

## Neuroprotective And Antihypoxic Effects Of Glial Cell Line-Derived Neurotrophic Factor (Gdnf) In Hypoxia Modeling

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**Summary.** The aim of the investigation was to assess antihypoxic and neuroprotective properties of the glial cell line-derived neurotrophic factor (GDNF) in hypoxia models *in vitro* and *in vivo*. *In vitro* experiments were carried out on primary hippocampal cultures. Hypoxia modeling was performed on day 14 of culture development *in vitro* (DIV) by replacing the normoxic cultural medium with a medium containing low oxygen for 10 minutes. Registration of extracellular action potentials was conducted by MEA systems (Multichannel Systems, Germany) application. Study the effect of GDNF on synaptic plasticity was performed using SmartFlare RNA Detection Probes (Merck Millipore, France) and fluorescent microscopy. *In vivo* experiments were carried out on C57BL/6j male mice. For acute hypobaric hypoxia a vacuum flow-through chamber was used at the ambient temperature of 20–22°C. We have investigated the resistance of animals to hypoxia and their spatial memory retention in Morris water maze test 24 hours after hypoxia. *In vitro* and *in vivo* data demonstrated that GDNF has strong antihypoxic and neuroprotective properties. Preventive GDNF application before hypoxia contributed to the animal survival and spatial memory retention as well as the maintenance of cells viability in primary hippocampal cultures.

**Key words.** Neuron-glia networks, glial cell line-derived neurotrophic factor (GDNF), hypoxia, primary hippocampal cultures, neuroprotection

### INTRODUCTION

Nowadays investigations concerning the searching endogenous factors for the nervous cells protection from hypoxic damage is one of the topical issues in modern neuroscience and medicine. Glial cell line-derived neurotrophic factor (GDNF) considered as a possible endogenous substance able to control cellular metabolic rates under low oxygen conditions and promotes neuronal survival. However, a question about the mechanisms of neuroprotective and antihypoxic actions of GDNF during hypoxia is still open. The aim of the investigation was to study antihypoxic and neuroprotective GDNF actions in hypoxia models *in vitro* and *in vivo*.

### MATERIAL AND METHODS

*In vitro* studies were performed using hippocampal cells dissociated from 18-day embryonic CBA mice. The cells were cultured on multielectrode arrays (Multichannel Systems, Germany). Hypoxia modeling was performed on day 14 of culture development *in vitro* by replacing the normoxic cultural medium with a medium containing low oxygen for 10 min. The oxygen was displaced from the medium in sealed chamber in which the air was replaced with an inert gas. The neurotrophic factor was added to the medium 20 min before hypoxia. In the control group hypoxia was induced without additional treatment. The viability of dissociated hippocampal cells was evaluated according to the percentage ratio between the number of dead cells stained by propidium iodide (Sigma, Germany) and the total number of cells stained by bisBenzimide (Invitrogen, USA) for 7 days after hypoxia. *In vivo* experiments were performed on 86 C57BL/6j sexually mature male mice weighing 18–20 g. For modeling of acute hypobaric hypoxia a vacuum flow-through chamber was used at the ambient temperature of

20–22°C. Mice were placed under conditions corresponding altitude 10 000–10 500 m (170–185 mm Hg) with a lifting speed 183 m/s [1]. The long-term memory retention test (Morris water maze re-test) was conducted 24 hours after hypoxia.

### RESULTS

*In vitro* experiments showed that 10-minutes acute hypoxia caused the decreasing of cellular viability in primary hippocampal cultures approximately 4.5 times ( $p < 0.01$ ). A preventive GDNF application reduced the number of dead cells in 2 times in comparison with control cultures ( $p < 0.01$ ). Electrophysiological data demonstrated that hypoxia led to spontaneous bioelectrical activity violations and to the destruction of pattern of spontaneous network activity. Preventive application of neurotrophic factor GDNF (1 ng/ml) partially neutralizes the negative hypoxic effects on the spontaneous bioelectrical activity. By the day 7 of the post-hypoxic period in group, which received preventive doses of neurotrophic factor, there was a restoration in the number of small network bursts and in the average number of spikes per burst up to the baseline. At the same time the parameters of spontaneous bioelectrical activity in control cultures, without preventive GDNF treatment, were significantly ( $p < 0.05$ ) lower than in experimental groups. To identify the possible GDNF influence on synaptic plasticity, the level of the expression of mRNA GluR2-subunits of AMPA-receptors in normoxic conditions and after acute oxygen deficiency was evaluated. The received data showed that hypoxia significantly decreased the number mRNA GluR2-positive cells. Preventive GDNF application contributed to the preservation the level of cells, expressing mRNA GluR2-subunits of AMPA-receptors, whether in normal conditions GDNF injection resulted in increased

expression of mRNA GluR2. The following step was to assess the effect of GDNF on animal survival to acute hypobaric hypoxia. It was shown that preventive intranasal application of the neurotrophic factor (4 µg/kg) increased animal resistance to acute hypobaric hypoxia which is manifested as significantly elevated the lifetime on the "height".

## CONCLUSIONS

Our data revealed that glial cell line-derived neurotrophic factor has strong antihypoxic and neuroprotective properties. Preventive GDNF application neutralizes the negative effects of oxygen deficiency by increasing the cell viability and maintaining of functional network activity in primary hippocampal cultures at a certain functional level.

## The Role Of The Medial Preoptic Area Glycinergic System In The Social Types Of Behavior Regulation

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**Summary.** The medial preoptic nucleus is critically involved in the social type of behavior regulation, such as parental behaviour, social recognition, sexual behaviour, ect.. A big amount of studies are focused on the role of glutamate, GABA, serotonin, and dopamine systems of medial preoptic area (mPOA) in the social types of behaviour regulation. However, the role of glycinergic system in this nucleus has not been investigated.

**Key words.** Medial preoptic nucleus, hypothalamus, glycine, sexual behaviour, social recognition

Medial preoptic area (mPOA) is critically involved in the regulation of male sexual behavior in all vertebrate species in which its role has been studied. Electric stimulation of this area determines consummatory phase of sexual behavior, while mPOA lesions in model experiments inhibit this type of behavior. Furthermore, the studies performed with mPOA slices of male rats showed that fast inhibitory responses in mPOA neurons depend on GABA and glycine. Also mPOA is involved in social recognition regulation. Social recognition supersedes any social type of behavior, particularly sexual behavior. Therefore, after the determining the mPOA glycine role in the male sexual behavior regulation, mPOA glycine role in social recognition regulation was examined. A microinjection technique and bilateral cannulas implantation into the mPOA were used in both series of experiments. For sexual behavior patterns like session duration, duration of postejaculatory period, number of intromissions and ejaculations were recorded using video registration in freely moving males in the presence of females and then analyzed. For social recognition were used the social recognition test box,

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divided into three compartments by partitions, and SMART v3.0.01 software for tracking. Bilateral microinjection of an inhibitory neurotransmitter glycine (1 mM) in male rat mPOA authentically decreases ejaculation latency period, the duration of the postejaculatory period and the number of intromission in session; bilateral microinjection of glycine antagonist strychnine (20 µM) increases the duration of the postejaculatory period. In this case, presumably, glycine depletes some mPOA inhibitory effect on sexual behavior. These results are consistent with the literature, according to which the concentration of glycine in the mPOA decreases after ejaculation and increases before the next session. An opposite effect of strychnine on this parameter also supports this hypothesis. After bilateral microinjection of strychnine (20 µM) male rats authentically prefer middle section rather than section A (with resident male rat) or section B (with intruder male rat). After bilateral microinjection of glycine (1 mM) male rats prefer section B (with intruder male rat) rather than section A and middle section. Obtained data allow to suggest that the mPOA glycine stimulation probably