoclastogenesis. These results may be considered in the therapy of different cancers especially in metastatic bone diseases.

P12.9

Sphingosine-1-phosphate enhances the contractile responsiveness of vascular smooth muscle via distinct S1P2 receptor mediated pathways

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We investigated the effect of S1P on the vascular smooth muscle (VSM) tone and the underlying signaling pathways. Thoracic aorta segments (TAs) were isolated from adult male wild type (WT), as well as S1P2-, S1P3-receptor, endothelial NO synthase (eNOS) and Gα12/13 knock-out (KO) mice and investigated in myographs. The endothelium was removed in some of the Gα12/13-KO vessels. First, the effect of S1P on the resting VSM tone was determined. Then we examined the changes in K+-induced vasoconstrictions during and after the incubation with S1P. Furthermore, eNOS mediated vasorelaxation to acetylcholine was determined in WT vessels before and after S1P treatment. When applied on the resting tension S1P (10 μM) induced weak contraction in WT and S1P3-KO but not in S1P2-KO and Gα12/13-KO TAs. This effect was sensitive to inhibition of Rho-kinase by Y-27632. Incubation with 10 μM S1P for 20 min enhanced the contractile effect of 20 mM K+ in WT segments. This early potentiation of contractility was present in S1P3-KO but not in S1P2-KO and Gα12/13-KO TAs. Interestingly, K+-induced contractions remained elevated even 3 hours after exposure to S1P in WT and S1P3-KO segments. This sustained potentiation was absent in S1P2-KO but not in Gα12/13-KO TAs. Y-27632 prevented the early but not the late phase of hyperreactivity. An opposite pattern of changes was noticed in eNOS-KO vessels: the potentiation developed after S1P incubation but disappeared after the second hour indicating that the late phase of enhanced contractility is related to impaired eNOS activity. In accordance, 20 min incubation with S1P decreased eNOS mediated vasorelaxation 2-3 hours after S1P exposure. Vessels lacking both endothelium and Gα12/13 showed neither early nor late potentiation after S1P. Our findings indicate, that S1P transiently enhances the contractile responsiveness of the VSM via Gα12/13 – Rho-kinase signaling, which is followed by a permanent inhibition of eNOS. Both effects of S1P appear to involve S1P2 receptors. The sustained hyperreactivity of the VSM may contribute to the development of vasospasm under conditions associated with enhanced S1P production.

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P12.10

Depletion of 14-3-3γ reduces the surface expression of Transient Receptor Potential Melastatin 4 (TRPM4b) channels and attenuates TRPM4b-mediated glutamate-induced neuronal cell death

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TRPM4 channels are Ca2+-activated nonselective cation channels which are deeply involved in physiological and pathological conditions. However, their trafficking mechanism and binding partners are still elusive.

We have found the 14-3-3γ as a binding partner for TRPM4b using its N-terminal fragment from the yeast-two hybrid screening. Ser88 at the N-terminus of TRPM4b is critical for 14-3-3γ binding by showing GST pull-down and co-immunoprecipitation. Heterologous overexpression of 14-3-3γ in HEK293T cells increased TRPM4b expression on the plasma membrane which was measured by whole-cell recordings and cell surface biotinylation experiment. Surface expression of TRPM4b was greatly reduced by short hairpin RNA (shRNA) against 14-3-3γ. Next, endogenous TRPM4b-mediated currents were electrophysiologically characterized by application of glutamate and 9-phenanthrol, a TRPM4b specific antagonist in HT-22 cells which originated from mouse hippocampal neurons. Glutamate-induced TRPM4b currents were significantly attenuated by 14-3-3γ shRNA in these cells. Finally, glutamate-induced cell death was greatly prevented by treatment of 9-phenanthrol or 14-3-3γ shRNA.

These results showed that the cell surface expression of TRPM4 channels is mediated by 14-3-3γ binding, and the specific inhibition of this trafficking process can be used as a potential therapeutic target for glutamate-induced neuronal cell death.

P12.11

Ruthenium red differentiates between closely related K2P channels

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Two-pore domain K+ (K2P) channels give rise to background potassium current, regulate excitability, adjust the resting membrane potential and influence the repolarization phase of action potentials. The functional channels are assembled from two, usually identical subunits. A large extracellular domain between the first transmembrane segment and the first pore domain is a characteristic feature of all K2P channels. According to crystal structure data of three K2P channels these domains of the two subunits constitute an extracellular cap above the ion selective pore. The cap forms a relatively narrow extracellular ion pathway (EIP) parallel to the plasma membrane restricting the access to the selectivity filter. This structure could be present in all K2P channels and may confer the resistance to distinct K+ channel blockers acting from the extracellular side.

On the other hand, the cap domain may serve as an interacting surface for extracellular factors modulating channel activity.
We have previously demonstrated that ruthenium red (RR) discriminates between TASK-1 and TASK-3 currents.
In the present study we examined the RR sensitivity of all functional K2P channels. K2P currents were measured by two-electrode voltage clamp in Xenopus oocytes and by whole-cell patch clamp technique in DRG neurons. TREK-2 current proved to be highly sensitive to RR (IC50=0.23 microM in mouse TREK-2). We identified aspartate 135 (D135), lining the „wall" of EIP in mouse TREK-2 as the target of the inhibitor. Using concatemer channels we also provided evidence that inhibition by RR requires both D135 residues of TREK-2 homodimer. TREK-1 was rendered sensitive to RR by a point mutation (I110D) at its residue corresponding to D135 of TREK-2.
DRG neurons have been reported to express predominantly TREK-2 and (RR-resistant) TRESK background K+ channels. Using RR as a pharmacological tool we demonstrated that a RR-sensitive background K+ current component is present in DRG neurons. We propose that RR may be useful for differentiating TREK-2 from other (RR-resistant) K2P channels even in native tissues.

P12.12
Effects of methane inhalation on rat liver mitochondria following partial hepatic ischemia

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Aims: Previously we have shown that increased methane (CH4) input has anti-inflammatory potential in experimental ischemia-reperfusion (I/R) injury, however intracellular targets of CH4 treatment remained elusive. As primary sources of intracellular reactive oxygen species (ROS) generation, we aimed at studying the effects of CH4 inhalation on the respiratory activity and ROS production of liver mitochondria.
Methods: 60 min partial hepatic warm I/R was induced in the presence or absence of CH4 inhalation with normoxic artificial air with 2.2% CH4, 5 min prior to the end of ischemia and during the 60 min reperfusion period (IR and IR+CH4 groups; n=5-5; each). Data were compared with those of sham-operated animals (n=5). For measuring the function of mitochondria, samples were harvested and homogenized then subjected to high-resolution respirometry (OROBOROS, Austria). After steady-state flux, the rate of respiration was determined by adding complex I inhibitor 0.5 μM rotenon with 10 mM succinate and 2.5 mM ADP. Cytochrome C release and whole blood superoxide and hydrogen-peroxide production were also measured.
Results: Significantly increased complex II basal respiration was found (~140 and 220%) at t=5 and t=30 and lower respiratory capacity (~60%) at t=60 min of reperfusion as compared with sham group. Methane inhalation preserved maximal respiratory capacity at the end of ischemic period (t=-5) and significantly improved the basal respiration during the reperfusion. In line with this, IR-induced cytochrome-C release together with ROS production was also significantly reduced.
Conclusion: IR injury was accompanied by the damage of the inner mitochondrial membrane as evidenced by the increased cytochrome-C release, and the dysfunction of electron flow in mitochondrial electron transport leading to elevated ROS production. Both the dysfunction of mitochondrial electron transport chain and mitochondria-related oxidative damage could effectively be modified by a normoxic methane inhalation protocol.

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P12.13
UVB-induced apoptosis signaling cascade and changes of molecular markers expression in a human dermal fibroblast line

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Aim: The present study was purported to assess morphological, phenotypic, and behavior characteristics of fibroblasts in vitro exposed to UVB.
Materials and methods: The effect of UVB radiation was tested in 2 groups of human dermal adult fibroblasts (HDFa), group 1 – cells in suspension and group 2 - adherent cells, exposed to UVB for 2 and 5 minutes, respectively. Morphological changes were assessed by light microscopy. The phenotypic surface markers (CD90, CD73, CD29, CD44, and CD26) and UVB-induced apoptosis (using the Annexin V/PI staining) were evaluated by flow cytometry, 72 hours after exposure.
Results: Morphology of both groups of UVB-exposed cells significantly changed throughout the study as compared to controls. Initially, the cells have a fibroblast-like shape, were adherent and rapidly expanded in culture and were positive for CD90, CD73, CD26, and CD29. The first morphological changes occurred 24 hours after exposure; at 48 hours cells became round-shaped and started to detach and these characteristics were more intense at 72 hours, when 60% of the cells were found in suspension for both study groups. Flow cytometry performed at 72 hours showed decreased expression of CD90, CD73, and CD26, and loss of CD29 adhesion molecule from the cellular surface. Two minutes exposure of HDFa induced an increase of early apoptotic cells in group 1 and 2 (52.85% and 35.11%) while 5 minutes exposure further augmented the early apoptotic population in both groups (67.24% and 54.73%) as compared to control cells (10%).
Conclusion: Two episodes of UVB induced early apoptosis in HDFa cells that was proportional with the duration of exposure. Cells in suspension appeared to be more sensitive to apoptosis signaling than the adherent cells. Both cells changed their phenotype, losing important adhesion molecules, and showed decreased viability after UVB exposure. The reversibility of these changes in the presence of therapeutical interventions will be further investigated.

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P12.14
Antioxidant effects and cytotoxicity of compounds of natural origin

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In spite of the intensive research, cardiovascular diseases, including angina pectoris myocardial infarction and heart failure, are the leader cause of death worldwide. Nowadays there is an increase in the application of natural materials for the prevention of different disorders. In our experiments we investigated the effects of beta-carotene (BC) and sour cherry seed kernel extract (SCSE) treatment on tissue antioxidant capacity (TAC). Rats were divided in four groups (control, BC treated, SCSE treated and combined). After 4 weeks, rats were sacrified and different organs were collected for TAC measurements. Our results show that in case of hearts and lungs all treatments significantly increased the values of TAC. However, the combined treatment did not show synergistic effect. Furthermore, the treatments had no effect on the TAC values of liver and kidneys. In further experiments we determined the cytotoxicity of the investigated compounds by MTT assay. SCSE did not show significant cytotoxic effects in the applied concentrations except the highest, 10 mg/ml concentration. In further experiments we investigated the effects of BC on H2O2 induced cell death. We found that 5 microM BC treatment significantly increased the viability while 10 and 20 microM BC had no protective effect on cell death in A549 cells. In contrast, in H9C2 cells 10 microM BC showed significant cytoprotection.
The results of these experiments presumably give us new insights of information about the pharmacological potential of natural compounds and their role in the reduction of oxidative stress; ischemia/reperfusion-induced damages. Based on the results, we could develop new phytochemical therapies to prevent and reduce the cardiovascular morbidity and mortality and due to this increase the lifespan and reduce the cost of medical care.

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P12.15
Mapping the subcellular localization and activity of the Nox4-p22phox enzyme complex

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Nox4 has been previously described to form a complex with p22phox, however little is known about the functional interaction between the endogenously expressed proteins. We have examined Nox4 and p22phox expression and H2O2 production in TGFbeta-stimulated dermal and pulmonary fibroblasts. TGFbeta increased Nox4 expression and stimulated H2O2 production in both cell types. Interestingly the p22phox protein was present in the absence of any detectable Nox/Duox expression and the level of p22phox was unaffected by TGFbeta. Epitope-labeled Nox4 and p22phox proteins localized to the endoplasmic reticulum and their distribution was not influenced by TGFbeta. We tried to detect the activity of the endogenously expressed Nox4-p22phox complex at different intracellular sites by using targeted Hyper constructs. Interestingly, Hyper was not oxidized at the sites examined, while Nox4-derived H2O2 was readily detected in the extracellular medium. The stimulatory effect of TGFbeta on H2O2 production was absent in both Nox4- and p22phox-deficient mouse fibroblasts, thus proving the role of the endogenously expressed Nox4-p22phox complex in TGF-beta-induced H2O2 production. Fibroblast to myofibroblast differentiation of both Nox4 – and p22phox-deficient cells was found to be normal, suggesting that H2O2 produced by the Nox4-p22phox complex is not essential for the differentiation process.

P12.16
Anti oxidative effect of Ozone on spinal cord injury

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Introduction: Spinal cord injury (SCI), is a critical event leading to oxidative stress and contributing to neurological disability. Oxidative stress reflects an imbalance between oxidants and anti oxidants in spinal cord injured animal model. Vascular injuries in SCI produce both hemorrhagic and ischemic damage. The microcirculation, especially venules and capillaries, appears to be damaged at the site of injury. Medical ozone treatment promotes an antioxidant capacity to oxidative stress, preventing the damage induced by reactive oxygen species. We aimed to identify the effect of ozone on oxidant and anti oxidant parameters and related microcirculation factors in SCI.
Material and Method: In this study, 28 two months old, Wistar albino male rats were used and divided into four experimental groups (in each group n=7): laminectomy (L) followed by for 24 h; underwent occlusion of spinal cord for 1 min followed by 24 h reperfusion (T); laminectomy than treated ozone/oxygen (O3/O2) mixture intraperitoneally (i.p.) (1,1 mg/kg) (L+O3) followed by 24 h reperfusion; underwent occlusion of spinal cord for 1 min followed by 1h reperfusion than treated O3/O2 mixture same as L+O3 group followed by 24 h reperfusion (T+O3). Serum levels of total antioxidant-oxidant status (TAS-TOS) were determined by colorimetric measurement. Plasma concentrations of endotelin-1 and eNOS were analyzed by rat ELISA assay kits.
Results: Decreased plasma level of eNOS in T+O3 was statistically significant compared to T group. There wasn't statistically significant difference for plasma level of endotelin-1 between all groups. Statistically significant difference was observed for TAS serum level between all groups. TOS serum level and OSI (TOS/TAS) parameters,

although not statistically significant, were found to be lower for O3 applied groups compared to L and T groups.
Discussion: Ozone treatment can reduce oxidative parameters without activating antioxidant system in spinal cord injured rats and this effect of ozone can be related to eNOS inhibition. This can be a clue for other possible processes of anti-oxidative effect of ozone in different physiological and pathophysiological conditions.

P12.17

Time- and dose-dependent characteristics of endogenous protoporphyrin IX production from delta-aminolevulinic acid and its derivatives

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Objective: Photodynamic therapy and Photodiagnosis based on the intracellular production of the photosensitizer protoporphyrin IX (PPIX) by administration of its metabolic precursor delta-aminolevulinic acid (ALA) achieved their breakthrough upon the clinical approval of MAL (ALA methyl ester) and HAL (ALA hexyl ester). For newly developed ALA derivatives or application in new tumor types, in vitro determination of PPIX formation involves multi-parametric experiments covering variable pro-drug concentrations, medium composition, time points of analysis and cell type(s).
Methods: Using a multimode microplate reader equipped with temperature controls and a gas control module which allows for precise adjustment of the CO2 partial pressure (to avoid pH shifts of the incubation medium), we recorded the PPIX formation kinetics during 72 hrs induced by up to ten different concentrations of ALA, MAL and HAL in A431 human epidermoid carcinoma cells stably transfected with green fluorescent protein (GFP) for correction of cellular proliferation. With this experimental setup, the PPIX fluorescence of cells treated with more than 40 different conditions (duplicate wells) can be automatically recorded for more than three days without any need to interfere with the measurement.
Results: The results indicate that the peak PPIX level is a function of both, incubation concentration and period: (i) the amount of active PS follows a three-phase kinetics, (ii) the concentration of the pro-drug determines the maximal level of intracellular PPIX and the point in time at which this maximum appears, (iii) the overall cellular PPIX concentration cannot exceed a certain value, independent of the amount of the pro-drug applied, and (iv) the more lipophilic HAL produces comparable amounts of PPIX at incubation concentrations about one order of magnitude lower compared to the hydrophilic ALA or MAL.
Conclusion: The results underline the need for detailed temporal analysis of PPIX formation to optimize ALA (derivative)-based PDT or Photodiagnosis and highlight the value of environment-controlled microplate readers for automated in vitro analysis.

P12.18

Effect of L-alpha glycerylphosphorylcholine on mitochondrial dysfunction and increased endogenous methane production caused by chronic whisky consumption

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Introduction: Chronic alcohol consumption generates intracellular NADH/NAD+ imbalance leading to oxido-reductive stress in target organs (Zakhari, 2013). Our previous studies demonstrated that blockade of the mitochondrial cytochrome c oxidase complex can lead to the production of non-bacterial methane in living aerobic systems (Ghyczy, 2008, Tuboly, 2013). Therefore, we investigated the in vivo effects of chronic ethanol ingestion on endogenous methane production and mitochondrial function in a rat model. In addition, we examined the effects of the potentially membrane protective compound L-α glycerylphosphorylcholine (GPC) in this setting.
Methods: SPRD rats were treated with whisky (per os 6.6 ml/kg/day for 10 days), which was combined with GPC-enriched diet (0.8% GPC in food) or antibiotics for the reduction of the potentially methanogen intestinal flora (p.o. rifaximin, 10 mg/kg/day), non-treated groups (n=6, each) were used as controls. The whole-body emission of methane was detected online in the unrestrained animals with photoacoustic spectroscopy, the oxygen consumption of the hippocampal and the liver mitochondria was measured from tissue biopsies using high resolution respirometry (Oroboros O2k).
Results: Chronic whisky consumption induced significant methane production by the 3rd day, compared to the controls ($p < 0.05$), and this level decreased remarkably by the 5th day in the GPC-treated group ($p=0.04$). The same tendency was observed after antibiotic treatment but only on the 8th day ($p=0.002$ vs control). The GPC treatment did not affect the altered mitochondrial oxygen consumption in the hippocampal samples, whereas the mitochondrial dysfunction was significantly decreased ($p=0.05$) in the liver.
Conclusion: Non-bacterial methane production might be an immediate indicator of the alcohol-generated dysfunction in the liver and brain mitochondria. Exogenous GPC provides protection against chronic alcohol consumption-caused mitochondrial dysfunction in the liver.

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P13

Microcirculation

P13.1

Application of local heat provocation test to assess vascular reactivity on healthy and inflammed human gingiva

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Local heat challenge is widely used provocation test to assess integrity of the skin microcirculation in various systemic diseases (e.g. diabetes mellitus, rheumatoid arthritis, polycystic ovary syndrome). Periodontal inflammation is associated with morphological changes in gingival blood vessels. However it is not known whether these alterations influence the regulatory mechanism of the microvasculature. Our aim was to implement heat provocation test on human gingiva in order to assess the gingival vascular reactivity. After installation of the test, the vascular reactivity was assessed at various degree of periodontal inflammation in a pilot study.

Method: The blood flow of marginal gingiva (GBF) was registered by Laser Doppler Flowmetry in healthy, non-smoking volunteers. First the effect of local heat on GBF was investigated in healthy periodontal tissue. The heat was generated either by warm saline (group A, n=9) or by halogen lamp (group B, n=13). Thereafter the later method was utilized for heat test in patients with various degree of periodontal inflammation (group C, n=18) classified by the amount of crevicular fluid production (CF). The percent changes were calculated after heat-test and were correlated to the CF by Spearman correlation test.

Results: GBF was not affected by mean arterial blood pressure. The application of local heat caused a rapid significant elevation ($p < 0.001$) of the GBF in both groups (Peak responses: 97±6%, 112±13% respectively) and remained elevated in the second minute (29±7%, $p < 0.01$, 40±9%, $p < 0.05$, respectively), but returned to the baseline in the third minute. In group C the basal GBF was not correlated significantly with the CF values ($r=-0.44$, NS), however higher CF values resulted in faster restoration of the GBF ($r=-0.64$, $p < 0.01$) after heating.

Conclusion: Both local heat provocation tests are compatible methods to assess gingival vascular reactivity. According to our pilot study the vascular response to thermal challenge of the gingiva attenuated with the severity of the inflammation.

P13.2

Hyperthyroidism reversibly impacts skin microvascular reactivity

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Endocrine thyroid diseases represent potential cardiovascular risk as thyroid hormones profoundly impact cardiovascular function. There's little data on the impact of hyperthyroidism (HT) and its treatment on microcirculation.

Therefore, our aim was to assess vascular reactivity in 25 patients (P) with newly diagnosed Graves disease (the most frequent cause of HT, confirmed by decreased levels of TSH, increased levels of T4 and T3 and the presence of TSAb – antibodies against TSH receptor) and to estimate the effect of medical treatment after establishing euthyroidism.

The model to study vascular function was skin microcirculation. We estimated skin blood flow by assessing laser Doppler flux (LDF) on the volar forearm (nonglabrous area) and the finger pulp (glabrous area) and performed a 3-min occlusion of the brachial artery to study postocclusive reactive hyperemia (PRH). Compared to 25 healthy age- and gender-matched controls (C), P had increased resting heart rate and increased baseline LDF at both measuring sites (t-test). As for the PRH, P exhibited significantly longer duration of the PRH ($p \leq 0.05$, Mann-Whitney test) and a smaller relative LDF increase ($p \leq 0.05$, Mann-Whitney test) on the volar forearm as compared to the C and a shorter time to peak LDF ($p \leq 0.05$, Mann-Whitney test) on the finger pulp. At both measuring sites, a trend toward larger area under the PRH curve was observed. After medical treatment with methimazole in appropriate dosage and establishement of euthyroidism (5.84±0.07 months), neighter of the measured LDF variables differed between the group of C and the treated euthyroid P.

The results have shown that hyperthyroidism induces reversible changes in vascular reactivity that might partly be explained by altered endothelial function or potentially direct effects of TSI on blood vessels. Different responses in glabrous and nonglabrous parts of skin point to different vascular tone regulation. Restoration of the PRH parameters after therapy speaks in favour of functional impact of hyperthyroidism rather that structural changes on microvessels and outlines the effectiveness of medical thyrostatic treatment also on skin microcirculation.

P13.3

Multimodal action of 5'adenosine monophosphate-activated protein kinase (AMPK) in reducing vascular tone of resistance arteries: Effects on calcium stores and membrane Potential

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Objective: Since insulin resistance in the metabolic syndrome (MS) can be alleviated by AMPK activation which may comprise direct vascular actions, we aimed to determine the effects of AMPK activation on microvascular tone and intracellular free calcium ([Ca2+]i) and the underlying mechanisms.
Methods: Influences of AMPK activation on microvascular tone and [Ca2+]i were assessed in pressure myography coupled with fluorimetric [Ca2+]i registration in isolated hamster resistance vessels. Electrophysiological analysis of channels and membrane potential changes were performed in freshly isolated vascular smooth muscle cells (VSMC) and isolated vessels. Further, protein levels and mRNA levels were studied by Western blot and qPCR from isolated arteries, respectively.
Results: Pharmacological activation of AMPK with two structurally different compounds (A769662 and PT-1) resulted in [Ca2+]i decrease and vasodilation. This effect was concentration-dependent and not mediated by the endothelium. Patch clamp studies in VSMC demonstrated activation of an iberiotoxin-sensitive outward current, i.e. stimulation of BKCa channels. Indeed, VSMC as well as isolated vessels exhibited hyperpolarization in the presence of an AMPK activator. However, in the organ bath setting AMPK-mediated vasodilation and [Ca2+]i decrease were only affected to a minor extent by iberiotoxin. Further investigation revealed that despite measurable BKCa activation, the bulk of the AMPK effects are effectuated by the parallel recruitment of the sarco/endoplasmic reticulum ATPase (SERCA). Combined inhibition of both AMPK targets eradicated any effect of AMPK on microvascular tone and [Ca2+]i.
Conclusion: Stimulation of AMPK in VSMC hyperpolarizes the cell membrane and activates SERCA. The resulting decrease of [Ca2+]i dilates the artery. These results underline that AMPK is indeed a potentially useful target in the treatment of the metabolic syndrome which has the power to modify systemic insulin resistance and arterial hypertension in parallel.

P13.4
NO-donating oximes relax corpora cavernosa through mechanisms other than those involved in arterial relaxation

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Erectile dysfunction as well as many cardiovascular diseases are associated with impaired NO-bioavailability. Recently, oxime derivatives have emerged as vasodilators due to their NO-donating capacities. However, whether these oximes offer therapeutic perspectives as alternative NO-delivery strategy for the treatment of erectile dysfunction is unexplored. This study aims to analyze the influence of formaldoxime (FAL), formamidoxime (FAM) and cinnamaldoxime (CAOx) on tension of isolated corpora cavernosa and to elucidate the underlying molecular mechanisms. Organ bath studies were carried out measuring isometric tension on isolated mice corpora cavernosa, thoracic aorta and femoral artery. After contraction with norepinephrine, cumulative concentration-response curves of FAL, FAM and CAOx (100 nmol/L–1 mmol/L) were performed. FAL-/FAM-induced relaxations were evaluated in the absence/presence of various inhibitors of different molecular pathways. FAL, FAM and CAOx relax isolated corpora cavernosa as well as aorta and femoral artery from mice. ODQ (sGC-inhibitor), DPI (non-selective flavoprotein inhibitor) and 7-ER (inhibitor of CYP450 1A1 and NADPH-dependent reductases) substantially blocked the FAL-/FAM-induced relaxation in the arteries, but not in corpora cavernosa. Only a small inhibition of the FAM response in corpora cavernosa was observed with ODQ. This study shows for the first time that NO-donating oximes relax mice CC. Therefore oximes are a new group of molecules with potential for the treatment of erectile dysfunction. However, the underlying mechanism(s) of the FAL-/FAM-induced corporal relaxation clearly differ(s) from the one(s) involved in arterial vasorelaxation.

P13.5
Mechanisms involved in resveratrol-induced relaxation of isolated mice corpora cavernosa

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For years moderate red wine consumption has been linked to cardiovascular benefits. This is partly due to the presence of the wine compounds resveratrol and quercetin. These are natural occurring polyphenols with known vasorelaxant capacity. Vasodilators often have a potential to regulate penile erection and could therefore be beneficial for the treatment of erectile dysfunction. The goal of this study was to determine the relaxant effect of the polyphenols resveratrol and quercetinr on mice corpora cavernosa and the mechanisms involved. Isometric tension measurements on isolated mice corpora cavernosa were performed in organ baths. Cumulative concentration-response curves (10–100 μM) were constructed for resveratrol and quercetin. Relaxation responses of corpora cavernosa to resveratrol and quercetin in the presence/absence of inhibitors of different molecular pathways were measured. Despite the fact that both polyphenols are potent vasodilators of mice aorta, only resveratrol relaxes mice corpora cavernosa. The relaxant effect of resveratrol on corpora cavernosa was significantly diminished by pretreatment with SQ 22,536, an adenylyl cyclase inhibitor and 8-Bromoadenosine-3′,5′-cyclic monophosphorothioate, Rp-isomer, a protein kinase A inhibitor. Also compound C, a 5’ adenosine monophosphate-activated protein kinase (AMPK) inhibitor significantly decreased the relaxant effect of resveratrol on corpora cavernosa. It is concluded that the red wine compound resveratrol, but not quercetin, relaxes mice corpora cavernosa in vitro through activation of adenylyl cyclase, leading to increased cAMP levels as well as through AMPK activation. Whether resveratrol could be beneficial for patients suffering from erectile dysfunction remains uncertain and requires further research.

P13.6

Foxo1 subcellular dynamic and its impact on redox homeostasis in endothelial cells

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FoXO1 is a transcriptional factor which has been considered a master integrator between metabolic signals and reduction/oxidation (redox) state. Endothelial cells (ECs) face daily fluctuating sceneries due to natural changes in the amount and quality of nutrients in plasma provoked by meal intake and postprandial period. However, subcellular traffic dynamics of FoXO1 and its impact on endothelial redox state have not been studied in ECs.

In this work, we were able to induce a nuclear accumulation of FoXO1 in cultured ECs by serum deprivation for 16-20 hours. Upon serum replacement, a rapid extrusion of nuclear FoXO1 was observed along with XBP-1 splicing. The efflux of FoXO1 from nucleus was not observed when IRE1-α was inhibited, the enzyme involved in the XBP-1 splicing, suggesting a role for XBP-1 activation in the nuclear extrusion of FoXO1. Long-term exposures to no-saturated fatty acids (e.g. palmitic acid) resulted in a loss of this phenomenon, with sustained nuclear presence of FoXO1, which in turns, promotes the upregulation of TXNIP, which is a negative regulator of thiorredoxins. In order to investigate whether this manipulation leads to a minor antioxidant capacity of ECs, we used a fluorescent hydrogen peroxide biosensor, called HyPer. ECs exposed to palmitic acid by 20 hours (palm) showed exacerbated responses of HyPer to a 50 μM H2O2 pulse compared to control cells (at basal, control: 0.57 ± 0.05 v/s palm: 0.65 ± 0.06; peak at 50 μM H2O2, control: 0.58 ± 0.05 v/s palm: 0.70 ± 0.07).

These findings suggest that nuclear location of FoXO1 is regulated by a mechanism dependent of XBP-1 in endothelial cells in the same manner as it occurs in hepatocytes when are exposed serum deprivation and then to serum replacement, an experimental maneuver that simulates a postprandial period. Long-term exposure to palmitate perturbs this phenomenon and is both, accompanied by an increase in TXNIP expression, a transcriptional target of FoXO1, and a diminished antioxidant capacity of treated ECs. Our data show that nutritional signals channelized by FoXO1 regulation have impact on redox balance in ECs.

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P13.7

Effect of systemic medical Ozone application on oxidative parameters in intestinal ischemia-reperfusion

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Introduction: Ischemia/reperfusion (I/R) injury is commonly seen in the field of surgery or transplantation. It has been accepted as one of the major cause of organ failure. Ozone protects the tissue against oxidative stress mediated by reactive oxygen species by increasing the activity of antioxidant enzymes. We aimed to investigate the effect of chronic systemic ozone treatment in intestinal I/R created rats.

Material and Method: In this study, 28 two-months-old, Wistar albino male rats were used and divided into 4 experimental groups (in each group n=7): Sham rats were underwent laparotomy (L); rats underwent occlusion of superior mesenteric artery for 30 min followed by 2h reperfusion (I/R); rats were treated ozone/oxygen mixture intraperitoneally (i.p.) (1,1 mg/kg) for 10 consecutive days than underwent laparotomy (O3+L); rats were treated ozone/oxygen mixture i.p. (1,1 mg/kg) for 10 consecutive days than underwent occlusion of superior mesenteric artery for 30 min followed by 2h reperfusion O3+I/R. Serum levels of TAS-TOS were determined by colorimetric measurement method. Plasma concentrations of endotelin-1 and eNOS were analyzed by rat ELISA assay kits.

Results: Plasma level of endotelin-1 in I/R and O3+L groups were significantly higher compared to L group, plazma level of endotelin-1 was significantly higher in O3+I/R group compared to I/R group. There was not statistically significant difference for serum level of eNOS between all groups. The decrease of serum TAS level in O3+L group was statistically significant compared to L group. Serum level of TOS, although not statistically significant, found to be lower for animal in O3+L group. Serum level of TAS, although not statistically significant, was found to be higher for animal in O3+I/R group.

Discussion: Chronic ozone therapy can activate antioxidant mechanisms at the acute phase of I/R. This can be a clue for the mechanism of ozone action which can include membrane receptors. Ozone can compensate microvascular circulation by activating endotelin-1 and inhibiting eNOS products especially in early phase of I/R. Another point is that ozone may reduce oxidative species by inhibiting eNOS activation.

P13.8

Model-based assessment of blood substitute-induced vasoactivity and red blood cell aggregation

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Introduction and aim: Hemoglobin-based oxygen carriers (HBOC) are designed to combat impaired tissue oxygenation. However, – similar to free hemoglobin (Hb) in plasma – HBOC may also scavenge NO thus impairing microcirculation due to vasoconstriction and red blood cell (RBC) aggregation. Our aim was to investigate these adverse effects of a pegylated HBOC molecule (PEGHbOx) in the brain cortex.

Methods: Hydroxyethyl-starch, HES (6 v/v%), was used as control and PEGHbOx (6 g/dl) as test molecule prepared endotoxin-free (Portoro et al., 2008). Isovolumetric blood-to-HES and blood-to-HBOC exchanges were performed acutely within 20 minutes in anesthetized male Wistar rats (n=7 and n=5; volume of 7 ml). The left femoral artery was cannulated

for measurement of blood pressure (P) and for assessment of hematocrit (H). Microregional RBC perfusion (mrF) was non-invasively measured in the parietal cortex by laser speckle contrast imaging method. Control period was defined at tc=-20 min, while test period between t1=65 min and t2=110 min. Microregional vascular resistance (mrR) and hematocrit (mrH) were estimated by a lumped microhemodynamic model based on quantitative relationship between its input (mrF, P, H) and output parameters (mrH and mrR). mrR was averaged within the region of interest and normalized by mrR(tc).

Results: Neither mrR nor mrH showed difference between PEGHbOx and HES groups (repeated measures ANOVA with Newman Keuls post-hoc test, p >0.05); PEGHbOx(t1): 0.34±0.3; HES(t1): 0.89±0.91; PEGHbOx(t2): 0.51±0.37; HES(t2): 0.67±0.39 for mrR and PEGHbOx(t1): 0.18±0.03; HES(t1): 0.17±0.04; PEGHbOx(t2): 0.19±0.04; HES(t2): 0.17±0.04 for mrH.

Conclusions: The time course and extent of change in microregional hemodynamics brought about by the acute exchange protocol is the same for the Hb-based oxygen carrier and the Hb-free plasmaexpander. Consequently, neither RBC aggregation nor vasoactivity was seen in the microvasculature of the brain cortex following blood-to-HBOC exchange. While scavenging of NO by HBOC molecules is a likely scenario, it may well depend on mechanisms not present in the monitored microregions of the brain cortex.

P14

Circadian Rhythm

P14.1

Effect of metabolic changes on the circadian clock

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Series of experimental and clinical data suggest an intensive interplay between metabolism and the circadian clock. To get a better insight into the molecular details of this interaction we used a model organism, the filamentous fungus Neurospora crassa. In Neurospora, a well detectable phenotype, i.e. the formation of asexual spores (conidia) is controlled by the circadian clock and the circadian oscillator is well characterized at the molecular level. We show that even physiological fluctuations in the generation of reactive oxygen species (ROS) can modulate the phase of conidiation via resetting the molecular oscillator. Our results suggest that among different ROS, superoxide anion plays a dominant role in this process, most probably by affecting the activity of protein phosphatase 2A, a regulator of the positive element of the circadian clock. In addition, we found that under entrained conditions, the phase of conidiation is dependent on the temperature and ROS may mediate this effect. The circadian period of Neurospora crassa is compensated for changes in glucose concentrations. However, under entrained conditions the phase is dependent on glucose. Glucose deprivation results in advanced phase and induces the expression of superoxide dismutase 1 (SOD-1). This latter event may help to compensate the enhanced ROS production during glucose starvation. We show that the control of sod-1 expression by glucose is dependent on the positive factor of the circadian clock.

P14.2

Social jetlag negatively affects academic performance in medical students

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Social jetlag (SJL), the discrepancy between sleep timing on workdays and weekends, affects the majority of the population and has been found to be associated with increased health risk and health-impairing behaviors. We investigated the relationship between SJL and academic performance in a sample of undergraduates of the Semmelweis University (n=247). We assessed SJL and other sleep-related parameters with the Hungarian version of the Munich ChronoType Questionnaire (MCTQ). Academic performance was measured by the average grade based on weekly test results as well as scores acquired on the final test.

In accordance with previous data, we found a positive correlation between chronotype and SJL and observed an earlier chronotype in females ($p < 0.001$). The average mid-sleep point on free days in the Hungarian sample fits well onto the regression line plotted for European sun times and chronotypes, confirming that sunlight has a major impact on chronotype. Multivariate analysis showed negative effect of SJL on the weekly average grade ($p=0.014$) during the lecture term with its highly regular teaching schedules, while this association disappeared in the exam period when students had no scheduled obligations and SJL was minimal. Furthermore, we found that students with later sleep times on free days achieved worse in the morning classes ($p=0.017$). We did not find significant association between academic performance on work days and sleep duration or sleep debt. Our data show that socially enforced sleep times can have a significant negative effect on academic performance and thus emphasize the importance of chronobiological findings for society and its timing.

P14.3

Effects of Apelin-13 administration on food and water intake in different photoperiod in male rats

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Apelin is a new adipokine that secreted from adipose tissue. Apelin and APJ receptor are found like as stomach, intestine and pancreas with hypothalamic nucleus (arcuat, paraventricular and supraoptic nuc.) that controlling fluid homeostasis, food intake and energy metabolism. Many studies showed conflicting results which questioning of the relationship apelin between food intake. In addition, the APJ receptor was determined on the pineal gland. Localisation of APJ on the pineal gland has been thouht apelin mediated food intake may be associated with light/dark phase.

The present study was designed to investigated the effects of chronic peripheral administration of Apelin-13 (AP-13) on food and water intake. The animals (Spraque Dawley rats, male, 180-220g) were adapted to metabolic cages for a period of 5 days before the experiments. AP-13 [30, 100 and 300 µg/kg (per group, n=8)] or vehicle was administrated i.p. for 10 days at the onset of the dark cycle. Metabolic measurements (food and water intake) 12 h/12 h dark/light photoperiod was determined. All data are reported as mean values ± SEM, compared by ANOVA.

Metabolic measurements revealed that AP administrated groups had significant increase food intake (8.13±0.2, 8.15±0.2g per 100g body weight, respectively; AP100, AP300) compared with the vehicle (7.45±0.3) in dark period but there was no statistically significant importance in light period. Additionally, AP treated groups had increased water intake (13.35±0.7, 13.34±0.5, 13.50±0.6 ml per 100g body weight, respectively) compared the vehicle (12.14±0.7) in dark period but there was no significant importance in ligt period.

Our data suggest that Apelin-13 is a peptide that stimulates food and water intake in dark period. These results showed that effects of Apelin-13 on food intake was associated hormones secreted from the pineal gland. However, this effect can be demonstrated by further studies are needed.

P14.4

Role of the Transient Receptor Potential Ankyrin 1 (TRPA1) ion channel in the acute and chronic inflammatory pain models using gene-deficient mice

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Introduction: Transient Receptor Potential Ankyrin 1 (TRPA1) is calcium-permeable non-selective cation channel, which is predominantly expressed on capsaicin-sensitive sensory nerve endings. Beside cold temperature (<17 °C) TRPA1 is stimulated by many exogenous irritants like mustard oil, cinnamaldehyde, allicin and endogenous agents like hydrogene sulfide (H2S) or bradykinin. Since few contradictory data are available on the involvement of this receptor in inflammation and nociception, we aimed to investigate the role of TRPA1 in acute and chronic inflammatory pain models.

Methods: Experiments were performed with male TRPA1 gene-deficient mice (TRPA1-/-) in comparison with their TRPA1+/+ wildtypes. Acute inflammation was evoked by intraplantarly injection of 3% carrageenan, while chronic inflammation was induced by Complete Freund's adjuvant (CFA) injected into the right hindpaw and the root of the tail. The noxious heat and the mechanonociceptive thresholds were measured with increasing-temperature hot plate or dynamic plantar aesthesiometer and the paw volume with plethysmometry. Cold allodynia was determined by the latency decrease of the nocifensive behaviours in ice cold water. The activity of neutrophil myeloperoxidase was investigated with luminol based luminescent in vivo imaging in the CFA-model.

Results: In TRPA1-/- animals there was no significant difference in the carrageenan-induced inflammatory hyperalgesia and the paw volume compared to their wildtypes. In contrast, mechanical hyperalgesia and paw edema were significantly lower in TRPA1-/- mice in the chronic inflammation model. The myeloperoxidase activity indicating neutrophil activation was not influenced by deficiency of TRPA1.

Conclusion: TRPA1 receptors might play an important role in chronic paw oedema and mechanical hyperalgesia. However it is not involved in the activation of neutrophil leukocytes and production of reactive oxygen species. This receptor is probably not involved in acute inflammation.

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P15

Systems Biology

P15.1

Insulin-like growth factor binding protein 3 in the brain of mother rats

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The postpartum physiological and behavioral changes are important parts of reproduction regulated by a complex neuronal network, which includes parts of the hypothalamus.

Lesions of the preoptic area abolish maternal behaviors while the arcuate nucleus plays a role in the regulation of lactation. In our previous microarray study, gene expressional alterations were described in the anterior hypothalamic areas of mother rat. The highest elevation was shown by the amylin, which we identified as a novel neuropeptide. The expression of insulin-like growth factor binding protein-3 (IGFBP-3) also increased significantly in lactating mothers as compared to mothers whose litter was taken away immediately after birth. This finding was also validated by RT-PCR. In the blood, IGFBP-3 is the major carrier molecule of insulin-like growth factors (IGFs), is a binding protein as a part of the IGF system (IGF-1,2, IGFR-1,2 and IGFBP-1-7). It forms a ternary complex with an acid-labile subunit of either IGF-1 or 2.
The distribution of the IGF-system including IGFBP-3 has not been described in the brain. Therefore, we developed in situ hybridization probes to map IGFBP-3. In the hypothalamus, IGFBP-3 mRNA was abundant in the anteroventral periventricular nucleus and the mediodorsal subdivision of the arcuate nuclei. In addition, we found significant IGFBP-3 mRNA levels in the choroid plexus. We could also confirm the elevation of IGFBP-3 expression in the hypothalamic sites of mothers, but a change was not seen in the choroid plexus suggesting a specific role of hypothalamic IGFBP-3 in adaptations of the female brain to motherhood. We also discovered co-localization of IGFBP-3 with tyrosine-hydroxylase (TH) positive neurons using a combination of in situ hybridization and immunohistochemistry. The time course of alterations in the IGFBP-3 mRNA levels during the reproductive cycle was also determined: IGFBP-3 mRNA level is low in control female rats, does not elevate by the end of pregnancy but is markedly induced by the first postpartum day.

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P15.2
Lifetsyle, hypertension and cancer - A modern reconsidering

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Cancer is the second comorbidity of modern society. However, cardiovascular diseases are far more frequent and despite all efforts show an ascending trend. Hypertension is almost being regarded as a constant, unsurprising parameter of patients. Still, hypertension has also been incriminated as a potential risk factor for renal cancer, by mechanisms that remain to be explained. In this sceneray, the aim of our study was to find the physiopathological mechanisms that link lifesyle, hypertension and renal cancer.
Patients and methods: We conducted a retrospective study, over a period of 13 years, on a group of patients with various types of tumors, including renal cancer, that were treated in the Bucharest Institute of Oncology "Prof. Dr. Al. Trestioreanu". We analysed the clinical abnormalities of these patients and correlated them with possible signalling pathways in order to discover the underlying physiopathological mechanisms of the studied correlation.
Results: Long time hypertension was found to be a statisticantly significant risk factor for renal cancer. Anti-hypertensive medication did not appear to influence the link between hypertensive patients and cancer. Possible mechanisms to explain this link were: certain genetic mutations, specific antihypertensive drugs, common lifestyle factors, such as abnormal diet and metabolic syndrome, that link through specific signalling pathways.
Conclusions: Renal cancer usually associates a poor prognostic, with low free-disease survival rates. As these type of tumors are usually diagnosed at advanced stages, an identification of risk factors and of their pathogenic mechanims is essential for their prevention, screening and targeted therapy. The explanation of the interlink between lifestyle- hypertension and certain types of cancer becomes a "must"of modern, effective therapy.

P15.3
Alterations in gene expression patterns of atopic dermatitis patients-derived lesional and non-lesional keratinocytes

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Clinical relevance of the cutaneous barrier functions (CBF) is very high, since their damages can lead to high prevalence diseases (e.g. atopic dermatitis, AD). The endocannabinoid system (ECS) has already been shown to modulate various elements of the CBF (e.g. via inhibiting keratinocyte differentiation); therefore, in our current study we aimed at investigating the putative expressional alterations of the members of the ECS in AD patient-derived lesional (AD-HEK) and non-lesional keratinocytes (AD-NHEK) in comparison with healthy individual-derived cells (NHEK). Moreover, we also intended to identify differences in the expression patterns (EP) of selected "barrier-" or "AD-relevant" genes during the differentiation of the above cells.
Keratinocytes were obtained by shave biopsy and isolated via enzymatic digestion. Alterations in the gene EPs during differentiation were investigated by comparing proliferating (harvested at ~70% confluence) and differentiated (harvested 2 days after confluence) cultures by RT-qPCR and Western blot.
We found that EP of several genes (keratin [K]-1, K15, loricrin [LOR], filaggrin [FLG] and aquaporin-3 [AQP3]) were altered in AD-patients as compared to healthy individuals. Moreover, we showed that EPs of Toll-like receptor [TLR]-2 and -3, as well as occludin (OCLN; a key protein of the barrier-forming cutaneous tight junctions) and the endocannabinoid degrading enzyme fatty acide amide hydrolase (FAAH) were different between AD-HEKs and AD-NHEKs. Moreover, Western blotting indicated that (despite expressing its mRNA) AD-HEKs were unable to express

FAAH at the protein level, and, upon differentiation, expressed less OCLN compared to AD-NHEKs or NHEKs.
These data argue that post-transcriptional loss of FAAH expression and subsequent elevation of the endocannabinoid tone (via inhibiting the physiological differentiation process) might contribute to the development of the barrier-disruption in AD.

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P15.4

Screening of differentially expressed microRNAs in TNBS induced colitis in rat colon

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Chronic inflammatory bowel diseases (IBD) such as Crohn's (CD) are associated with differential expression of genes and miRNAs. Moreover, gasotransmitters such as nitric oxide (product of nitrogen monoxide synthase, NOS), carbon monoxide (produced by heme oxigenase, HO) and hydrogen sulphide (synthetized by cystathionine γ-lyase, CSE) also play important role in CD pathogenesis. MicroRNAs (miRNAs) are small 22-nucleotide, noncoding, single-stranded RNA molecules which directly degrade mRNA, influence a number of disease processes. However, the miRNAs expression changing and the link between gasotransmitters have not been established so far.
The aim of our study was to clarify the differentially expressed microRNAs in TNBS induced colitis in rat colon and discover the link between gasotransmitters and miRNAs.
Male Wistar rats received TNBS (10 mg/0.25 ml in 50% ethanol) rectally to induce colitis. Groups are: absolute control (no treatment), vehicle (50%ethanol) and TNBS. The miRNAs expression profile was assayed by nanoString nCounter® miRNA Expression Assay after 3 days of TNBS treatment.
Totally 228 miRNA were screened: 94 showed upregulation and 63 downregulation. The upregulated miRNAs can be clastered into 8 groups based on their target and function: hypoxia regulators, apoptosis, and angiogenesis, cell cycle regulators, tumor suppressors and oncogens, inflammatory mediators, haematopoiesis regulators. The upregulated miRNAs were divided into 7 clasters: anti- and proinflammatory, tumor supressors and oncogens, apoptosis, immune and haematopoesis regulators. Between the downregulated miRNAs 4 were observed that regulate gasotransmitters mRNA: miRNA26-; miRNA98 and miRNA122-HO miRNA21-CBS.
This comprehensive approach to quantifying miRNAs expression provides insights into the pathogenesis of IBD by elucidating distinct gasotransmitters' signaling pathway in CD. Furthermore, this is the first study demonstrating that miRNA expression profiling in colon might be a practical and effective tool in the diagnosis and prognosis of IBD and may help identify molecular markers that can predict and monitor response to individualized therapeutic treatments.

P16

Aging

P16.1

The evolution of K+-evoked spreading depolarization shortly after carotid occlusion in young and aged rats

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Spreading depolarization (SD) is a wave of transient, intense cellular depolarization that propagates across the cerebral grey matter, and is accompanied by typical cerebral blood flow (CBF) changes. In brain ischemia, altered CBF responses associated with spontaneously occurring SDs exacerbate the ischemic injury. Aging is the most important risk factor for the incidence of cerebral ischemic stroke; yet, it is not clear how age alters SD evolution. Our aim was to compare the typical features of SD during cerebral ischemia in young and aged rats. Male Sprague-Dawley or Wistar rats were anesthetized with halothane or isoflurane in N2O:O2. Transient global forebrain ischemia was induced by bilateral occlusion of the common carotid arteries (2VO) for 40 min in young (8-9 weeks old, n=8) and old (2 years old, n=6) animals. Sham-operated rats served as control (n=6). DC potential and CBF were acquired via a small craniotomy above the parietal cortex. SD was elicited by topical application of 1 or 3M KCl through a second craniotomy distal to the recording site. Ischemia reduced the amplitude of the SD-related shift in DC potential (19.58± 1.67 vs. 26.11± 1.07 mV), the slope of depolarization (-4.34± 1.02 vs.-14.52±4.64 mV/s), while it increased the DC shift duration at half amplitude (66.18±8.07 vs. 21.36±1.66 s). Six types of hemodynamic responses were identified, ranging from dominating hyperemia to prolonged cortical spreading ischemia with intermediate forms. Ischemia reduced the maximal deflection of the long lasting oligemic phase of the CBF response (7.26±2.51 vs. 31.95±4.4 %), and increased the duration of hyperemic phase (237.66±34.39 vs. 36.66±4.82 s). Inverse neurovascular coupling evolved only in the aged ischemic group (4 of the 6 animals). While ischemia clearly compromised the kinetics of SDs and associated CBF response, age exerted an additional shift to more injurious CBF response types involving inverse neurovascular coupling. We propose that structural and functional (mal)adaptation of the cerebrovascular system with aging serves as a potential basis for compromised vascular reactivity.

P16.2

The effect of recreational physical exercise, caloric restriction and high triglyceride diet in experimental menopause

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Introduction: The incidence of cardiovascular diseases are significantly higher after occurrence of estrogen deficiency in menopausal age. Augmented level of pro-inflammatory and activity of myeloperoxidase (MPO) enzymes and decreased activity and expression of heme-oxygenase (HO) are accompanying factors of heart and coronary diseases.
Aims: We investigated the effects of hormone deficiency after surgical menopause as well as the recreational physical exercise (RPE) and nutrition on levels of TNF-α and HO-1 and activity of MPO and HO enzyme systems.
Methods: Female Wistar rats were divided into 12 groups. The two main groups were the ovariectomized (OVX) and sham-operated (SO) groups. Both of the OVX and SO groups were divided into trained and control (without exercise) groups. We separated high trigliceride (HT), caloric restriction (CR) and normal (CTRL) diet groups within running and control groups. The feeding and training period were monitored over 12 weeks. TNF-α and HO-1 level were measured by ELISA while the activity of HO and MPO enzymes were detected by spectrophotometric assays.
Results: We found that the HO activity and HO-1 expression were significantly decreased in OVX CTRL LV comparing with SO CTRL rats, which could be normalized via CR and running. The HT diet reduced significantly the level of HO-1 in case of SO animals and this changes might be prevented by RPE. The concentration of plasma TNF-α and MPO activity of heart were significantly higher in OVX females as compared to the SO groups. The level of TNF-α and MPO were reduced by CR diet while the activity of MPO was significantly decreased via RPE. The HT diet caused significant increase in TNF-α and MPO of SO animals and this rising could be improved by RPE.
Conclusion: The OVX and HT diet are responsible for cardiovascular risk which might be associated with inflammatory processes and the decreased function of antioxidant systems, which could be improved by RPE.

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P16.3

Comparison of exfoliated human mammary cells count with demographical and nutritional parameters of lactating mothers

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Mammary epithelial cells are exfoliated into breast milk during lactation. Exfoliated cells can provide valuable information about change in gene expression, DNA methylation, DNA damage, protein expression for mammary epithelial tissue. In the present study, we have investigated any correlation between the number of exfoliated cells with demographical and nutritional habits of lactating women. We collected milk samples from 50 healthy lactating mothers living in Istanbul. Sample collection procedure was approved by the local ethics committee. During collection, subjects were asked to answer a questionnaire form including information on demographical, nursing, smoking and nutritional parameters. We also compared enriched and depleted cell counts recovered from breast milk with demographical and nutritional information of mothers. Before cell isolation procedure, total cell count in the breast milk was determined by using 0.4% tryphan blue and hemocytometer. Epithelial cells were isolated using magnetic human epithelial anti-body. After isolation, epithelial enriched cells (EEC) and epithelial depleted cells (EDC) were collected separately and counted with hemocytometer.

Mean age of the mothers in this study was 28.3±4.9. Forty-one of the subjects were postpartum (0-4 weeks). Number of previously smoking mothers was 8. Mean body mass index (BMI) of the women was 26.3±4.8. The mean of total cell count, EEC and EDC count were 1.46E+06, 2.28E+05 and 3.40E+05, respectively. We determined a significant correlation between the age and EEC ($p<0.05$). More exfoliated epithelial cells were observed at older age. There was also a significant correlation between BMI and EEC. The number of exfoliated cells increased with higher BMI. No significant correlation was observed between number of nursed children, other nutritional information (e.g. fish, meat, poultry consumption etc) and cell counts. Exfoliation is a natural process to remove external cells from the luminal surface of the breast epithelium; our findings suggest that the number of exfoliated cells increases with age and accumulation of high fat.
This study also confirms that sampling and retrieval of epithelial cells from breast milk provides a valuable and non-invasive tool to study epigenetic changes in mammary cells.

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P16.4

Quercetin treatment reverse endothelial dysfunction and oxidative stress in patients with rheumatoid arthritis

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Background: Rheumatoid arthritis (RA) is a chronic inflammatory disease associated with premature atherosclerosis. Endothelial dysfunction, which represents the earliest stage of atherosclerosis, has been observed in patients with RA with high inflammatory activity. The endothelial dysfunction increases cardiovascular mortality for this disease. Oxidative stress (OS) plays a significant role in the pathogenesis of endothelial dysfunction in RA. The purpose of this study is to investigate the effects of Quercetin (Que) treatment in inflammation, endothelial dysfunction and serum levels of OS in patients with RA.
Patients and methods: We studied 45 consecutive patients with RA (33 women, 12 men; mean age, 57 years [23-75 years]) with active disease (mean Disease Activity Score 28 [DAS28]), without clinically overt cardiovascular disease and 45 control subjects matched for age, sex, hypertension, blood cholesterol and glucose. The patients with RA were treated with Que for four weeks (500 mg Que/day). Blood samples were collected at the beginning and at the end of the treatment. We assessed the serum levels of endothelin-1 (ET-1), intercellular adhesion molecule 1 (ICAM-1), the marker of oxidative stress (malondialdehyde, MDA and carbonylated proteins, CP) and the activity of erythrocytaire antioxidant enzymes (superoxide dismutase, SOD and catalase, CAT).
Results: The serum levels of ET-1 and ICAM-1 were significantly higher in patients with RA than controls ($P < 0.05$) and were significantly lower in patients with RA treated with Que. The serum levels of MDA and CP of patients with RA increased significantly ($P < 0.05$) and SOD and CAT activities decreased significantly ($P < 0.05$) compared to those of the controls. Treatment with Que of RA patients also resulted in significant reduction ($P < 0.05$) of ET-1, ICAM-1, lipid peroxidation and protein carbonilation. These reductions were observed after four weeks of treatment. However, Que administration significantly increased ($P < 0.05$) SOD and CAT activities in the RA patients.
Conclusions: In patients with RA, treatment with Que contributed significantly to the reduction of biochemical markers of endothelial dysfunction.

P16.5

The effects of body mass on CMRgluc-related metabolic activity in mouse joints investigated by in vivo dynamic PET/MRI

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CROmed Ltd

Aim: In the case of Glucose Cellular Metabolic Rate (CMRgluc) the uptake of 18F-Fluoro- Deoxy-Glucose (FDG) is theoretically to be quantified insensitively from the body mass of the subject. Our aim was to test the validity of CMRgluc to express joint metabolic activity using PET/MRI measurements in healthy mice. Our hypothesis was that higher body mass induces a higher burden on joints thereby increasing their metabolic activity and provides a body mass-dependent CMRgluc.
Methods and materials: We imaged three healthy c57bl6 mice with a body mass of 27.00±0.35 g and another three animals weighing 38.80±1.10 g. An activity of 8.9±1.6MBq FDG was injected intravenously, and we performed a dynamic whole-body PET scan in each of them. We then determined all knee joint and ankle joint PET dynamic quantitative analyses. We then expressed CMRgluc-s of all joints as well as performed Logan, Patlak and RE-plotting on the data using our own code written under Octave to determine kinetic constants in the joints.
Results: There is a significant difference between low mass and high mass animals in both ankle and knee CMRgluc values. We also found a correlation between FDG (CMRgluc) and body mass (both cases $p < 5\%$). In all cases of pharmacokinetic models, k-values also significantly differed between groups.
Discussion – Conclusion: We observed a body mass dependence in FDG PET values both in ankle and knee joints. The study points to a translational setting where higher wear and tear by higher body mass did increase CMRgluc values in joints therefore it is expected that even without inflammatory or degenerative changes a patient's body mass is a variance factor in FDG- PET joint inflammation studies.

P16.6

Exposure to static magnetic field induces decrease of antioxidant oligoelements in aging heart

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Introduction: With rapid technological development, everyday exposure to static magnetic fields (SMF) is increasing. The list of potential sources of SMF is wide and their effects could be both, beneficial and adverse. However, a response to certain stimuli could be changed in aging. The aim of this study was to examine SMF effects on status of cardioprotective antioxidant oligoelements Selenium (Se), Manganese (Mn), Cooper (Cu) and Zinc (Zn) in elderly rats.
Material and methods: Eighteen male Wistar rats, 36 months old, were randomly divided into two groups: Control (n=9) and Magnet (n=9). Horseshoe shaped iron magnets were placed directly beneath cage with Magnet group, and rats were moving freely inside the cage. Intensity of static magnetic field was 30mT. Control group was not exposed to magnetic field. After 10 weeks, animals were sacrificed, hearts were collected, and concentration of Selenium (Se), Manganese (Mn), Copper (Cu) and Zinc (Zn) was determined.

Results: Concentration of Selenium and Copper in heart significantly decreased in Magnet group when compared to Control group. There was no significant difference in Manganese and Zinc concentration between Control and Magnet group.
Conclusion: Results of our study indicated that 30 mT SMF exposure in elderly rats induced decrease of antioxidant oligoelements Selenium and Copper in heart.

Keywords: static magnetic field, aging, heart, antioxidant oligoelements

P16.7
The projected increase of rheumatoid diseases due to an aging population in Austria from 2012 to 2050

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Purpose: The aim of this study is to present projected numbers of Austrians in 4 different age classes with rheumatoid diseases in 2012 to 2050 and to discuss possible implications for currently used therapeutic strategies in the future. Methods: The work is based on 2 data sources: Population projections in Austria and age dependent prevalences for rheumatoid diseases provided by STATISTICS AUSTRIA for subjects at the age of 15-30, 30-45, 45-60, 60-75 years and at the age of 75 years or older.
Results: Whilst the absolute numbers of diseased subjects remain stable for the age classes from 15-75 years, the number of diseased subjects at the age of 75 years or older will increase from approx. 90.000 to 211.000 (lower limit:183, upper limit:237) which corresponds to a relative increase of 235% (203%-262%).
Conclusions: The dramatically increasing number of aged individuals aggravates the health economic issue of rheumatic diseases especially for non-inflammatory, degenerative rheumatic disorders. From a health economic point of view, cost effective therapies in the prevention or management of rheumatic diseases are of great importance since rheumatic disorders state a relevant cost factor due to the need for long term medication, frequent hospitalizations, joint arthroplasty and sick leave. The long term intake of medication causes severe side effects that result in additional medical interventions and dramatically reduce the life quality of the affected individual. Some years ago, the mortality rate caused by gastro-intestinal side effects due to NSAID intake was about 2000 per year in Germany and 16.500 per year in the USA. Despite the additional intake of gastro-protective drugs, the ratio of NSAID-consumers suffering from gastro-intestinal ulcers was 1 in 400 and the ratio of those who died was 1 in 8000. The generation of COX-2 inhibitors diminished the risk for gastro-intestinal complications, however, the elevated risk for cardio-vascular events remained. Taken together, from the patient´s as well as from the socio-economic point of view there is an urgent need for new therapeutic strategies that allow for a reduction of medicamentation.

P16.8
Beta-herpesviruses related to aging and frailty

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The comprehension of the physiological mechanisms of aging in human subjects has been the target of many efforts. Immunosenescence is an age-related diminution of immune system capacity associated with frailty which can lead to severe consequences in older persons. Cytomegalovirus (CMV) and Epstein-Barr (EBV) were associated with aging and immunosenescence. CMV, Human Herpesvirus-6 (HHV-6) and Human Herpesvirus-7 (HHV-7) are beta-herpesviruses which are ubiquitous and can cause latent infectious. Latent infection is a well-controlled infection resulted of a balance between minimal viral replication and activity of specific T-CD8 cells. This study aimed to compare the EBV, CMV, HHV-6 and HHV-7 viral loads between elderly and young (control group) as well as subgroups with frailty. Sera were separated from blood and DNA extracted using standard protocol. Real-time PCRs were carried out for CMV, HHV-6, HHV-7 and EBV and viral loads were determined. Among total of elderly, 59.1% presented positive to CMV in contrast to 8.3% of young individuals. Elderly classified as frail, pre-frail and non-frail presented 81.8%, 56.5% and, 47.8% of positivity, respectively. The viral load was significantly higher in elderly than control group ($p < 0.0001$) and, higher in elderly with frailty than without frailty ($p=0.01$). HHV-6 was found in 4.2% of elderly and was not detected in control group. HHV-7 was found in 47.9% of elderly and in 8.3% of control group. Elderly classified as frail, pre-frail and non-frail presented 77.2%, 43.5% and, 26.9% of positivity, respectively. The viral load was significantly higher in elderly than control group ($p < 0.0001$) and, higher in elderly with frailty than without frailty ($p=0.01$). EBV was found in 29.6% of elderly and in 25% of control group. No difference was found among subgroups. CMV was associated with aging and frailty and could act driving the differentiation of T cells and accelerating the immunosenescence as an antigenic stressor such as described on the literature. HHV-7 was also associated with aging and frailty, however, the physiopathological mechanisms remains to be elucidated but hypothetically it could act similarly.

P16.9

Poor correlation between handgrip strength and isokinetic performance of knee flexor and extensor muscles in community-dwelling elderly women

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In scientific research and clinical practice, instruments such as the handgrip dynamometer and isokinetic dynamometer can be used to assess muscle function. The handgrip dynamometer is used to evaluate handgrip strength (HGS), and consists of a hydraulic measurement system and is recommended by the American Society of Hand Therapists. Low HGS values are associated with falls, disability, and mortality. Alternatively, the isokinetic dynamometer is currently the gold standard for muscle function assessment, providing accommodative resistance and a predetermined angular velocity, which allows the muscle to exert its maximum capacity. Previous studies showed an association between HGS and global muscle strength. However, there are no data published about correlation between HGS with a gold standard instrument for the analysis of muscle function.

The aim of this study was to investigate the correlation between handgrip strength and performance of knee flexor and extensor muscles determined using an isokinetic dynamometer in community-dwelling elderly women. This was a cross-sectional study. Subjects consisted (by convenience) of 221 (71.07 ± 4.93 years) community-dwelling elderly women. Knee flexor and extensor muscle performance was measured using an isokinetic dynamometer Biodex System 3 Pro®. The isokinetic variables chosen for analysis were peak torque, peak torque/body weight, total work/body weight, total work, average power, and agonist/antagonist ratio at the angular velocities of 60°/s and 180°/s. Assessment of handgrip strength was performed using the Jamar® dynamometer. Spearman correlation coefficient was calculated to identify inter-variable correlations. Only knee flexor peak torque (60o/s) and average power (60o/s), and knee extensor peak torque (180o/s) and total work (180o/s) were significantly ($p < 0.05$) yet poorly correlated with handgrip strength ($r < 0.30$). The majority of analyses did not reveal any correlation between variables assessed by isokinetic dynamometer and handgrip dynamometer. Caution is required when generalizing handgrip strength as a predictor of global muscle strength in community-dwelling elderly women.

P16.10

Aging exacerbates hypertension-induced intracerebral microhemorrhages in mice

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Aging is associated with high prevalence of cerebral microbleeds (CMBs) in the elderly. In the ageing population, more than 80% of individuals are not successful in maintaining a normal blood pressure. The combination of aging and high blood pressure leads to the formation of numerous small vessel ruptures which contribute to the age-related cognitive decline. Despite its clinical significance, the pathophysiology of CMBs is poorly understood. To elucidate the mechanisms by which aging exacerbates the deleterious cerebrovascular effects of high blood pressure, hypertension was induced in young (3 mo) and aged (24 mo) C57/BL6 mice (Ang II plus L-NAME). Neurological examination and gait analysis, followed by histological studies of serial brain sections (DAB staining of CMBs), showed that aged mice exhibit a significantly earlier onset and increased incidence of hypertension-induced CMBs (incidence; young:27%, aged:90% of the animals; average number of CMBs: young:15±3, aged:28±2). Aging exacerbated hypertension-induced cerebral oxidative stress, NADPH oxidase expression (Nox2 mRNA: 2±0.1 fold increase in aged vs. young) and activation of MMPs (6±0.9 fold increase in aged vs. young). Treatment of aged mice with the dietary polyphenol resveratrol (200 mg/kg for 20 days) significantly attenuated oxidative stress, down-regulated NADPH oxidase, decreased MMP activity, and prevented/delayed the development of CMBs without affecting blood pressure.

Collectively, aging exacerbates hypertension-induced intracerebral microhemorrhages in mice likely by increasing vascular oxidative stress and MMP activation. Therapeutic strategies to reduce vascular oxidative stress and MMP activity should be considered for the prevention of CMBs in the elderly.

P16.11

Changes in norepinephrine induced vasomotor response and vascular α1-receptor expression as a function of age

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Introduction: Norepinephrine (NE) released from postganglionic neurons of the sympathetic nervous system plays an important role in the regulation of vascular resistance, primarily via α1-receptors. Interestingly, NE-induced vasomotor responses have obtained mostly in young animals thus the potential effect of aging on them have not yet not yet been explored.

Hypotesis: We hypothesized that aging increases the magnitude of vasomotor contractions of isolated arteries to NE, which correspond to increases in α1-receptor protein expression.

Methods: Thus carotid arteries were isolated from newborn (8days: 8d), young (2month: 2m), adult (6m) and adult (12m) and senescence (24 and 30m) rats and placed in a wire myograph Danish MyoTechnology 610M) to measure changes in their isometric tension. Two dose response curves to NE were obtained in a sequential manner. The vascular α1-receptor mRNA expression was measured by quantitative

reverse transcription polymerase chain reaction (qRT-PCR), the protein expression by Western blot.

Results: Contractions to first administration of NE increased to the age of 2m (8d: 0.7±0.3 and 2m: 6.9±0,7 mN), then it did not change (6m: 5.9±0.5, 12m: 5.7±0.9, 24m: 6.0±0.8mN, 30 m: 6.3±0.6mN, respectively, n=12, p <.05). Compared to these, contractions to the second administration of NE did not change significantly (8d: 0.3±0.2mN, 2m: 6.4±0.8; 6m: 5.4±1.2, 12m: 5.0±1.7, 24m: 6.3±0.4mN, 30 m: 6.5±0.4mN, respectively, n=12, p <.05). Also, α1-receptor mRNA expression increased from 8d to18m, than decreased to the age of 30m (8d: 1.5±0.9 c/m vs. 18m: 8.3±0.6c/m vs. 30m: 2.8±0.6c/m, n=12, p <.05), whereas protein expression continuously increased from 8d to 30m (8d: 1.0±0; 2m: 0.9±0.2, 12m: 1.6±0.7 30m: 3.8±0.6c/m, respectively, n=4, p <.05).

Discussion: In conclusions, the magnitude of NE-induced contractions increased in young age during development, and then did not change further in older age and did not exhibited tachyphylaxis. In contrast, the α1-receptor mRNA expression exhibited a „bell shape" curve; while the protein expression increased as a function of age. These findings suggest that the magnitude of contractile response of arterie

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P16.12

Age-related impairment of myogenic adaptation to pulsatile pressure in cerebral arteries of Mus musculus

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Clinical and experimental studies provide ample evidence in support of the concept that stability of myogenic tone of cerebral arteries, is essential for adequate control over penetration of pressure (P) waves into the distal portion of the cerebral microcirculation (the resistance to flow is dominated by the fourth power of the internal vessel radius). Because aging promotes cerebromicrovascular injury, we tested the hypothesis that aging alters the myogenic response. P-induced constriction of cannulated middle cerebral arteries (MCA) isolated from young (3 mo) and aged (24 mo) mice was assessed. Both young and aged MCAs developed similar myogenic tone in response to stepwise, steady-state increases in intraluminal P. Young MCAs exhibited significant myogenic adaptation to sinusoidal pulsatile P (amplitude: 40 mmHg, freq:450/min). While in myogenically inactive MCAs each P pulse elicited a ~7% distension in synchrony with the pulsatile P, in young myogenically active MCAs the amplitude of the diameter changes induced by the P pulses in the autoregulated P range was significantly attenuated (~2%). The mean P-myogenic tone curve was similar in young MCAs exposed to constant and pulsatile P. In aged MCAs the cyclic changes in diameter induced by the P pulses were increased (~4%) and development of myogenic tone in response to pulsatile P was impaired. Collectively, aging impairs myogenic adaptation of cerebral arteries to pulsatile P, which likely promotes the development of cerebral microbleeds and BBB disruption by allowing high P to penetrate the distal portion of the cerebral microcirculation.

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