Intracellular Ca^{2+} signals are key triggers of aerobic glycolysis in primary astrocytes

Anemari Horvat[1,2], Marko Muhič[1], Tina Smolič[1], Ena Begić[1], Robert Zorec[1,2], Marko Kreft[1,2,3], Nina Vardjan[1,2]

[1]Laboratory of Neuroendocrinology – Molecular Cell Physiology, Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
[2]Laboratory of Cell Engineering, Celica Biomedical, Ljubljana, Slovenia
[3]Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

MCP Laboratory of Neuroendocrinology - Molecular Cell Physiology & Carl Zeiss Reference Center for Confocal Microscopy

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Introduction

During intense brain activity, astroglial aerobic glycolysis supports high-energy-demanding neurons by converting D-glucose to L-lactate that is transported to neurons, where it can be used as an energy fuel. Astroglial aerobic glycolysis is a highly regulated process that can be augmented via plasmalemmal receptors coupled to intracellular Ca^{2+}- and cAMP-signals, but their individual role in regulation of aerobic glycolysis is not clear.

Aim of the study

To determine the role of Ca^{2+}- and cAMP-signals in regulation of astroglial aerobic glycolysis.

Methods

Experiments were performed on primary rat cortical astrocytes expressing genetically encoded D-glucose and L-lactate fluorescence resonance energy transfer-based nanosensors, reporting changes in intracellular free D-glucose ($[glc]_i$) and L-lactate ($[lac]_i$) concentrations, respectively, using real-time microscopy. Astrocytes were treated with α_1-/β-adrenergic, β-adrenergic and purinergic receptor agonists to selectively activate intracellular Ca^{2+}/cAMP-, cAMP-, and Ca^{2+}-signals, respectively.

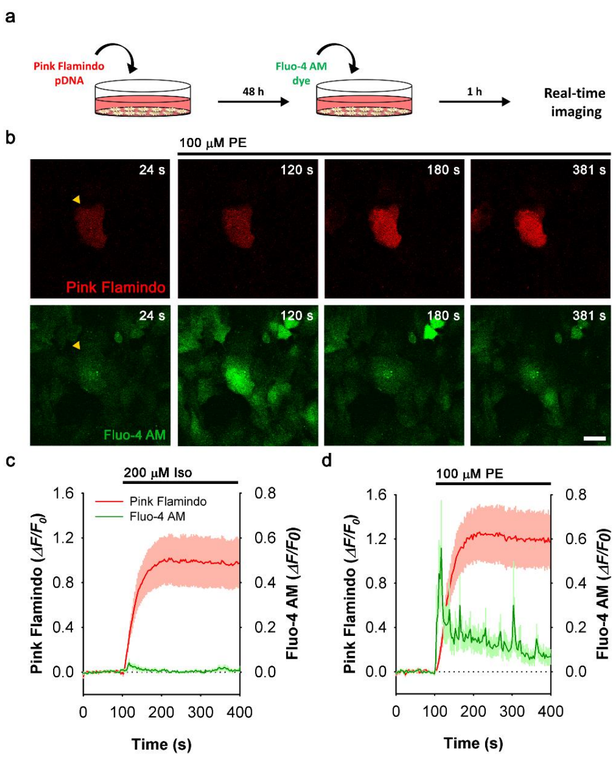


Fig. 1 Simultaneous real-time measurements of α_1- and β-AR-induced Ca^{2+} and cAMP signalling in single astrocytes. (**a**) Schematic representation of the experimental procedure for the simultaneous measurement of cAMP and Ca^{2+} signals. (**b**) Representative fluorescence images of astrocytes labelled with genetically encoded cAMP indicator Pink Flamindo (upper panels, red) and Ca^{2+} indicator Fluo-4 AM dye (lower panels, green). (**c** and **d**) Mean time-dependent changes in the Pink Flamindo (red) and Fluo-4 (green) fluorescence intensity signals ($\Delta F/F_0$) after stimulation with (c) 200 µM isoprenaline (Iso) and (d) 100 µM phenylephrine (PE). Yellow arrowheads point to the cell of interest expressing Pink Flamindo (red) and labeled with Fluo-4 (green). Note that the addition of 100 µM PE leads to an exponential increase in the $[cAMP]_i$ along with a transient increase in $[Ca^{2+}]_i$. Data are presented as means ± SEM.

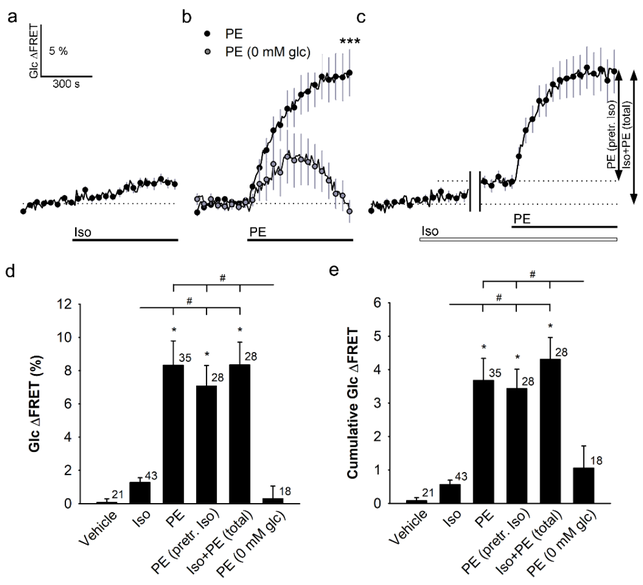


Fig. 2 Activation of α_1-/β-adrenergic signalling increases $[glc]_i$ in astrocytes. (**a–c**) Mean time-dependent changes in the FLII^{12}Pglu-700 µδ6 FRET signal (Glc ΔFRET), reporting $[glc]_i$, after stimulation with (a) 200 µM isoprenaline (Iso), (b) 100 µM phenylephrine (PE) in 3 mM glucose (black circles) or 0 mM glucose (grey circles (0 mM glc)), and (c) 100 µM PE after pre-treatment with 200 µM Iso. Note that the addition of PE, but not Iso, leads to a significant exponential increase in the FRET signal, indicating α_1-AR-mediated increase in $[glc]_i$. ***P=0.001; comparison between the last three data points, Student's t test. (**d, e**) Mean (d) amplitude (Glc ΔFRET (%)) and (e) cumulative change (Cumulative Glc ΔFRET) in the FRET signal after the addition of various stimuli. Numbers adjacent to the error bars represent the number of cells analysed. Data are presented as means ± SEM. *P=0.05 *vs.* control (Vehicle); #P=0.05 comparison between different stimuli, Kruskal–Wallis one-way ANOVA on ranks, followed by Dunn's test.

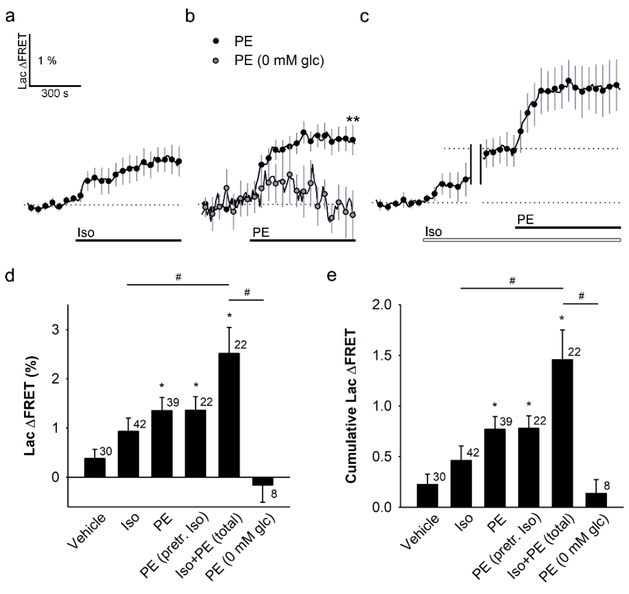


Fig. 3 Activation of α_1-/β-adrenergic signalling increases $[lac]_i$ in astrocytes. (**a–c**) Mean time-dependent changes in the Laconic FRET signal (Lac ΔFRET) after stimulation with (a) 200 µM isoprenaline (Iso), (b) 100 µM phenylephrine (PE) in the presence (3 mM, black circles) or absence of extracellular glucose (0 mM glc; grey circles), and (c) 100 µM PE after pre-treatment with 200 µM Iso. Note that the addition of PE, but not Iso, increased the FRET signal significantly *vs.* control, indicating α_1-AR-mediated increase in $[lac]_i$. **P=0.01; comparison between last three data points, Student's t test. (**d-e**) Mean (d) amplitude (Lac ΔFRET (%)) and (e) cumulative change (Cumulative Lac ΔFRET) in the FRET signal after addition of various stimuli. Numbers adjacent to the error bars represent the number of cells analysed. Data are presented as means ± SEM. *P=0.05 *vs.* control (Vehicle); #P=0.05 comparison between different stimuli, Kruskal–Wallis one-way ANOVA on ranks, followed by Dunn's test.

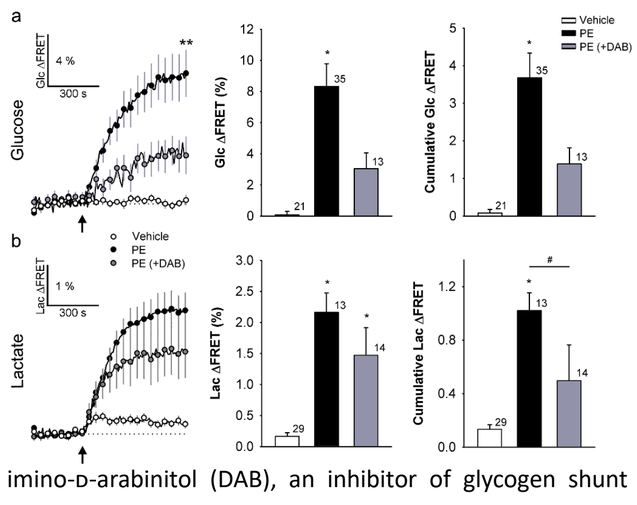


Fig. 4 Inhibition of glycogen shunt lowers $[glc]_i$ and $[lac]_i$ increase in astrocytes upon stimulation with α_1-/β-AR agonist. (**a,b**) Mean time-dependent changes in FRET signal reporting $[glc]_i$ and $[lac]_i$ (Glucose; Lactate; left panels), amplitude (ΔFRET (%); middle panels), and cumulative change (Cumulative ΔFRET; right panels) in the FRET signal in astrocytes expressing (a) nanosensor FLII^{12}Pglu-700 µδ6 (Glucose) or (b) nanosensor Laconic (Lactate) in control (Vehicle) and cells stimulated with phenylephrine (PE; 100 µM) in the presence (PE (+DAB); grey) and in the absence of 1,4-dideoxy-1,4-imino-D-arabinitol (DAB), an inhibitor of glycogen shunt activity (PE; black). Black arrows indicate the time of stimulation. Note that the presence of DAB reduced the α_1-AR-mediated increase in FLII^{12}Pglu-700 µδ6 and Laconic FRET signals. Numbers adjacent to the error bars represent the number of cells analysed. Data are presented as means ± SEM. In (a) and (b), left: **P=0.01 and P=0.26 comparison between last three data points (PE *vs.* PE (+DAB)), Student's t test, respectively. In (a) and (b), middle and right: *P=0.05 *vs.* control (Vehicle); #P=0.05 comparison between different stimuli; Kruskal–Wallis one-way ANOVA on ranks, followed by Dunn's test.

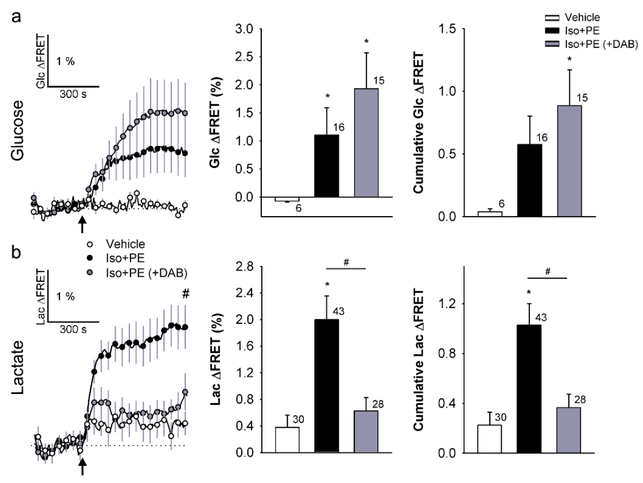


Fig. 5 Inhibition of the glycogen shunt abolishes the increase in $[lac]_i$ upon simultaneous activation of astrocytes with β-AR and α_1-/β-AR agonists. (**a,b**) Mean time-dependent changes in FRET signal reporting $[glc]_i$ and $[lac]_i$ (Glucose; Lactate; left panels), amplitude (ΔFRET (%); middle panels), and cumulative change (Cumulative ΔFRET; right panels) in the FRET signal in astrocytes expressing (a) nanosensor FLII^{12}Pglu-700 µδ6 (Glucose) or (b) nanosensor Laconic (Lactate) in control (Vehicle) and in cells stimulated with isoprenaline (Iso; 200 µM) and phenylephrine (PE; 100 µM) in the presence (Iso + PE (+DAB); grey) and absence of 1,4-dideoxy-1,4-imino-D-arabinitol (DAB), an inhibitor of glycogen shunt activity (Iso + PE; black). Black arrows indicate the time of stimulation. Note that the presence of DAB inhibited α_1- and β-AR-mediated increase in the Laconic but not in the FLII^{12}Pglu-700 µδ6 FRET signal. Numbers adjacent to the error bars represent the number of cells analysed. Data are presented as means ± SEM. In (a) and (b), left: P=0.3 and #P=0.05, comparison between the last three data points (Iso + PE *vs.* Iso + PE (+DAB)), Student's t test, respectively. In (a) and (b), middle and right: *P=0.05 *vs.* control (Vehicle); #P=0.05 comparison between different stimuli, Kruskal–Wallis one-way ANOVA on ranks, followed by Dunn's test.

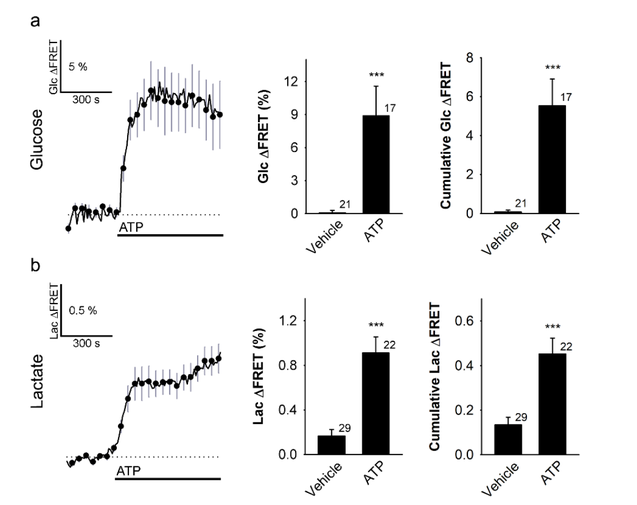


Fig. 6 Stimulation of purinergic P_2R/Ca^{2+} signalling increases $[glc]_i$ and $[lac]_i$ in astrocytes. (**a,b**) Mean time-dependent changes in FRET signal reporting $[glc]_i$ and $[lac]_i$ (Glucose, Lactate; left panels), amplitude (ΔFRET (%); middle panels), and cumulative change (Cumulative ΔFRET; right panels) in the FRET signal in astrocytes expressing (a) nanosensor FLII^{12}Pglu-700 µδ6 (Glucose) or (b) nanosensor Laconic (Lactate) upon stimulation with ATP (100 µM). Numbers adjacent to the error bars represent the number of cells analysed. Data are presented as means ± SEM. ***P=0.001, Mann–Whitney U test.

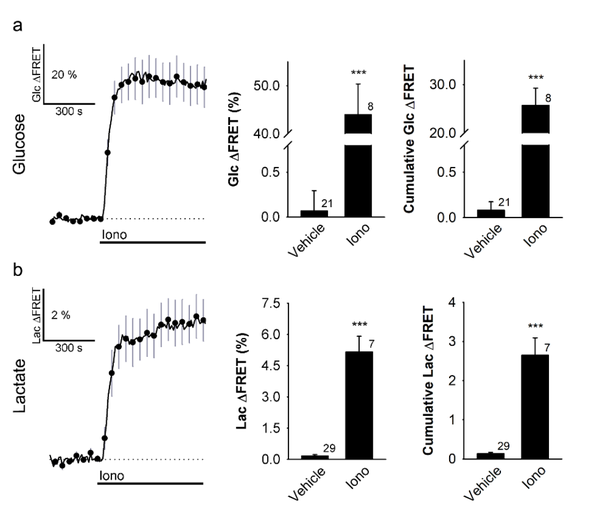
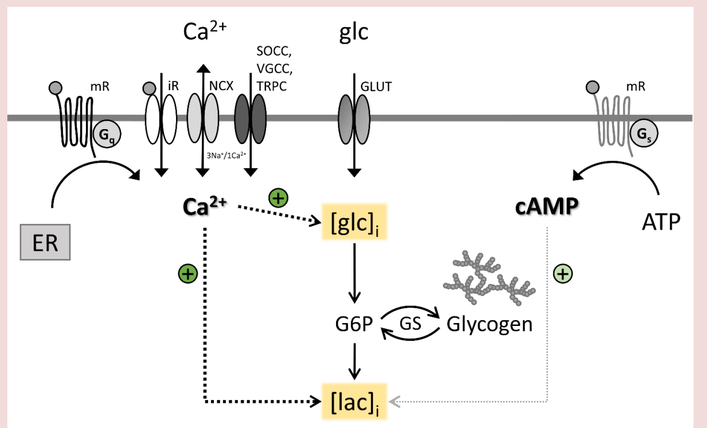


Fig. 7 Increase of cytosolic Ca^{2+} by ionomycin, a Ca^{2+} ionophore, increases $[glc]_i$ and $[lac]_i$ in astrocytes. (**a,b**) Mean time-dependent changes in FRET signal reporting $[glc]_i$ and $[lac]_i$ (Glucose; Lactate; left panels), amplitude (ΔFRET (%); middle panels), and cumulative change (Cumulative ΔFRET; right panels) in the FRET signal in astrocytes expressing (a) nanosensor FLII^{12}Pglu-700 µδ6 (Glucose) or (b) nanosensor Laconic (Lactate) upon stimulation with ionomycin, a Ca^{2+} ionophore (Iono; 10 µM). Numbers adjacent to the error bars represent the number of cells analysed. Data are presented as means ± SEM. ***P=0.001, Mann–Whitney U test.

Conclusions

- **Ca^{2+} signals are key triggers** of augmented **aerobic glycolysis** in astrocytes.
- **cAMP aids** to Ca^{2+}-driven increase in aerobic glycolysis in astrocytes.
- Aerobic glycolysis in astrocytes **depends on extracellular D-glucose** and **glycogen shunt activity**.



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ACKNOWLEDGEMENTS

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