of DI in prenatal development are correlated with longevity among mustelids [*Thom et al., 2004*]. We suggest that presence of DI also may have effects on animal behavior. We first made whole genome sequence of three mustelids with delayed implantation stage in prenatal development - mink (*Neovison vison*), marten (*Martes martes*) and sable (*Martes zibellina*). Using the data from whole genome sequencing of these animals and assembled genome of ferret (*Mustela putorius furo*) which is closely related specie that does not display diapause we analyzed the genes involved in melatonin pathway.

CONCLUSIONS

We described a set of genetic alteration in genes of melatonin pathway in mink, marten and sable. The data imply the genetic alteration may lead to changes of quantity and regulation level of melatonin in animals with DI stage in prenatal development.

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The Role Of Cannabinoid Receptors (Type 1 And Type 2) In Implementation Of Neuroprotective And Antihypoxic Effects Of N-Arachidonoyldopamine In Acute Hypoxia *In Vitro*

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Summary. The aim of the investigation was to study a role of cannabinoid receptors type 1 (CB1) and type 2 (CB2) in implementation of antihypoxic and neuroprotective effects of N-ADA in hypoxia model *in vitro*. The experiments were carried out on primary hippocampal cultures. N-ADA effect on the spontaneous bioelectrical and calcium network activity in dissociated hippocampal cultures in normal and hypoxic conditions as well as the role of CB1 and CB2 in the implementation of these effects were investigated. Registration of extracellular action potentials was conducted by MEA systems (Multichannel Systems, Germany) application. For the detection of patterns of spontaneous calcium oscillations we used fluorescent calcium dye Oregon Green 488 BAPTA-1 AM (Invitrogen) and a confocal laser scanning microscope (Zeiss LSM510, Germany). Study the expression of mRNA CB1 receptors was performed using SmartFlare RNA Detection Probes (Merck Millipore, France) and fluorescent microscopy. Our data demonstrated that N-ADA has strong antihypoxic and neuroprotective properties associated with activation of cannabinoid receptors type 1.

Key words. Neuron-glial networks, endocannabinoid system, N-arachidonoyldopamine, hypoxia, primary hippocampal cultures, neuroprotection

INTRODUCTION

Nowadays ischemic stroke is one of the main causes of death and severe disability of the population in Russia and around the world. Hypoxia considered as a key factor of brain cells damage during ischemic stroke. The endogenous cannabinoid system plays an important role in the modulation of synaptic transmission, plasticity and maintaining the normal functioning of the nervous system. The neuronal activity regulation by cannabinoids receptor's activation in ischemia has shown in a number of studies on different models *in vivo* and *in vitro*. A recently discovered and synthesized endocannabinoid N-arahidonoildofamin (N-ADA) is a perspective substance for hypoxic damages correction. N-ADA was described as an agonist both cannabinoids (CB1 and less CB2). In our recent studies neuroprotective and antihypoxic effects of N-ADA were shown [1]. However, a question concerning the molecular mechanisms of N-ADA neuroprotective and antihypoxic actions during hypoxia is still open. The aim of the investigation was to study the role of cannabinoid receptors type 1 and type 2 in antihypoxic and neuroprotective effects of N-ADA in hypoxia model *in vitro*.

MATERIAL AND METHODS

In vitro studies were conducted using hippocampal cells dissociated from 18-day embryonic CBA mice. Hippocampal cells were plated on multielectrode arrays (MEA60, Multichannel Systems) or coverslips. 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. An application of N-ADA (10 mcM) or N-ADA with CB1 or CB2 antagonist was conducted into hypoxic

cultural medium. We used SR151716 (SR1) 1 mcM (Sanofi) as antagonist of CB1 receptors, SR 141716A (SR2) 1 mcM as antagonist of CB2 receptors. The main parameters of spontaneous neural and calcium activity as well as the viability of cells were investigated. For the detection of patterns of spontaneous calcium oscillations we used fluorescent calcium dye Oregon Green 488 BAPTA-1 AM (Invitrogen) and a confocal laser scanning microscope (Zeiss LSM510, Germany). 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. Study the expression of mRNA CB1 receptors was performed using SmartFlare RNA Detection Probes (Merck Millipore, France) and fluorescent microscopy.

RESULTS

The effect of N-ADA on the neural network activity and cellular viability in primary hippocampal cultures during hypoxia were investigated. Our experiments showed that 10-minutes acute hypoxia caused the significantly decrease in cellular viability of primary hippocampal cultures (in 4.5 times, $p<0.01$) in the posthypoxic period. N-ADA application maintained the viability of cells at level appropriate normoxic conditions (no significant differences from sham). Blockade of CB1 receptors by SR1 eliminated the N-ADA neuroprotective effect. Electrophysiological and Ca^{2+}-imaging data demonstrated that hypoxia causes a catastrophic reduction of spontaneous bioelectrical and calcium activity in primary hippocampal cultures and changes in activity patterns. Cannabinoid receptors activation via N-ADA applications during hypoxia partially preserved the spontaneous bioelectrical and calcium activity of neural networks for 7 days after hypoxia modeling. N-ADA neuroprotective effect associated with cannabinoid receptors type 1, as their blockade by using antagonist SR141716A (SR1) exterminate the neuroprotective effects of N-ADA. CB2 blocking by application of antagonist SR144528 (SR2) had no substantially effect. In this regard, we evaluated the level of CB1 receptors expression in hypoxic conditions. Our data revealed that mRNA CB1 is actively synthesized both neurons and glial cells. N-ADA was significantly ($p <0.05$, ANOVA) reduced the number of mRNA CB1 positive cells in primary hippocampal culture at normoxia. In the control group mRNA CB1 expression was detected in 37,7 ± 5,41% of cells and in 24,4 ± 7,26% of cells 48 hours after N-ADA (10 mM) application. Thus, it was shown that exogenous N-ADA application leads to changes the level of mRNA expression of cannabinoid receptor type 1. Hypoxia caused the increase of mRNA CB1 expression especially in astrocytes.

CONCLUSIONS

Therefore, it was shown that N-ADA has strong antihypoxic and neuroprotective properties. The protective N-ADA effect primarily implemented through CB1 receptors.

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Growing Unidirectional Synaptic Architecture In Dissociated Neuronal Cultures Using Microfluidic Methods

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Summary. In this study we developed microfluidic structure with two neuronal cultures grown in separated chambers and connected by microchannels for axon outgrowth. We estimated bursting activity transfer characteristics between chambers in relation toculture development and determine rate of axonal growth in chambers.

Key words. Microfluidic system, microchannels, microelectrode array.