THE P2X3 RECEPTOR BLOCKER AF-353 (Ro-4) REDUCES BIOENERGETIC INDEX OF A PRIMARY MIXED CULTURE OF HIPPOCAMPAL NEURONS

A.S. Zelentsova, M.Yu. Skorkina*, A.V. Deykin

Belgorod State National Research University,
85 Pobedy str., Belgorod, 308015 Russia; *e-mail: marinaskorkina0077@gmail.com

In clinical studies, the purinergic receptor P2X3 is considered as a molecular target for pain correction in spinal sensory neurons by highly selective antagonists based on diaminopyrimidine derivatives. In the CNS, P2X3 receptors are involved in synaptic plasticity underlying memory and learning. Currently, potent and selective allosteric modulators of P2X3 and P2X2/3 receptors have been recognized among diaminopyrimidine derivatives. These include 5-(5-iodo-2-isopropyl-4-methoxyphenoxy)pyrimidine-2,4-diamine (Ro-4 or AF-353), gefapixant, which have a good pharmacokinetic profile and are less active with respect to a wide range of kinases, receptors, and ion channels. Although the therapeutic value of P2X3 receptor blockade in CNS neurons has not been studied, however, certain evidence exists in the literature that this receptor could represent a new target in the search for antiepileptic drugs, as well as drugs that reduce anxiety and stress. The aim of the work was to study the effect of the P2X3 receptor antagonist AF-353 (Ro-4) on the neuronal bioenergetic health index (BHI) in a primary mixed hippocampal culture. The P2X3 receptor blockade in embryonic and postnatal mouse hippocampal neuron cultures increased non-mitochondrial respiration by 27.5% and 15.8%, respectively, proton loss by 31.0% and 61.4%, and decreased basal respiration by 89% and 39% compared to the control. The neuronal BHI decrease in the postnatal culture was 68% compared to the control. The obtained results indicate the effect of AF-353 on mitochondrial respiration of a primary mixed culture of hippocampal neurons; this reveals the potential of the P2X3 receptor as a pharmacological target in hypoxic conditions of the brain.

Keywords: P2X3 receptor; AF-353 antagonist (Ro-4); mitochondrial respiration; hippocampus; neurons

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INTRODUCTION

Intercellular communication in the nervous system includes the family of purinergic P2 receptors, which are divided into two classes: ligand-gated P2X channels and metabotropic P2Y receptors associated with G-protein [1]. Each of the families is divided into subclasses P2X1-7, P2Y 1,2,4,6,11-14 [2]. It is known that the P2X3 receptor of sensory neurons is involved not only in the occurrence and development of neuropathic and inflammatory pain [3, 4], but it also plays a neuroprotective role after damage to the integrity of the nerve fiber [5, 6], participating in the process of regeneration of nerve endings [7, 8]. In the central nervous system (CNS), P2X3 receptors are involved in the regulation of synaptic plasticity due to their high permeability to calcium [9]. Researchers suggest its participation in fast excitatory synaptic transmission via zinc-sensitive ATP-dependent channels on the pyramidal cells of the CA3 hippocampus [10]. P2X3 and P2X1 can modulate synaptic transmission of mossy fibers in the hippocampus at the pre- and postsynaptic level [11]. P2X3 receptors are significantly activated in temporal epilepsy, and their inhibition leads to relatively low-frequency discharges [12]. Mice lacking the P2X3 receptor exhibit abnormalities in hippocampal synaptic plasticity with impaired long-term depression at CA1, CA3, and the dentate gyrus of the hippocampus. Calcium influx through postsynaptic P2X3 channels is thought to play a key role in the induction of long-term depression [13]. Despite the deficit in hippocampal long-term depression, P2X3 receptor knockout (P2X3KO) mice performed normally spatial learning tests in a water maze, provoking the authors to speculate that the P2X3 gene knockout improved learning. Additionally, P2X3KO mice performed better than wild-type mice on a task that involved visually locating and swimming to a platform [13]. To date, a number of diaminopyrimidine derivatives acting as potent and selective allosteric modulators of P2X3 and P2X2/3 receptors have been developed. These include 5-(5-iodo-2-isopropyl-4-methoxyphenoxy)pyrimidine-2,4-diamine (Ro-4 or AF-353, IC_{50}=3.16 nM), gefapixant (IC_{50} of 0.03 μM for P2X3 and 0.250 μM for P2X2/3 receptors), which have a good pharmacokinetic profile [14–16]. AF-353 (Ro-4) is 100 times less active against a wide range of kinases, receptors, and ion channels [15]. Diaminopyrimidine class P2X3 receptor antagonists have improved

physicochemical properties compared to earlier developed P2X3 antagonists and are likely to bind to the allosteric site of the channel [16, 17]. The therapeutic significance of P2X3 receptor blockade in CNS neurons has not been studied yet; however, certain evidence exists in the literature, that this receptor may be a new target in the search for antiepileptic drugs [12]. Although various signaling pathways involving the P2X3 receptor have been described in neuropathic and inflammatory pain [3, 5], the involvement of this receptor in mitochondrial respiration mechanisms has not been studied. However, activation of P2X3 channels mediates the influx of extracellular calcium [18], which can trigger various signaling cascades.

The aim of the work was to study the effect of the P2X3 receptor antagonist AF-353 (Ro-4) on the neuronal bioenergetic health index (BHI) of a primary mixed hippocampal culture.

MATERIALS AND METHODS

Animals

Two-month-old CD1 mice weighing 23–25 g were used in this study. The animals were purchased from the Stolbovaya branch of the Scientific Center for Biomedical Technologies of the Federal Medical and Biological Agency (certificate No. 18980 dated 23.05.2023). The animals were kept in the conventional vivarium of the Belgorod State National Research University (NRU "BelSU") with a 12 h : 12 h day/night cycle and a temperature of 22–26°C with free access to food and water.

Isolation of a Primary Mixed Culture of Hippocampal Neurons

To obtain a mixed culture of hippocampal neurons, mice were euthanized using the cervical dislocation method. For preparation of an embryonic culture (E18), hippocampal tissue from 24 well-formed embryos on day 18 of gestation was taken. A postnatal primary mixed culture of hippocampal neurons (P2) was prepared using ten 2-day-old newborn CD1 mice. All manipulations were performed on ice. The abdominal cavity was opened, the uterus was excised with embryos, which were placed in a Petri dish with cooled Hanks' solution (PanEco, Russia). The embryos were separated from the uterine wall and washed in cooled Hanks' solution. The embryos were sequentially transferred to a dry Petri dish. In newborn mice, the upper part of the skull was dissected from the skin and fascia, the skull bones were removed, exposing the brain, which was then extracted and placed in a Petri dish with cooled phosphate-buffered saline (PBS). The brain was isolated from embryos and newborn mice using a Leica Microsystems EZ4 stereomicroscope (Leica, Germany). The hippocampus was transferred to a glass well with cooled PBS pH 7.4 (PanEco), PBS was removed, the hippocampus was divided into 4–5 parts with a scalpel and transferred to a test tube with 1 ml of 0.25% trypsin-ethylenediaminetetraacetic acid (trypsin-EDTA; PanEco) [19].

Trypsinization of E18 and P2 Hippocampal Tissue

The brain tissue was trypsinized in 0.25% trypsin-EDTA solution for 20 min at 37°C, 5% CO_2 in a Binder CB150 incubator (Binder, Germany). After trypsinization, 1 ml of ice-cold Dulbecco's solution (PanEco) was added, and then the cell suspension was washed three times with PBS (pH 7.4). After that, 2 ml of neurobasal medium (PanEco), 2% neuromax supplement (PanEco), 0.5 mM L-glutamax (Thermo Fisher Scientific, USA), 1% penstrep (PanEco) were added to the resulting suspension.

Seeding and Cultivation of a Primary Mixed Culture of Hippocampal Neurons

The primary mixed culture of hippocampal neurons was cultivated using pre-prepared 8-well plates for the Seahorse XF HS mini cell metabolism analyzer (Agilent, USA). 400 μl-Aliquots of sterile distilled water were added to the grooves around the wells. Culture wells B-G were coated with 10 μl of 0.01 mg/ml poly-D-lysine (Thermo Fisher Scientific), and 180 μl of distilled water were added for blank correction in wells A and H. The plates were left for 1 h in a laminar flow hood under a UV lamp. Then the plates were washed three times with distilled water and dried in a laminar flow hood. Each well of the Cell Culture Miniplates (Agilent) was coated with 80 μl of cell suspension containing 2×10^4 hippocampal cells. The number of cells for seeding and the selection of the optimal FCCP concentration for performing the Mitostress test were determined by pre-calibrating the device to optimize the number of cells and finding the FCCP concentration for a given cell type according to the manufacturer's instructions. The cells in the plates were grown for three days with a daily change of half of the medium portion.

Pharmacological Blockade of P2X3 Receptor

Pharmacological P2X3 receptor blockade was achieved by adding the highly selective blocker, 12 nM AF-353 (Ro-4) (5-(5-iodo-2-isopropyl-4-methoxyphenoxy)pyrimidine-2,4-diamine monochloride salt, Selleckchem, USA). The choice of the final concentration (12 nM) was based on the known half-maximal inhibitory concentration (IC_{50}) of 3.16 nM for this receptor ligand [14]. The blocker was prepared in a culture neurobasal medium and introduced into the culture plates 24 h after the start of cultivation in the experimental wells (B, C, D) during medium change. Wells (E, F, H) with a culture medium without the inhibitor served as a control.

The Mitostress Test

Mitochondrial respiration was measured using a Seahorse XF HS mini cell metabolism analyzer (Agilent). The Agilent Seahorse XFp sensor cartridge (Agilent) was hydrated 24 h before analysis by filling it with a calibrant (200 μl per well). The cartridge was placed in a CO_2-free incubator for 24 h at 37°C. The analytical medium was prepared using Seahorse XF DMEM Media (Agilent) containing 10 mM glucose (Glucose XF 1.0 M; Agilent), 1 mM pyruvate (Pyruvate XF 100 mM; Agilent), and 2 mM L-glutamine (L-Glutamine XF 200 mM; Agilent) according to the manufacturer's recommendations. Mitochondrial functioning was assessed using a Seahorse XF Cell Mito Stress Test Kit (Agilent). Stock solutions were prepared according to the manufacturer's instructions. The kit includes oligomycin, FCCP, a mixture of rotenone and antimycin A. In the experiment, working solutions were prepared in the final concentration per well: oligomycin — 1 μM, FCCP — 2.5 μM, rotenone/antimycin A — 0.5 μM. Mitostressors were injected into cell cultures through the ports of the Cartridge Agilent Seahorse XFp sensor cartridge (Agilent). The cartridge was calibrated, and then the calibration plate was replaced with a plate with cells and the oxygen consumption rate (OCR), reflecting the degree of aerobic mitochondrial respiration in the cell, was measured. In each experimental and control well, 3 technical measurements were made. The obtained experimental data were processed using Wave 2.6 software (Agilent), the data were normalized by the number of cells using the "Normalization" tab in the program. Basal respiration, proton leak, maximum respiration, spare respiratory capacity, non-mitochondrial respiration, ATP production, respiratory coupling coefficient (Fig. 1), and the bioenergetic index of cell health were calculated [20].

The bioenergetic index of neuronal health was calculated according to formula (1):

$$BHI = \frac{SRC \times ATP}{nonMit \times H^{+}leak} \quad (1),$$

where BHI is the bioenergy health index in arbitrary units; SRC is the spare respiratory capacity in pmol/min; ATP is the ATP production in pmol/min; nonMit is the non-mitochondrial respiration in pmol/min, H^{+}leak is the leak of protons in pmol/min.

Oxygen consumption rate (OCR) curves of E18 embryonic culture and postnatal hippocampal culture were obtained in real time. The measured OCR data were used to calculate:

– non-mitochondrial respiration rate (minimal OCR value after injection of the rotenone/antimycin A mixture);

– basal respiration rate (last measured OCR value before the first injection minus non-mitochondrial respiration);

– maximal respiration rate (difference) between maximum OCR values after FCCP injection and non-mitochondrial respiration;

– proton leakage as minimal OCR value after oligomycin injection minus non-mitochondrial respiration;

– ATP production (after addition of oligomycin by the difference between the last OCR value);

– spare respiratory capacity (difference between maximum respiration and basal respiration);

– coefficient of respiratory coupling efficiency (ratio of ATP production to basal respiration).

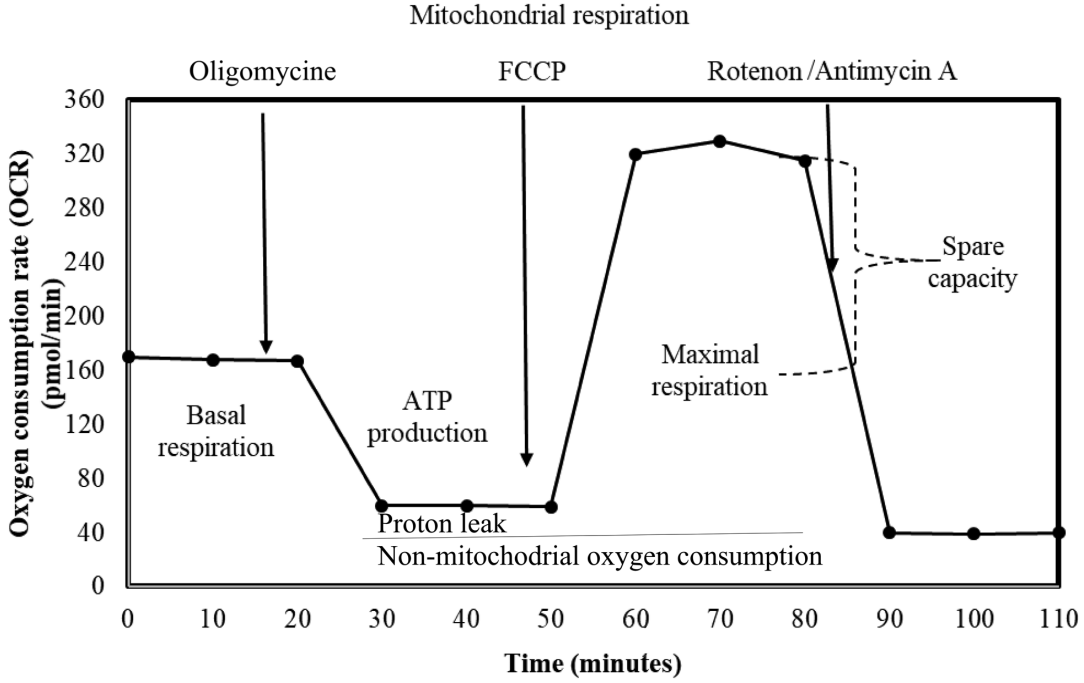

Figure 1. The Wave 2.6 software profile.

Statistical Analysis

Experimental results were processed using Wave 2.6 software (Agilent) and the Excel 10.0 descriptive statistics package. The experimental data are presented as median and minimum and maximum values. The hypothesis about the type of distribution of the obtained data was tested using the Pearson goodness-of-fit criterion. Considering that all the obtained numerical data do not follow the hypothesis of normal distribution, the Mann-Whitney criterion for samples with non-normal distribution and the number of measurements n≤20 was used to assess the reliability of the obtained results. The critical level of significance when testing statistical hypotheses in this study was taken at p=0.05

RESULTS AND DISCUSSION

Results are given in Table 1.

In the E18 hippocampal neuron culture, the AF-353 blocker, increased non-mitochondrial respiration and proton loss by 27.5% and 31%, respectively (p<0.05), while ATP production decreased by 25% (p<0.05) compared to the control. The other parameters of the mitochondrial bioenergetic status insignificantly differed from the control.

Analysis of the mitochondrial functioning in the P2 neuron culture treated with the P2X3 receptor blocker revealed an increase in non-mitochondrial respiration by 15.8% (p<0.05) and proton loss by 61.4% (p<0.05) compared to the control. At the same time,

Table 1. Parameters of mitochondrial respiration of primary mixed hippocampal culture of neurons under conditions of P2X3 receptor blockade

Parameter, pmol/min/cells×10^3		E18 culture		P2 culture	
		Control	AF-353 blocker	Control	AF-353 blocker
Non-mitochondrial respiration	M	888.0	1224.0*	1731.9	2058.3*
	min	610.0	1141.4	1616.2	2003.6
	max	1030.8	1331.4	1912.6	2199.8
Basal respiration	M	3298.0	2948.0	1065.7	1158.6
	min	2602.2	2407.2	659.0	591.2
	max	3786.6	3612.2	1706.4	1661.0
Maximal respiration	M	4316.2	3982.7	3710.2	4040.5
	min	3270.8	2804.8	2465.0	3581.8
	max	5134.2	4959.2	4290.6	4764.2
Proton leakage	M	2146.5	3104.6*	496.5	1286.8*
	min	1665.0	3835.8	232.0	1072.2
	max	2796.2	3467.0	642.4	1696.8
ATP production	M	4433.6	3544.2*	1530.5	1405.2
	min	3454.0	2520.0	1177.4	1124.6
	max	5598.0	4590.0	1738.8	1717.4
Spare respiratory capacity	M	1024.2	1032.6	2644.2	2892.6
	min	213.6	128.3	1451.4	1990.8
	max	1454.6	1910.6	3405.6	3700.6
Respiratory efficiency coefficient, %	M	1364.2	1472.6	3188.6	2772.6
	min	1103.6	440.6	1664.8	1716.4
	max	1653.4	2752.0	4547.6	5215.2
Bioenergetic health index, %	M	0.9	0.8	5.0	1.6*
	min	0.5	0.1	2.8	1.0
	max	3.1	4.7	9.7	2.7

* – statistically significant difference (p<0.05) versus control, M – median value, max – maximal value, min – minimal value.

no significant differences were found in ATP production, the efficiency of coupled respiration and spare respiratory capacity between the experimental and control groups.

Based on the data obtained for E18 and P2 hippocampal cultures, BHI was calculated for normal conditions and under P2X3 receptor blockade. According to the data obtained, P2X3 receptor blockade led to a decrease in the BHI both for E18 and P2 cultures. The P2 culture was characterized by a significant BHI decrease by 68% (p<0.05) compared to the control (Table 1).

In this study, the effect of the highly selective P2X3 receptor antagonist AF-353 (Ro-4) on the BHI was studied in the primary mixed culture of hippocampal neurons. The hippocampus has been chosen because it is a key brain structure involved in the formation of spatial navigation, emotional memory and learning, and at the same time, it is an ideal model for creating primary high-frequency neuron cultures to study pathophysiological processes in the nervous system [21]. At the cellular level, the hippocampus is represented by pyramidal cells with a minimal number of interneurons and glial cells compared to other areas of the brain [22]. In the hippocampus of rodents, the P2 family receptors are represented by the following subtypes: P2X2 [23], P2X3 [24, 25], P2X4 and P2X7 [26], as well as P2Y1 and P2Y11-13 [27–29]. The P2Y1 and P2X1-3 receptors are assigned a specific function in the processes of learning and memory [5, 30]. Purinergic receptors are expressed both on glial cells and on true neurons of the hippocampus; the presence of P2X2, P2X3, P2X4, P2X7 has been described [31]. The study of two hippocampal cultures at the embryonic and postnatal stages is due to the different function of the P2X3 receptor at different stages of the nervous tissue formation. In the early stages of embryonic development, the P2X3 receptor plays a key role in neurogenesis; researchers indicate that it may be the only receptor subtype involved in fast ATP-mediated excitatory signaling [32]. In addition, embryonic cultures are characterized by high resistance to chemical dissociation and survival after mechanical impact on tissue. Embryonic hippocampal cultures are characterized by a high degree of purity, a smaller number of complex neurites, lower connectivity between neurons and less dependence on culture substrates compared to postnatal cultures [33, 34].

Postnatal hippocampal culture is a convenient model to study the physiological processes underlying learning and memory, as well as the P2X3 receptor involvement in the adaptation of neural networks during the development of anxiety and motivation processes [13]. In contrast to embryonic tissue, postnatal brain tissue is relatively sensitive to the cultivation process and has reduced viability due to physical degradation of neurons and removal of intraneuronal trophic support [33]. Postnatal cultured neurons become more susceptible to glutamate-mediated excitotoxicity during their development [35] and exhibit increased caspase activation and apoptosis [36].

Treatment of both E18 and P2 cultures with AF-353 (Ro-4) increased OCR due to an increase in non-mitochondrial respiration and proton leakage under conditions of decreased basal respiration. The decrease in basal respiration indicates the development of cellular stress upon receptor blocker addition. It has been proven that oxidative stress changes bioenergetic parameters, increases oxygen consumption associated with proton leakage [37]. The loss of protons and an increase in non-mitochondrial respiration under conditions of a significant decrease in ATP production indicates a shift in intracellular homeostasis and the generation of free radicals. In this context it should be mentioned that non-mitochondrial oxygen consumption usually increases in the presence of stressors, including reactive oxygen (ROS) and nitrogen species [37, 38]. At the same time, the increase in proton loss induced by the AF-353 (Ro-4) treatment can be considered as a protective mechanism that reduces mitochondrial damage by negative feedback. This is consistent with the data obtained on the mitochondrial spare respiratory capacity and the respiratory efficiency coefficient, which were maintained at the control level in the experimental group. Currently, increasing evidence exists for the presence of a protective feedback loop, where increased ROS generation activates mechanisms that trigger proton leakage, and they, in turn, reduce ROS production, thereby limiting damage to mitochondrial function [39, 40]. The fact that the respiratory reserve is not depleted under conditions of the P2X3 receptor blockade in both E18 and P2 cultures indicates that the AF-353 (Ro-4) antagonist does not cause the development of severe oxidative stress in the cell. Previously, using cardiac cells exposed to severe oxidative stress it was shown that the respiratory reserve depleted under conditions of decreased basal respiration threshold, thus leading to cell death [41, 42]. In general, researchers note that the spare respiratory capacity is a parameter that depends on a cell type. Mitochondria in excitable cells such as cardiomyocytes and neurons function in conditions of high calcium and other ion flows that will use the proton gradient and thus increase the rate of oxygen consumption regardless of the need for ATP [43]. The higher the values of the reserve respiratory capacity, the more effectively the mitochondria can meet the need for ATP and cope with metabolic stress [37].

The treatment with AF-353 (Ro-4) revealed a difference in ATP production by E18 and P2 cultures. In the E18 culture, ATP production was sharply reduced, while in the P2 culture no significant differences were found. This indicates high plasticity and the presence of compensatory-adaptive mechanisms

of the P2 culture, most likely due to complex neuroglial connections and synaptic exchange mechanisms [44]. The P2 culture is characterized by a high respiratory reserve compared to the embryonic E18 culture.

Analyzing the BHI of neurons in the embryonic hippocampal culture, it is evident that initially it is 5 times lower compared to the postnatal culture; this indicates low mitochondrial activity under physiological conditions. Treatment with the P2X3 receptor antagonist AF-353 increased non-mitochondrial respiration and proton loss in the embryonic culture under conditions of decreased ATP production. It is known that respiration in the absence of ATP synthesis is caused by proton leakage across the inner mitochondrial membrane [45]. Surin et al. demonstrated that in embryonic cultures the neuronal mitochondrial potential was significantly lower than in postnatal cultures, due to the increased permeability of the inner mitochondrial membrane for protons [46]. It has been shown that in 84% neurons of rat E17-E18 cultures mitochondrial ATP synthase does not produce ATP, but consumes it to maintain the mitochondrial membrane potential. In embryonic cultures, ATP synthesis is predominantly glycolytic [46]. Thus, blockade of the P2X3 receptor under conditions of uncoupled mitochondrial respiration leads to a decrease in ATP production and an increase in non-mitochondrial respiration. It is possible that such changes are associated with increased proton conductivity of the inner mitochondrial membrane in embryonic cultures, compared with postnatal ones, as well as features in the energy phenotype of the cell. According to our previously obtained results, blockade of the P2X3 receptor does not affect the metabolic profile of the embryonic culture [47]. Embryonic hippocampal culture is characterized by aerobic respiration both in the control and with the blockade of the P2X3 receptor, most likely due to glial cells [47]. Astrocytes, which are synthesizers and exporters of bioenergetic molecules (lactate/ketone bodies) for oxidative phosphorylation, can play a significant role in this case [48]. Blockade of the P2X3 receptor function in embryonic cultures under conditions of reduced mitochondrial function can negatively affect cell morphology, the formation of processes and synaptic contacts between neurons. In a mouse model of Alzheimer's disease, impaired hippocampal neurogenesis, a decrease in proliferation and underdevelopment of neurites in the brain under conditions of inhibition of the *p2rx3* gene were found [49].

CONCLUSIONS

In postnatal hippocampal culture, blockade of the P2X3 receptor with the highly selective antagonist AF-353 (Ro-4) leads to a decrease in the BHI index and ATP production, but increases OCR and preserves the spare respiratory capacity of the neuron. This is important in terms the cell energy sources and its ability to cope with metabolic stress. Embryonic hippocampal culture initially has a fairly low BHI value compared to postnatal culture; this indicates the prevalence of glycolytic processes in ATP synthesis. P2X3 receptor blockade in embryonic culture leads to increased proton loss and decreased ATP production, which can have further negatively impact on the formation of interneuronal connections in the culture. The data obtained indicate the potential of the P2X3 receptor as a pharmacological target in hypoxic conditions of the brain during neurogenesis.

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COMPLIANCE WITH ETHICAL STANDARDS

All experiments were performed in compliance with ethical standards of the Helsinki Declaration on the Humane Treatment of Animals (Helsinki Declaration of Ethical Principles, 2008), EU Directives on the protection of animals used for experimental and other scientific purposes, as well as the ethical standard adopted by the expert councils of the RSF “Position on the Ethics of the Use of Animals in Research Supported by the Russian Science Foundation” dated 09.08.2022 and approved by the Committee for the Control of the Care and Use of Laboratory Animals of the National Research University “BelSU” (expert conclusion No. 01i/23 dated 23.01.2023).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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БЛОКАТОР P2X3 РЕЦЕПТОРА AF-353 (Ro-4) СНИЖАЕТ БИОЭНЕРГЕТИЧЕСКИЙ ИНДЕКС ПЕРВИЧНОЙ СМЕШАННОЙ КУЛЬТУРЫ НЕЙРОНОВ ГИППОКАМПА

А.С. Зеленцова, М.Ю. Скоркина*, А.В. Дейкин

Белгородский государственный национальный исследовательский университет,
308015, Белгород, ул. Победы, 85; *эл. почта: marinaskorkina0077@gmail.com

В клинических исследованиях пуринергический рецептор P2X3 рассматривается как молекулярная мишень для коррекции боли в спинальных сенсорных нейронах с использованием высокоселективных антагонистов на основе производных диаминопиримидина. В ЦНС P2X3 рецепторы участвуют в синаптической пластичности, лежащей в основе памяти и обучения. В настоящее время известны мощные и селективные аллостерические модуляторы рецепторов P2X3 и P2X2/3 — производные ряда диаминопиримидина. Среди них 5-(5-йод-2-изопропил-4-метоксифенокси)пиримидин-2,4-диамин (Ro-4 или AF-353), гефапиксант, которые имеют хороший фармакокинетический профиль и менее активны в отношении широкого спектра киназ, рецепторов и ионных каналов. Терапевтическое значение блокирования рецепторов P2X3 в нейронах ЦНС не изучалось, однако, судя по литературным данным, этот рецептор может стать новой мишенью при поиске противоэпилептических препаратов, а также препаратов, снижающих тревожность и стресс. Целью работы было изучить влияние антагониста P2X3 рецептора AF-353 (Ro-4) на биоэнергетический индекс здоровья (BHI) нейронов первичной смешанной культуры гиппокампа. В условиях блокады P2X3 рецептора в эмбриональной и постнатальных культурах нейронов гиппокампа мыши немитохондриальное дыхание увеличилось на 27,5% и 15,8% соответственно, потеря протонов — на 31,0% и 61,4%, а базальное дыхание снизилось на 89% и 39% по сравнению с контролем. Снижение BHI в постнатальной культуре составило 68% по сравнению с контролем. Полученные результаты указывают на влияние AF-353 на митохондриальное дыхание первичной смешанной культуры нейронов гиппокампа, что раскрывает потенциал P2X3 рецептора в качестве фармакологической мишени при гипоксических состояниях головного мозга.

Полный текст статьи на русском языке доступен на сайте журнала (http://pbmc.ibmc.msk.ru).

Ключевые слова: P2X3 рецептор; антагонист AF-353 (Ro-4); митохондриальное дыхание; гиппокамп; нейроны

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