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Research Article

Primary Screening of Selective Agonists and Adrenoreceptor Blockers on A New Paramecium Caudatum Model

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Abstract:

The study investigated the impact of selective agonists and antagonists of adrenergic receptors at different concentrations on the behavior of the unicellular organism Paramecium caudatum. It was demonstrated that exposure to methoxamine at a concentration of 10-3 M increases the movement speed of Paramecium caudatum cells but reduces the number of motile cells by activating various intracellular processes. The presence of phenylephrine at a concentration of 10-3 M in the environment decreases motor activity and exhibits similar effects to adrenaline.

The introduction of terbutaline at a concentration of 10-3 M leads to a sharp and prolonged increase in cell movement speed and overall activity, while the addition of dobutamine at a concentration of 10-3 M reduces motor activity. The study also showed the effects of selective blockers prazosin and atenolol at a concentration of 10-5 M, which manifested in the complete correction of the effects induced by the corresponding selective agonists on all investigated parameters of motor activity.

Key words: paramecium caudatum; comparative physiology; cellular model; neurotransmitters; adrenergic receptors

1.Introduction

As of today, amidst the tightening of bioethical control over preclinical studies, the use of various cell cultures for screening pharmacological compounds has become more popular in the scientific community compared to research involving higher vertebrates and endeavors focused on predicting effects through computer modeling. However, pharmacological studies on cell cultures, particularly on unicellular organisms, are characterized by the diversity of employed methods and are complex in the interpretation of obtained results, leading to significant debates and discussions within this field [1].

Previously, in pioneering studies aimed at developing new methods for utilizing Paramecium caudatum as a model organism for primary screening of pharmacological compounds, we demonstrated and described a method that allowed the registration of changes in swimming behavior in response to the administered drug. This method is based on the variability of the motor activity of eukaryotic cells of Paramecium caudatum [2]. This method further confirmed its effectiveness in subsequent studies involving agonists and blockers of GABA-A receptors [3].

In our recent study, we decided to present the results obtained after optimizing our method, enabling the parallel comparison of investigated drugs, specifically selective agonists of adrenergic receptors and their blockers widely used in medical cardiological practice. This significantly accelerated data processing time and improved the quality of the results obtained, as simultaneous registration of movements placed all experimental groups in equal conditions.

2.Materials and methods

The study was conducted using a sterile culture of live Paramecium caudatum cells. The organisms were maintained in conditions approximating their natural habitat, with a temperature of 21 degrees Celsius, pH ranging from 6.8 to 7.2, under a 12-hour light cycle, and a cell density of 600 cells/mL. Feeding with infusoria was performed daily

with 200 μL of a Saccharomyces cerevisiae yeast solution per colony in 100 mL. To maintain the sterility of the environment, monthly filtration of the colony through a fine-dispersed sponge was carried out. Infusoria feeding was halted on the day of the experiment.

Our study comprises two series of experiments. In the first series, the effect of adrenergic receptor agonists on the motor activity of Paramecium caudatum was investigated. Selective α-1 adrenergic receptor agonists included phenylephrine (Sigma) and methoxamine (Sigma) at concentrations of 10-3 and 10-5 M. clonidine (Sigma) at concentrations of 10-3 and 10-5 M was used to activate α-2 adrenergic receptors. dobutamine (Sigma) and terbutaline (Sigma) at concentrations of 10-3 and 10-5 M, respectively, were chosen as selective β-1 and β-2 adrenergic receptor agonists.

The second series explored the possibility of correcting the effects of selective agonists with selective blockers such as prazosin (Sigma) at a concentration of 10-5 M for α-1 adrenergic receptors and atenolol (Sigma) at a concentration of 10-5 M for β-1 adrenergic receptors. Each experiment was replicated eight times and accompanied by its own control. The intact group received the Lozina-Lozinsky solution in the same volume.

The movement of cells was recorded using a trinocular stereoscopic microscope, Olympus SZ6, equipped with a 24-well plate. The volume of the investigated cell culture was one milliliter. For the analysis of motor activity based on the obtained video recordings, the ImageJ (Fiji) program with the "Track Mate" plugin was employed [4].

Statistical data processing: The statistical analysis was performed using the "Statistica 10" program with a non-parametric Two-Way ANOVA test. Significance levels were assessed at $p < 0.05$.

3.Results and discussion

Prior to working with selective adrenergic receptor agonists, it was observed that the introduction of adrenaline at concentrations of 10-3 M into the medium with Paramecium caudatum cells reduces the motor activity of Paramecium caudatum (Fig. 1a, b).

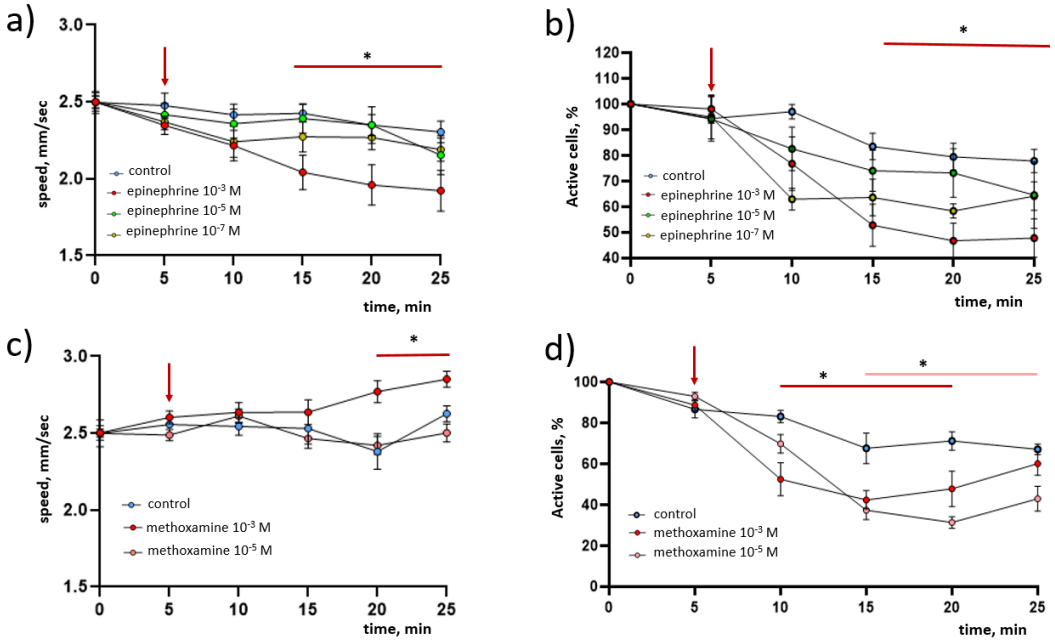

Figure 1: Changes in the speed of movement and activity of Paramecium caudatum in response to the administration of adrenaline and methoxamine. a – Changes in the average speed of cell movement under the influence of adrenaline at concentrations of 10-3, 10-5, 10-7 M during a 25-minute registration period. The arrow indicates the moment of adding the drugs to the well (fifth minute of registration). Cell concentration - 600±50 cells/mL. * - significant difference from the speed of cells in the intact group, $p < 0.05$, two-way ANOVA; b – Changes in the relative number of motile cells under the influence of adrenaline at concentrations of 10-3, 10-5, 10-7 M during a 25-minute registration period. Cell concentration - 600±50 cells/mL. * - significant difference from the relative number of motile cells in the intact group, $p < 0.05$, two-way ANOVA; c – Changes in the average speed of cell movement under the influence of methoxamine at concentrations of 10-3, 10-5 M during a 25-minute registration period. The arrow indicates the moment of adding the drugs to the well (fifth minute of registration). Cell concentration - 600±50 cells/mL. * - significant difference from the speed of cells in the intact group, $p < 0.05$, two-way ANOVA; d – Changes in the relative number of motile cells under the influence of methoxamine at concentrations of 10-3, 10-5 M during a 25-minute registration period. Cell concentration - 600±50 cells/mL. * - significant difference from the relative number of motile cells in the intact group, $p < 0.05$, two-way ANOVA.

The concept of motor activity encompasses three parameters: swimming speed, the number of actively moving cells, and the smoothness of Paramecium trajectories. Thus, the presence of adrenaline at a concentration of 10-3 M in the medium reduces the swimming speed of Paramecium caudatum by 0.7 mm/s, while lower concentrations of adrenaline did not lead to changes in this parameter (Figure. 1a). The alteration in swimming speed may be associated with a disruption in the sequence and rhythmicity of cilia beating in Paramecium. [5]. In response to the activation of adrenergic receptors by administered adrenaline, there is a manifestation of intermittent jerky movements in different directions. It is also noteworthy that there is a 50% decrease in the number of motile cells in the experimental wells (Figure. 1b). Such a reduction may be attributed to the activation of feeding behavior or sexual behavior in Paramecium. [6] In response to the administration of adrenaline into the

medium, the examination of movement trajectories allows for the assessment of the alternation of exploratory behavior (cells spreading throughout the well) with the formation of cell clusters. These clusters are formed through chemotaxis, induced by the local secretion of various chemoattractants by Paramecium cells in response to endocytosis stimulation [7]. The activation of α-1 adrenergic receptors by the selective agonist methoxamine at a concentration of 10-3 M resulted in an increase in the speed of Paramecium movement by 0.2 mm/s. The development of the effect was delayed by 15 minutes after exposure, and lower concentrations of the drug did not differ in their effect on this parameter compared to the intact group (Figure. 1c). However, the number of motile cells in the well decreased by 50% at both concentrations of 10-3 M and 10-5 M (Figure. 1b). Notably, there is a tendency to restore motility after 20 minutes of exposure, which is attributed to the selective action of α-1 adrenergic receptors, unlike adrenaline, which has a broader spectrum of action. On the contrary, a different selective agonist of α-1 adrenergic receptors, phenylephrine, at a concentration of 10-3 M, exhibits an opposite effect on the swimming speed of Paramecium caudatum (Figure. 2).

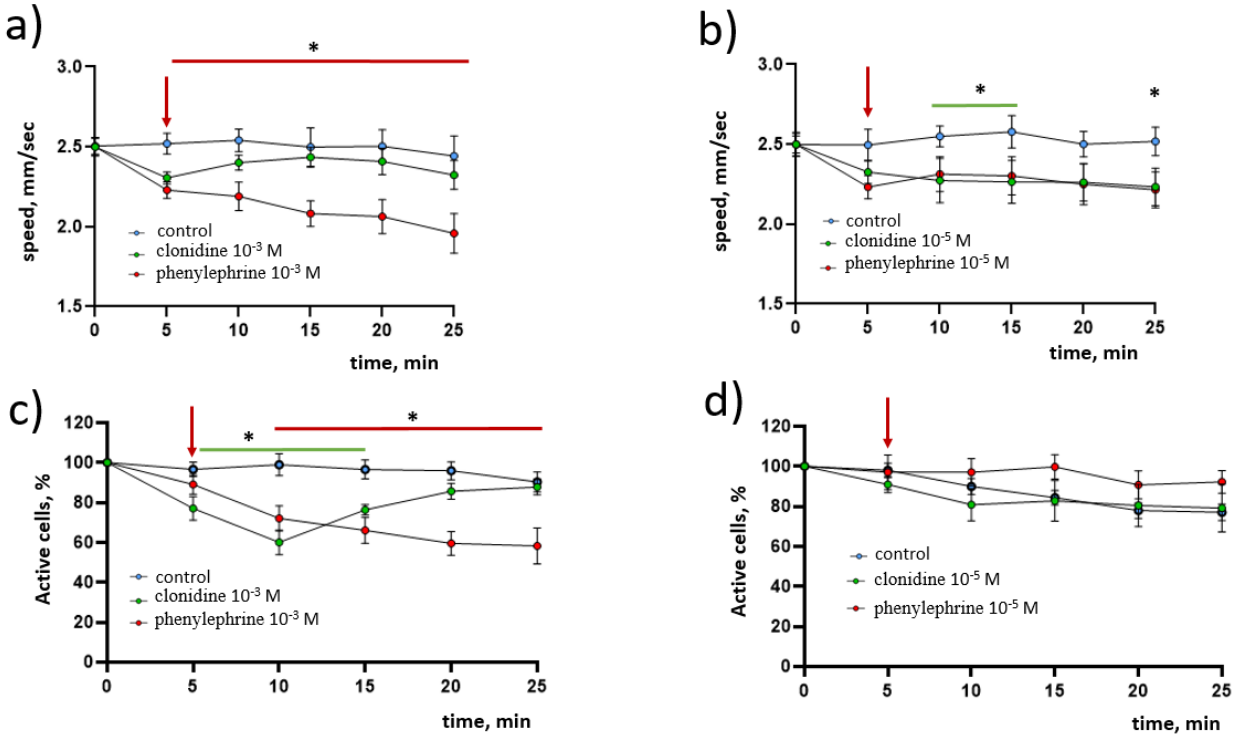

Figure 2: Changes in the speed of movement and activity of Paramecium caudatum in response to the administration of selective agonists of α-1 and α-2 adrenergic receptors. a – Changes in the average speed of cell movement under the influence of phenylephrine and clonidine at a concentration of 10-3 M during a 25-minute registration period. The arrow indicates the moment of adding the drugs to the well (fifth minute of registration). Cell concentration - 600±50 cells/mL. * - significant difference from the speed of cells in the intact group, $p < 0.05$, two-way ANOVA; b – Changes in the average speed of cell movement under the influence of phenylephrine and clonidine at a concentration of 10-5 M during a 25-minute registration period. The arrow indicates the moment of adding the drugs to the well (fifth minute of registration). Cell concentration - 600±50 cells/mL. * - significant difference from the speed of cells in the intact group, $p < 0.05$, two-way ANOVA; c – Changes in the relative number of motile cells under the influence of phenylephrine and clonidine at a concentration of 10-3 M during a 25-minute registration period. Cell concentration - 600±50 cells/mL. * - significant difference from the relative number of motile cells in the intact group, $p < 0.05$, two-way ANOVA; d – Changes in the relative number of motile cells under the influence of phenylephrine and clonidine at a concentration of 10-5 M during a 25-minute registration period. Cell concentration - 600±50 cells/mL.

In this case, the action of phenylephrine on Paramecium is consistent with the action of adrenaline at the same concentration, resulting in a decrease in the speed of cell movement by 0.5 mm/s (Figure. 2a) and a reduction in the number of motile cells by about 50% (Figure. 2c). The opposite effects on speed induced by the activation of one type of receptor by phenylephrine and metaxamine might seem peculiar at first glance. However, in studies on the inotropic effects of atria, the administration of adrenaline and phenylephrine led to a positive inotropic response, while metaxamine induced negative inotropic responses under normal and low regulation purity [8]. The force and frequency of heart contractions, as well as the change in the speed of Paramecium movement, directly depend on electrochemical interactions. The presence of phenylephrine in the medium at a concentration of 10-5 M did not lead to significant differences from the parameters of the intact group (Figure. 2b, d). Activation of α-2 adrenergic receptors using the selective agonist clonidine resulted in less pronounced effects. The strength of the induced effect is likely to be associated with different receptor quantities on the membrane of Paramecium cells.

Opposite effects can also be observed when introducing selective β-agonists of adrenergic receptors, dobutamine and terbutaline, into the medium (Figure. 3).

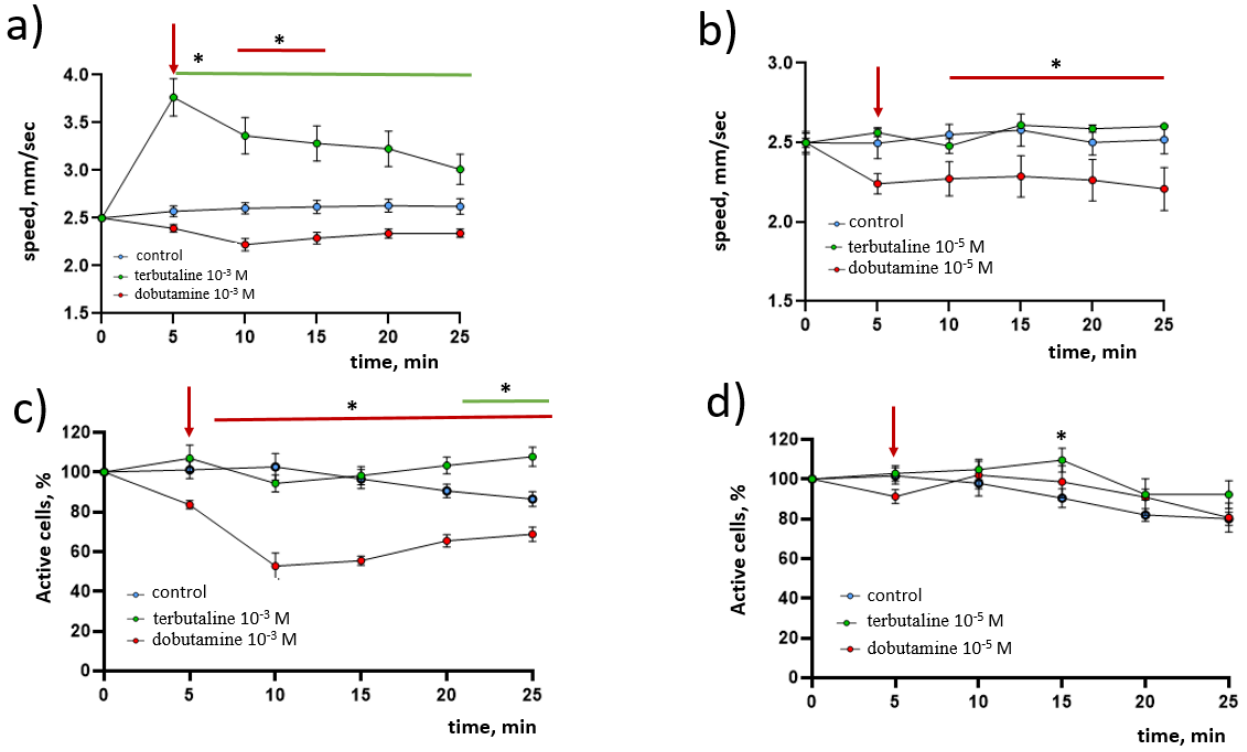

Figure 3: Changes in the speed of movement and activity of Paramecium caudatum in response to the administration of selective β-1 and β-2 adrenergic receptor agonists. a – Changes in the average speed of cell movement under the influence of dobutamine and terbutaline at a concentration of 10-3 M during a 25-minute registration period. The arrow indicates the moment of adding the drugs to the well (fifth minute of registration). Cell concentration - 600±50 cells/mL. * - significant difference from the speed of cells in the intact group, $p < 0.05$, two-way ANOVA; b – Changes in the average speed of cell movement under the influence of dobutamine and terbutaline at a concentration of 10-5 M during a 25-minute registration period. The arrow indicates the moment of adding the drugs to the well (fifth minute of registration). Cell concentration - 600±50 cells/mL. * - significant difference from the speed of cells in the intact group, $p < 0.05$, two-way ANOVA; c – Changes in the relative number of motile cells under the influence of dobutamine and terbutaline at a concentration of 10-3 M during a 25-minute registration period. Cell concentration - 600±50 cells/mL. * - significant difference from the relative number of motile cells in the intact group, $p < 0.05$, two-way ANOVA; d – Changes in the relative number of motile cells under the influence of dobutamine and terbutaline at a concentration of 10-5 M during a 25-minute registration period. Cell concentration - 600±50 cells/mL.

It is demonstrated that the introduction of the selective β1-adrenergic receptor agonist dobutamine at a concentration of 10-3 M briefly reduces the speed of Paramecium movement by 0.3 mm/s and also decreases the number of motile cells during the registration period by 40%. On the other hand, the presence of the selective β2-adrenergic receptor agonist terbutaline in the medium significantly and persistently increases the speed of cell movement by 1.2 mm/s. Moreover, the activity of cells in the well increases by 20% relative to intact cells after 15 minutes of registration (Figure. 3a, c). It is noteworthy that dobutamine is more effective at a concentration of 10-5 M than at a concentration of 10-3 M in terms of the speed parameter, while not reducing the number of motile cells in the well (thus, no local cell accumulations are observed). The presentation of terbutaline at a concentration of 10-5 M did not have a strong effect, neither on the speed parameter nor on the activity of Paramecium caudatum cells (Figure. 3b, d).

The divergent effect on the activation of β-adrenergic receptors can likely be explained by the evolutionary differences in these receptors among mammals. The β1-adrenergic receptor predominates in cardiac muscle tissues, and its activation leads to subsequent contraction, while the β2-adrenergic receptor predominates in smooth muscles, as well as in the ciliated epithelium of the lungs and bladder [9]. Activation of β2-adrenergic receptors results in the relaxation of these organs. Additionally, Paramecium caudatum cells, based on their electrophysiological characteristics, resemble cells of the ciliated epithelium in organs such as the trachea and seminal vesicle. This organism often serves as a model in the study of these structures. Activation of β2-adrenergic receptors also stimulates rapid, rhythmic beating of cilia, which subsequently helps propel fluid [10]. Thus, the effects of dobutamine will be analogous to the effects of adrenaline and phenylephrine, while the effects of terbutaline will be distinctly different. In the second part of the study, we investigated the action of α-1 and β-1 adrenergic receptor agonists by introducing methoxamine and phenylephrine at a concentration of 10-3 M against the background of prazosin at a concentration of 10-5 M (Figure. 4a, b), as well as the introduction of dobutamine at a concentration of 10-5 M against the background of atenolol at a concentration of 10-5 M (Figure. 4c, d).

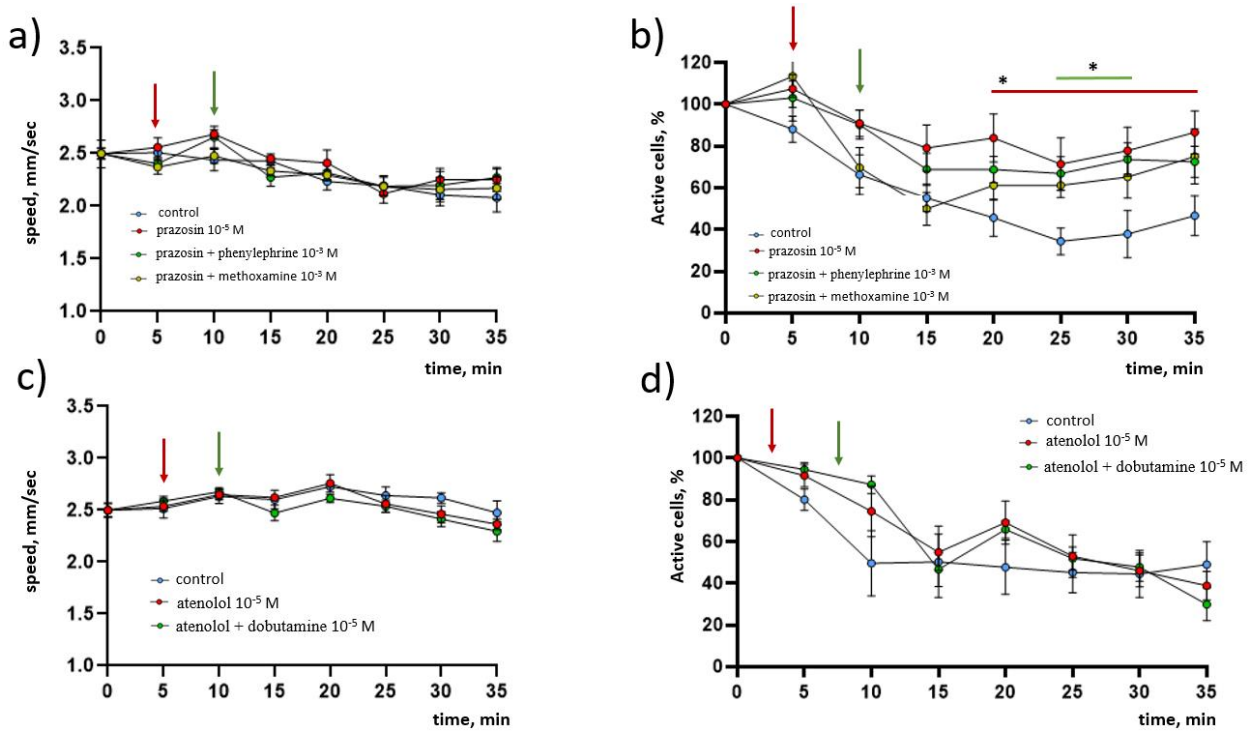

Figure 4: Changes in the movement speed and activity of Paramecium caudatum in response to the presentation of selective α-1 and β-1 adrenergic receptor agonists against the background of the action of selective α-1 and β-1 adrenergic receptor blockers, prazosin, and atenolol, at a concentration of 10-5 M. a – Changes in the average movement speed of cells under the action of methoxamine and phenylephrine at a concentration of 10-3 M against the background of prazosin at a concentration of 10-5 M during 25 minutes of recording. The arrow indicates the moment of adding prazosin to the wells (fifth minute of recording) and the introduction of the corresponding agonists (tenth minute of recording). Cell concentration - 600±50 cells/ml.; b – Changes in the relative number of mobile cells under the action of methoxamine and phenylephrine at a concentration of 10-3 M against the background of prazosin at a concentration of 10-5 M during 25 minutes of recording. The arrow indicates the moment of adding prazosin to the wells (fifth minute of recording) and the introduction of corresponding agonists (tenth minute of recording). Cell concentration - 600±50 cells/ml, * - significance of the difference from the relative number of mobile cells in the intact group, $p < 0.05$, two-way ANOVA; c – Changes in the average movement speed of cells under the action of dobutamine at a concentration of 10-5 M against the background of atenolol at a concentration of 10-5 M during 25 minutes of recording. The arrow indicates the moment of adding atenolol to the wells (fifth minute of recording) and the introduction of dobutamine (tenth minute of recording). Cell concentration - 600±50 cells/ml.; d – Changes in the relative number of mobile cells under the action of dobutamine at a concentration of 10-5 M against the background of atenolol at a concentration of 10-5 M during 25 minutes of recording. The arrow indicates the moment of adding atenolol to the wells (fifth minute of recording) and the introduction of dobutamine (tenth minute of recording). Cell concentration - 600±50 cells/ml.

Despite the fact that the effects of methoxamine are aimed at increasing the movement speed of Paramecia, while phenylephrine, in turn, reduces this parameter, the introduction of these agonists 5 minutes after adding prazosin to the medium results in complete blockade of the effect (Figure. 4a). In addition to correcting the speed of Paramecia to normal values, it is worth noting an increase in the number of mobile cells compared to the intact group (Figure. 4b). This indicates that prazosin equally effectively mitigates the effects of methoxamine and phenylephrine.

When dobutamine was introduced into the medium with cells 5 minutes after the presentation of atenolol, a blocking effect was also observed (Figure. 4c). In this case, the swimming speed of the experimental group's Paramecia does not differ from the movement speed of the intact group. It is also worth noting that the number of mobile cells in the well, both in the group containing atenolol in the medium and in the group receiving dobutamine on the background of atenolol, remains at the level of values in the intact group. The blocking effect of prazosin and atenolol can be explained by the high degree of selectivity of these agonists for the investigated adrenoreceptors and their agonists. [11].

4.Conclusion.

The use of the primary screening method of pharmacological agents on the cellular model of Paramecium caudatum has demonstrated its effectiveness in determining the most active concentration of adrenoreceptor agonists. Analysis of changes in cell motility revealed a divergent effect upon activation of β-1 and β-2 adrenoreceptors in relation to the two investigated parameters. Activation of α-adrenoreceptors leads to a reduction in the number of mobile cells, corresponding to the cellular response to the presentation of adrenaline. Additionally, the study demonstrated that the introduction of selective blockers, prazosin and atenolol, completely blocks the action of selective adrenoreceptor agonists. This suggests the targeted action of these drugs on the investigated adrenoreceptors.

Author Contribution

Gruzdev G. A "conducting an experiment, obtaining and processing data, writing a draft of the article"; Karpukhina O. V. "supervision and

explanation of results"; Inozemtsev A. N. "writing – review and editing"; Povarnina P. Yu "project administration".

Compliance with ethical standards

All applicable international, national, and/or institutional principles for the care and use of animals were followed. This article does not contain any studies with human participants as subjects.

Sources of financing

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Conflict of interest

The authors declare no explicit or potential conflicts of interest associated with the

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