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The Effect of the Bicyclononan Derivative on the Conditioned Reflex of Passive Avoidance in Rats with Cerebral Ischemia

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***Abstract* — The aim of this study was to develop the effect of substance Z–109 - a bicyclononan derivative, presumably belonging to the class of ampakines on the memory of rats undergoing cerebral ischemia. The evaluation of mnestic functions was carried out on the model of the conditioned passive avoidance reflex. Ischemic brain damage was reproduced by 200 in rats of both sexes - occlusion of the middle cerebral artery, photoinduced thrombosis in the prefrontal cortex, complete ligation of both carotid arteries. The test substance was found to have a positive effect at doses of 2.5 mg / kg, 13 mg/kg and 25 mg / kg in the treatment of various types of ischemic brain damage, the substance had a maximum effect at a dose of 2.5 mg / kg. The investigated substance is possible for prospective study as a neuroprotective anti-stroke agent.**

Keywords — bicyclononan, nootropics, memory, cerebral ischemia, antiamnestic effect, rats

I. INTRODUCTION

Acute stroke is one of the leading causes of morbidity and mortality worldwide. According to the WHO, acute cerebrovascular accident (stroke) is the second leading cause of death [1, 2]. In Russia, more than 450,000 strokes are registered annually, of which ischemic account for 80-85% [3, 4]. Pathogenetic therapy of ischemic stroke has two main directions: reperfusion and neuroprotection. Reperfusion in acute focal cerebral ischemia gives the greatest effect in the first minutes of the development of stroke and can be used in the first 3-6 hours from the onset of the disease after mandatory visualization of the nature of the stroke [2, 3]. Unfortunately, its possibilities are small if the time of the therapeutic effect is missed. Neuroprotection can be used both at the prehospital and hospital stages of treatment without the use of neuroimaging and, undoubtedly, is one of the most promising areas of treatment for stroke [3, 4, 5]. Currently, there is a wide arsenal of drugs recommended in the treatment of cerebrovascular disorders, but their effectiveness does not fully meet the requirements of clinicians [1-5].

Therefore, the search for drugs reducing the degree of neurodegeneration and improve mnemonic functions in brain ischemia is an urgent pathogenetic problem.

Many compounds with a different mechanism of action have a neuroprotective effect. Based on the pathogenetic mechanisms of stroke and known data the processes of preserving and restoring the viability of nerve tissue, special attention is paid to substances acting on the glutamatergic system [6-8]. In the works of recent decades, the leading role of glutamate receptors in the processes of information transfer to the central nervous system, their role in the processes of learning, and the formation of memory have been convincingly shown and proved. There is a wide representation of glutamatergic receptors in the hippocampus and the new cortex - structures directly related to the formation, storage and extraction of a trace of memory and learning mechanisms [9]. According to the data of Grigoryev (2009), for the manifestation of the antiamnestic and neuroprotective properties of drugs with a glutamatergic mechanism of action, it is necessary to be an NMDA receptor antagonist or an AMPA receptor agonist or modulator [10].

The creation of substances that are positive modulators of AMPA receptors (PAM AMPA), the so-called “AMPakins” (ampakines), is currently one of the promising directions in the development of psychopharmacology [10, 11]. Our attention was attracted by the substance with the chemical name 6- [4-methoxy-3- (1H-pyrazol-1-ylmethyl) benzyl] -1,11-dimethyl- 3,6,9 - triazatricyclo [7.3.1.1] tetradecane -4.8,12-trion (Z–109) [12] The structure of its molecule was selected by the developers using the molecular modeling method based on the analysis of the spatial structure of the AMPA receptor, its complexes with the known AMPA receptor PAM and the results of their molecular docking [13, 14]. It is assumed that Z–109 belongs to a new class of ampakines - derivatives of 3,7-diazabicyclo [3.3.1] nonane with a tricyclic skeleton, possessing the properties of PAM AMPA.

The aim of this research was to develop the effect of compound Z–109 on the memory of rats with ischemic brain damage.

II. EXPERIMENTAL

The object of the study is a derivative of 3,7-diazabicyclo [3.3.1] nonane. The structural formula of this drug is presented on Fig. 1.

In the work was used a pharmaceutical substance synthesized on the basis of the Chemical Faculty of Moscow State University M.V. Lomonosov.

Piracetam (200 mg / kg) was used as a reference drug. The control group received a suspension of starch in the same volume. The drugs were administered orally. The test substance (at doses of 2.5 mg / kg, 13 mg / kg and 25 mg / kg) and the reference drug Piracetam (200 mg / kg) were administered to experimental animals 4 hours after modeling of the ischemic state and then every 24 hours for 21 days 200 ischemic brain damage was reproduced in rats of both sexes (SD line, weight 180-220 g) under anesthesia (Zoletil 100, 0.05 g / kg intramuscularly). Each group of animals consisted of 10 animals, 5 females and 5 males. All surgical operations were performed under sterile conditions.

Occlusion of the middle cerebral artery (OSMA) was performed using a coagulator proximally to the place of its bifurcation on the frontal and parietal branches. In the field of view of the microscope, a cessation of blood flow through the middle cerebral artery above the site of occlusion was observed [15].

To reproduce one-sided photochemical damage to the cerebral cortex of the rat, the animal's head was fixed in the head holder of the SR-5R stereotactic device (Narishige, Japan). A one-sided focal ischemic focus in the prefrontal (fields Fr1 and Fr2) cortex of rat brain using the atlas G. Paxinos and C. Watson was created by photochemical thrombosis using a TTG001-100 laser with a luminosity of 100 mlV / cm^2. A 532 nm light beam was brought to the skull surface at a distance of 0.5 cm. Immediately prior to irradiation, anesthetized animals were injected intravenously (into the jugular vein) with a 3% solution of the Bengal Pink dye, at a dose of 40 mg / kg. The exposure time was 15 minutes. As a result of the interaction of the fluorescent dye with the light beam, free oxygen is released, which damages the vascular endothelium, which leads to adhesion and aggregation of platelets, the formation of blood clots and

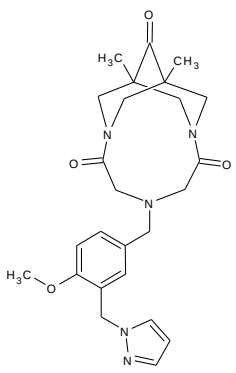

Fig. 1. The chemical structure of compound Z–109 according to IUPAC6-[4methoxy-3- (1H-pyrazol-1-ylmethyl) benzyl] -1,11 dimethyl-3,6,9-triazatricyclo [7.3.1.1] tetradecane-4.8, 12-trion.

impaired local blood flow. False operation was carried out according to the same scheme, with the exception of intravenous administration of the dye "Bengal pink" [16, 17].

Global cerebral ischemia was performed by complete ligation of both animal carotid arteries [17].

To create a reperfusion model of ischemic damage, clips were applied to both common carotid arteries for 1 hour, after which the blood flow to the common carotid arteries was restored, achieving reperfusion.

To evaluate the cognitive functions of rats, animals were trained 2 hours before the intervention for the conditioned passive avoidance reaction [17]. The reflex was reproduced on days 1 and 21 after modeling ischemia. In this test, we used an installation consisting of two compartments: darkened and illuminated, connected by a door. During training, the animal was placed in a bright compartment (tail to door). As soon as the animal passed into the dark compartment, the door was closed, and in the compartment along the mesh metal floor an electric current was applied to the paws, with a force of 0.5 mA and duration of 3 seconds. After that the door was opened. Playing the test, the latent period was measured - the period when the animal passively avoided the dark compartment. During the day, animals were trained. A control test was performed 24 hours after training. The animal was in the installation for 180 seconds. All manipulations with experimental animals were performed in accordance with regulatory documents relating to the humane treatment of animals and standard operating procedures (SOP) of the Sechenov University Center for Preclinical Studies. The conduct of experiments with animals was approved by the Commission on Biomedical Ethics of the Sechenov First Moscow State Medical University.

The obtained data were processed statistically.

III. RESULTS AND DISCUSSION

None of the models showed any differences between the indicators of the state of males and females, therefore, the article presents the average data for each group. Occlusion of the middle cerebral artery (OSMA) allows obtaining indices of focal ischemic damage to the brain necessary for experimental evaluation [17]. When simulating OSMA, all animals that underwent surgery lived for more than 21 days. On the first day after the operation, control animals with OSMA were in the bright compartment for 46.9 ± 3.4 sec., which indicates a loss of reflex and a decrease in memory. Animals receiving piracetam before the operation better remembered the developed reflex: they left the light compartment after 75.6 ± 1.8 sec. Compound Z–109 contributed to memory preservation: when the substance was administered in doses of 2.5 mg / kg, 13 mg / kg and 25 mg / kg, the latent time in all groups was increased and amounted to 117.4 ± 3.7 sec., 79.9 ± 2.3 sec. and 57.9 ± 2.8 sec., respectively ($p < 0.05$ for all groups). None of the animals with OSMA, both in the control and in the experimental groups, retained the conditioned reflex in full. Passive avoidance reaction is a relatively simple defensive reaction, with the help of which it is possible to study the basic laws of the formation, storage and reproduction of conditioned reflexes [17].

After 21 days' surgery, the cognitive functions of control rats partially recovered: the time spent in the bright compartment increased by 8% and amounted to 50.6 ± 1.6 sec., but this change was not statistically significant. Against the background of the introduction of piracetam, memory recovery was more successful: on the 21st day after the operation, the animals were in the bright compartment for 84.2 ± 2.9 sec., that is 11% longer ($p < 0.05$). Against the background of the administration of compound Z–109 at a dose of 25 mg / kg, the residence time in the bright compartment increased by 47%, amounting to 85.2 ± 3.5 sec. ($p < 0.05$). Against the background of the administration of compound Z–109 at a dose of 13 mg / kg 21 days after surgery, the rats left the light compartment after 122.7 ± 4.2 sec., that is, they were 1.5 times longer in it than on the first day after modeling OSMA and 2.4 times longer than the animals of the control group ($p < 0.05$). The animals were the longest in the bright compartment, receiving compound Z–109 at a dose of 2.5 mg / kg - 151.5 ± 4.6 sec., which was 3 times higher than the corresponding indicator of the control group ($p < 0.05$). However, the time spent in the light part increased by only 29%, possibly because it was in this group that the best results were observed on the first day after the operation (Fig. 2).

The method of photostimulated cerebral vascular thrombosis (Watson B. et al, 1985) differs from OSMA in that the animal's brain not only undergoes local ischemia, but also reproduces some of the pathogenetic factors of stroke in humans (endothelial function disorders, changes in blood coagulation and others). The prefrontal cortex, together with the hippocampus, plays a key role in learning and memory processes, and its local damage leads to selective impairment of cognitive functions of the brain without concomitant disorders of muscle tone, motor coordination and general animal behavior [18]. Therefore, it was interesting to evaluate the effect of compound Z–109 on this model.

Studying memory in animals subjected to photoinduced thrombosis, results were obtained (Fig. 3), and similar to those observed on the OSMA model. A one-sided focal ischemic focus in the prefrontal cortex of the brain caused significant memory impairment in animals. On the first day, all rats rushed into the dark compartment, the latent time was 43.8 ± 1.4 sec. The memory was gradually recovering, 21 days after thrombosis, the time spent in the bright compartment increased by 26% ($p < 0.05$) to 55.4 ± 1.3 sec. Thus, the restoration of mnemonic abilities of animals after photoinduced thrombosis occurred to a greater extent than in animals with OSMA.

With the introduction of piracetam, the process of extracting information improved: the time spent in the bright compartment on days 1 and 21 after thrombosis was longer than the control ($p < 0.05$) and amounted to 65.3 ± 1.9 sec. and 79.0 ± 2.0 sec. respectively. Thus, the introduction of piracetam for 21 days contributed to an increase in the latent period by 20%.

The administration of Z–109 to animals after photochemical thrombosis also improved the process of reproducing the memorial trail. Against the background of administration of a substance at a dose of 25 mg / kg to rats in the acute period, on the first day, the animals were in the bright compartment for 70.3 ± 1.5 sec., while using the substance at a dose of 13 mg / kg - 76.5 ± 3.2 sec. and at a dose of 2.5 mg / kg - 126.4 ± 3.8 sec. In all three groups, an improvement was observed after three weeks. So, against the background of the introduction of the substance Z–109 at a dose of 25 mg / kg on the 21st day, the rats did not enter the dark compartment for 100.8 ± 4.5 sec. (an improvement of 43% compared with the data on day 1). Daily administration of Z–109 at a dose of 13 mg / kg contributed to an increase in latent time by 33% (up to 102.5 ± 3.6 sec.). Against the background of the administration of the substance Z–109 at a dose of 2.5 mg / kg, the residence time in the bright compartment increased by 28%, but the final indicator was the best: 160.9 ± 1.1 sec. Nevertheless, the latent time during treatment was better than the control for all groups ($p < 0.05$), therefore, the severity of cognitive deficit in animals with focal ischemia treated with substance Z–109 was less.

In the next part of the experiments, the memory status of rats undergoing brain ischemia was studied, which was reproduced by bilateral occlusion of the carotid arteries (Fig. 4). The damage caused was more significant than in previous experiments, which was accompanied by significant disturbances of conditioned reflexes worked out. This may indicate not only cognitive impairment, but also an increase in anxiety in animals with cerebral ischemia. After 21 days, the memory of animals that did not receive treatment did not improve: they left the light compartment almost at the same time, after 26.5 ± 2.9 sec.

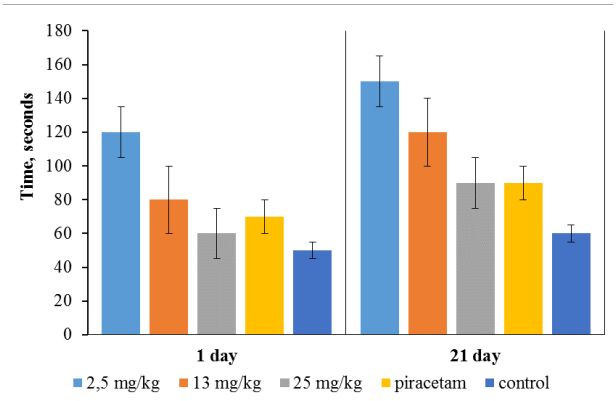

Fig. 2. The time spent in the bright compartment when checking the conditioned reflex of passive avoidance in animals undergoing occlusion of the middle cerebral artery.

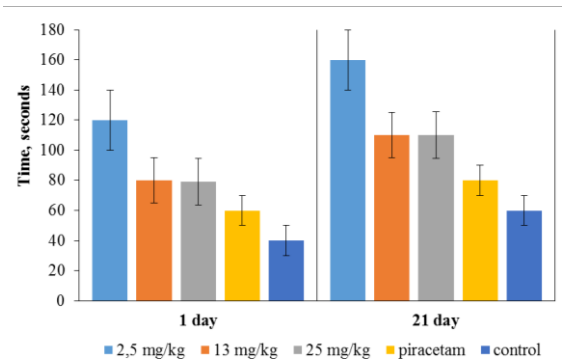

Fig. 3. The time spent in the bright compartment when checking the conditioned reflex of passive avoidance in animals undergoing photoinduced thrombosis.

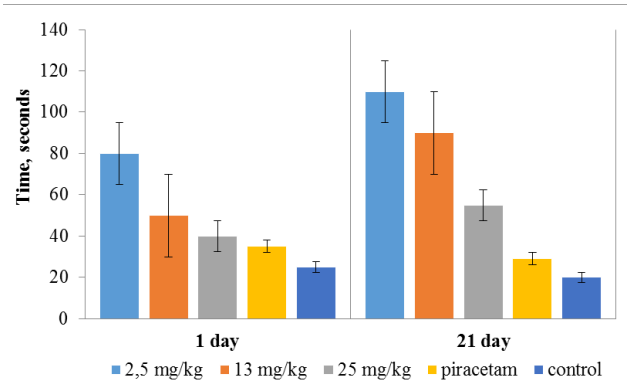

Fig. 4. Time spent in the bright compartment when checking the conditioned reflex of passive avoidance in animals undergoing bilateral occlusion of the carotid arteries.

The use of piracetam for the treatment of animals on the first day increased the time spent in the bright compartment by 18% (35.0 ± 1.4 sec., the difference from control was p <0.05). Over the course of three weeks, the reflex not only did not recover under the influence of piracetam, but also faded away, reaching by day 21 parameters that were not distinguishable from the control group (29.3 ± 1.5 sec.).

Against the background of substance Z–109 at a dose of 25 mg / kg, on the first day the rats reproduced the reflex better than in the control: the residence time in the bright compartment was 40.0 ± 1.5 sec., which is 1.35 times longer than in the control animals. The indicators did not improve by day 21, but did not deteriorate: the latent time was 44.0 ± 2.6 sec. Using Z–109 at a dose of 13 mg / kg on the first day, rats remained outside the dark compartment longer than in the control, 1.7 times (51.2 ± 2.1 sec., p <0.05). This group of animals after 21 days was in the bright compartment for a longer time than on the first day - 80.0 ± 3.3 sec., that is 56% more (p <0.05). Even more pronounced was the effect of the substance Z–109 at a dose of 2.5 mg / kg. On the first day, the rats were in the bright zone for 80.7 ± 2.8 sec., that is 2.7 times longer than control animals. The rats refrained from entering the dark compartment on day 21 for 110.1 ± 4.3 sec.

IV. Conclusion

Studying the properties of the drug Z–109, it was found that it eliminated cognitive deficit in animals that simulated various types of ischemic brain damage. The maximum effect was observed in the study drug group at a dose of 2.5 mg / kg. The antiamnestic effect of substance Z -109 at a dose of 2.5 mg / kg significantly exceeded the antiamnestic effect of piracetam (200 mg / kg). The basis of the pathogenesis of cognitive impairment in ischemia is damage and degeneration of neurons, leading to learning and memory disorders. Probably, the substance Z -109 promotes faster recovery of neurons, thereby exerting a neuroprotective effect. Given the peculiarities of the chemical structure, the substance Z–109, created by molecular modeling, is believed to be a positive modulator of AMPA receptors for the glutamatergic system. Apparently, affecting these receptors, Z -109 promotes the activation of neuronal processes in the ischemic zone and prevents the development of pathological changes that lead to impaired reproduction of the memory area.

One of the properties of stimulation of AMPA receptors is the so-called synaptic plasticity, the result of which is the effect of prolonged potentiation [19, 20]. In addition, AMPKins are able to cause depolarization of the postsynaptic membrane, significantly increasing the expression of neurotrophic factors - nerve growth factor NGF (nerve growth factor) and brain neurotrophic factor BDNF (brain-derived neurotrophic factor). This, in turn, is associated with improved cognitive functions and is a powerful mechanism for the restoration of nerve cells [13].

Presumably, using of compound Z–109 for neuroprotection patients with ischemic stroke is able to more quickly restore the integrative activity of the brain, as evidenced by the studies.

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