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Calcium activity of neuron-glial networks upon HIF prolyl hydroxylase inhibition in hypoxia *in vitro*

Savyuk M.[1]*, Poloznikov A.[2], Vedunova M.[1], Mitroshina E.[1]

[1] *National Research Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia*

[2] *Higher School of Economics, Moscow, Russia*

** mary.savyuk@bk.ru*

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Motivation and Aim: Hypoxia is one of the main pathogenetic components of ischemic stroke. It also accompanying various neurodegenerative diseases, for example, Alzheimer's disease, Parkinson's disease, Huntington's disease, and brain traumas [1]. So, it is important to find a way to reduce neuronal damage and adapt nerve cells to hypoxia. One of the main molecular mechanisms of cell's hypoxic adaptation is hypoxia-inducible factor-1 (HIF-1), which controls many genes associated with angiogenesis and cells survival. HIF activity is regulated by oxygen-dependent HIF prolyl hydroxylase (PHD). Pharmacological inhibition of PHD by highly selective inhibitors is promising idea for hypoxic damage correction [2].

Methods and Algorithms: Primary mouse hippocampal cultures were used for *in vitro* studies [3]. Modeling of acute hypoxia was carried out by complete replacement of the culture medium with a low oxygen for 10 min on 14 DIV. In order to characterize the functional calcium dynamics of neuron-glial networks, the functional calcium imaging was carried out. Fluorescent calcium sensitive color dye Oregon Green 488 BAPTA-1 AM (OGB-1) (Invitrogen, USA) was used as a calcium sensor. Visualization was performed using an LSM 800 confocal laser scanning microscope (Carl Zeiss, Germany) [4]. Previously developed software was used for calcium activity analysis [5]. Statistical analysis was performed using GraphPad Prism v.9.3.1.471.

Results: We studied the effects of the novel D'014-0038 PHD inhibitor on network calcium activity of hippocampal cultures. Inhibitor was applied in cultural medium immediately after hypoxia simulation in concentration 1 μM. The following calcium characteristics were analyzed: the duration and frequency of calcium events, the number of working cells, the level of correlation of the calcium signal in pairs of cells, the speed of signal propagation, and the number of existing functional connections relative to the maximum possible mathematically calculated connections number. It has been shown that hypoxia causes a significant decrease in all calcium activity characteristics compare to the intact group. Our data show that PHD blockades by D'014-0038 (1 μm) preserve the activity of nervous cells, but did not restore connectivity of the neuron-glial network in post-hypoxic periods. The frequency of calcium oscillations in the "PHD inhibitor" group was significantly higher than the "Hypoxia" group (0.24 ± 0.07 and 0.12 ± 0.01 osc/min respectively. Moreover, a similar effect was shown for the parameter "Number of active cells" ("PHD inhibitor – 71.35 ± 5.93 % active cells and "Hypoxia" $42.76 \pm$ ± 7.17 % active cells). However, there is no significant difference in the level of calcium signal correlation and the speed of signal propagation.

Conclusion: It was shown that the use of the PHD inhibitor D'014-0038 maintain calcium activity of neuronal cells in hypoxia modeling, but does not preserve network connectivity

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