DOI: 10.17816/KMJ611074

Activity of enzymes destroying extracellular nucleotides in the tissues of rats with the valproate model of autism

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Abstract

Background. Ectonucleotidases hydrolyze extracellular nucleotides and thus can control the effect of these substances on purinergic P1 and P2 receptors.

Aim. To evaluate the activity of ectonucleotidases in the smooth muscle tissues of internal organs of 9-month-old rats with the valproate model of autism using high-performance liquid chromatography.

Material and methods. Autism was modeled in outbred Wistar rats by administering valproic acid (500 mg/kg) subcutaneously to pregnant females on days 12–13 of pregnancy. The born offspring were used in the study when the rats reached 270±8 days. Animals were guillotined under light ether anesthesia, the bladder, uterus, vas deferens, and duodenum were isolated, and smooth muscle tissue samples were prepared. Total ectonucleotidase activity was determined by incubating tissue samples with adenosine triphosphate (reaction substrate) for 10 minutes with further assessment of the content of the substrate and reaction products (adenosine diphosphate, adenosine monophosphate) in the incubate using high-performance liquid chromatography. Mathematical and statistical processing of the results was carried out using Microsoft Excel and IBM SPSS Statistics 26.0 software. Group comparisons were made using the nonparametric Mann–Whitney U test. Differences were considered significant at $p < 0.05$.

Results. In rats with the valproate model of autism, the activity of ectonucleotidases in the smooth muscle tissues of the vas deferens (609.5±153.9) and uterus (232.7±2) was significantly lower than control values (2114.6±524.3, p=0.040; 539.6±63.5, p=0.010, respectively). In the duodenum (1808.4±184.5) and bladder (1021.3±280.7) we did not find a significant difference compared to the control values (2115.0±393.3, p=0.712; 2302.3±615.8, p=0.274, respectively). This study allows us to evaluate the possible contribution of purinergic transmission to the changes we found earlier in the contractile activity of smooth muscle tissue in rats with the valproate model of autism.

Conclusion. In 9-month-old rats with a model of autism, the activity of ectonucleotidases in the smooth muscle tissues of the reproductive organs is reduced; no such changes were found in the tissues of the intestines and bladder.

Keywords: autism, valproic model, rats, ectonucleotidases, purinoceptors.

For citation: Ivanova DV, Khabirov RA, Ziganshin AU. Activity of enzymes destroying extracellular nucleotides in the tissues of rats with the valproate model of autism. *Kazan Medical Journal.* 2024;105(1):84–89. DOI: 10.17816/KMJ611074.

Background

Ectonucleotidases are ectoenzymes that hydrolyze extracellular nucleotides to nucleosides. This mechanism regulates nucleotide concentration, which can have biological effects. It can significantly alter the effect of endogenous ligands on purinergic P2 and adenosine receptors [1].

Extracellular nucleotide regulation and P2-receptor signaling involve four main types of ectonucleotidases: ecto-nucleoside-5′-triphosphate diphosphohydrolase, ectonucleotide pyrophosphatase/phosphodiesterase, glycosylphosphatidylinositol (GPI)-linked ecto-5′-nucleotidase (ecto-5′-NT), and GPI-linked alkaline phosphatase [2].

Ecto-nucleoside-5′-triphosphate diphosphohydrolase-1 (CD39) and ecto-5'-nucleotidase (CD73) are related ectonucleotidases that play crucial roles in immune system regulation and nucleotide metabolism. CD39 is a membrane glycoprotein that converts adenosine triphosphate (ATP) and other nucleotides into adenosine diphosphate (ADP) and adenosine monophosphate (AMP) and corresponding nucleosides.

CD39 hydrolyzes ATP and other nucleotides in the extracellular space to ADP and AMP, resulting in decreased ATP levels. This decrease is a potent stimulator of thrombosis and inflammation. Similarly, CD73 serves as a membrane glycoprotein that

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Submitted 20.10.2023; accepted 03.11.2023; published 28.12.2023.

converts AMP to adenosine. CD73 is expressed in various tissues and is densely distributed in glioblastoma cell membranes.

CD39 and CD73 are enzymes that regulate the release of adenosine, a potent immunomodulator, and work together to control adenosine levels in tissues and organs. CD39 breaks down ATP and ADP to AMP, which is then converted to adenosine by CD73 [2, 4]. Ectonucleotidase is a critical antigen of immunocompetent cells, specifically the CD73 receptor, which regulates the maturation and activity of T- and B-lymphocytes and their adhesion to the endothelium. Hydrolysis of the phosphatidylinositol bond causes ecto-5'-nucleotidase to dissociate from the cell, leading to disturbances in the biosynthesis of nucleic acid precursors and maturation processes in lymphocytes [5].

In a study on mice with altered CD73 gene, CD73 is believed to regulate learning, memory formation, and psychomotor coordination [6]. Furthermore, CD39 and CD73 enzyme activity may serve as potential markers for diagnosing schizophrenia [7].

Regulation of extracellular nucleotide levels through the use of ectonucleotidases is crucial in understanding the mechanisms of purinergic transmission in tissues and its potential implications in developmental disorders, such as autism [8].

The most commonly used animal model for studying autism is the valproate model of autism (VMA) [9].

Aim

This study aimed to assess ectonucleotidase enzyme activity in smooth muscle tissues obtained from 9-month-old rats with valproate-induced autism using high-performance liquid chromatography.

Materials and methods

The present study used 9-month-old outbred Wistar rats that were housed in a room for experimental animals with a temperature of 22°C–24°C and relative humidity of 40%–50%. The rats were fed a complete balanced diet according to GOST 33215-2014 and were kept in compliance with the laboratory practice rules for preclinical studies in the Russian Federation (GOST 351,000.3-96 and 1000.4-96) and the international recommendations of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (1986).

The study was approved by the Ethical Committee of Kazan State Medical University (minutes no. 2; February 15, 2022).

To model autism, female rats were injected with sodium salt of valproic acid at a dose of 500 mg/kg subcutaneously in the withers on days 12–13 of gestation, and the experiments were conducted using male and female offspring of such rats at 9 months old. Controls were rats of similar age and sex born from intact rats, i.e., not exposed to any drug effect. The VMA was validated in the laboratory of Kazan State Medical University [10].

The study began when the rats reached 270 ± 8 days of age. Under light ether anesthesia, rats from both the control (N = 8) and experimental (N = 10) groups were guillotined, and tissue samples (2–4 mg) from the bladder, uterus, seminal ducts, and duodenum were prepared. One tissue sample (n) comprised up to four smooth muscle samples from each animal (N).

The total ectonucleotidase activity was determined by incubating rat smooth muscle tissue samples with ATP, the reaction substrate, for 10 min. The incubated substrate and reaction product (ADP and AMP) contents were further estimated using high-performance liquid chromatography.

Experiments were conducted at 37°C ± 0.5°C in a buffer with the following composition (in mM): HEPES, 10; NaCl, 135; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 2; and glucose [hydrogen exponent (pH), 7.4], 10. In a series of control experiments, buffer with ATP (300 μM) was used without the addition of tissue. The reaction was stopped by adding 300 μl of 3% perchloric acid.

The incubation liquid was centrifuged, and the resulting centrifugate was frozen for subsequent chromatographic analysis. The analysis was performed using a Shimadzu chromatograph with Supelco silica gel columns (4.6-mm inner diameter, 150-mm length) and the LC Solution program. The mobile phase consisted of 0.2 M KH_2PO_4 and 3% methanol (pH = 6.0), with a flow rate of 1.5 mL/min, wavelength of 260 nm, and sample volume of 20 μL. Complete separation occurred within 5.5 min.

ATP concentration was determined by comparing the area under the curve with that of the control sample. Ectonucleotidase activity was expressed as the amount of ATP (pmol) degraded per 1 mg of tissue per 1 minute.

The results were mathematically and statistically processed using Microsoft Excel and IBM SPSS Statistics 26.0 software. The groups were compared using the nonparametric Mann–Whitney U test. The data are presented as mean ± error of mean (M ± m), with n representing the number of muscle preparations used. $P < 0.05$ indicated significant difference.

Results and discussion

The duodenal samples of rats with 9 months VMA did not show a significant difference in ectonucle-

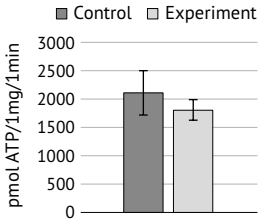

Fig. 1. Duodenal ectonucleotidase activity of rats in a valproate model of autism at 9 months of age. Data are presented as M ± m. Control (n = 12); experiment (n = 11); p = 0.712

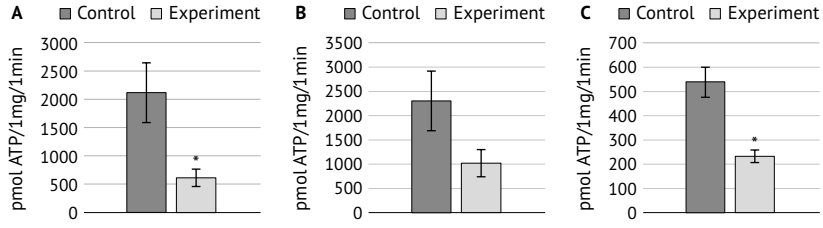

Fig. 2. Ectonucleotidase activity in the urogenital system of 9-month-old rats in a valproate model of autism. Data are presented as M ± m. A. Seminal duct: control (n = 15), experiment (n = 10), *p = 0.040. B. Bladder: control (n = 19), experiment (n = 12), p = 0.274. C. Uterus: control (n = 6), experience (n = 19), *p = 0.010 compared to the control group

otidase activity compared with the control group (p = 0.712) (Fig. 1).

In previous studies, the mechanical activity of the intestine in rats with 9 months VMA was evaluated [11]. Carbacholine induced contractions in the duodenum and ileum in rats with VMA for 9 months, and no significant differences were observed between the control and experimental groups in samples of both smooth muscle tissues. The present study found that ATP did not have a significant effect on the relaxation of the duodenum and ileum of 9-month-old rats with VMA, nor did the P2Y-receptor agonist 2-methylthio-ATP. These results show that purinergic transmission in the duodenum of 9-month-old rats with VMA was not significantly different from that in controls.

In 9-month-old VMA rats, the ectonucleotidase activity in seminal duct samples was 3.5 times lower than that in controls (p = 0.040) (Fig. 2A). In a previous study, the contractile activity of the seminal duct in response to the enzymatically persistent P2 receptor agonist α,β-methylene-ATP in controls was not different from that in 9-month-old VMA rats [11]. The results indicate an ambiguous effect of the purinergic system on the contraction of the seminal duct of rats with VMA at 9 months of age.

In 9-month-old rats with VMA, the ectonucleotidase activity in bladder samples was not significantly different from the enzyme activity values in tissue samples of control animals (p = 0.274) (Fig. 2B). The contractile activity of the isolated bladder of rats with 9 months VMA was evaluated, and carbacholine induced more significant bladder contractions in 9-month-old VMA rats than in controls. The absence of significant differences was found in the action of α,β-methylene-ATP on the contraction of smooth muscle tissue of rats in the experimental and control groups [11]. Purinergic transmission in the bladder tissues of 9-month-old rats is of the same significance as that in control animals.

In uterine samples of rats with VMA at 9 months, ectonucleotidase activity was significantly lower than that in controls (p = 0.010) (Fig. 2C). A pharmacological study of isolated smooth muscle samples from the uterus of rats with 9 months VMA revealed that contractions induced by the P2 receptor agonists α,β-methylene-ATP and β,γ-methylene-ATP were significantly lower than those in controls. Electric field stimulation induced contractions in isolated uterine specimens of rats with VMA at 9 months, which were not statistically different from those in controls [12]. Chromatographic analysis data shows that the changes in contractile activity in the uterus of 9-month-old VMA rats are related to disturbances that occur in the postsynaptic membrane.

Previous studies have demonstrated that valproic acid administration in pregnant females can model autism. The mechanical activity of the duodenum, ileum, bladder, ejaculatory duct, and uterus in the offspring of 3-month-old [13] and 9-month-old [11, 12] rats differed from that in control animals.

The valproate model was selected as a model of autism because valproic acid is the most commonly used variant [14–17]. High doses of valproic acid block histone deacetylases, which are enzymes involved in the deacetylation of histone proteins, affect gene expression. Therefore, when pregnant females are administered valproic acid, the offspring of laboratory animals develop fetal valproate syndrome, which is considered a model of autism [18].

This study indicates that 9-month-old rats with VMA exhibit distinct ectonucleotidase activity in the smooth muscle tissues of the urogenital system and gastrointestinal tract. Furthermore, rats with VMA showed significantly lower ectonucleotidase activity in the smooth muscle tissues of the vas deferens and uterus compared with the controls; however, no significant difference was observed in the duodenum and urinary bladder.

According to [3], a decrease in ectonucleotidase activity results in decreased ATP metabolism, leading to increased strength and duration of agonist action on the receptor. Conversely, an increase in enzyme activity reduces the effect of ATP on the receptor.

A previous study has demonstrated that P2-receptor agonists do not significantly affect the mechanical activity of the intestine, ejaculatory duct, and bladder of 9-month-old VMA rats [11]. However, contractions in the isolated uterus were significantly lower than the control values [12].

This study confirms that the purinergic signaling system contributes to the mechanical activity of the intestine, bladder, and seminal duct of 9-month-old VMA rats. Additionally, the results support the concept that changes in uterine contractility are mediated by postsynaptic disturbances.

Conclusions

The smooth muscle tissues of the vas deferens and uterus exhibited significantly lower ectonucleotidase activity in 9-month-old VMA rats than in controls. However, no significant difference was found in the tissues of the duodenum and bladder compared with those in controls.

Authors' contributions. DVI, biochemical study and writing the article; RAH, biochemical study and literature review; AUZ, editing the text and final approval of the article.

Funding source. This study was supported by RNF (project no. 22-25-00030).

Conflict of interest. The authors have no conflict of interest to declare.

Acknowledgments. The authors would like to thank DO Nikitin and AV Nikitina for their help in modeling autism.

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