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Creation of a New Combined Drug Based on Gamma-Aminobutyric Acid and Thiotriazoline

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Abstract

General anesthesia, especially ketamine, can cause damage to the central nervous system (CNS) in the postoperative period, among which a special place is occupied by postoperative cognitive dysfunction (POCD), which can develop in patients of different age groups, including in cases with an unburdened neuropsychiatric history. Neuroprotective therapy becomes essential for preventing neuronal damage or eliminating already existing cognitive dysfunction in the early postoperative period, when these changes are still potentially reversible. Influencing the course of cerebral metabolism, it prevents or interrupts pathological cascades that cause dysfunction or death of neurons.

A special place in neuroprotection is occupied by neurometabolic drugs - nootropics. Studies in neurochemistry have established that the use of general anesthetics leads to disruption of the GABA-ergic system of the brain, a decrease in the affinity of GABA receptors. All this directs the search for pharmacologists in the development of nootropics effective in POCD towards structural analogs of gamma-aminobutyric acid (GABA). Thiotriazoline, being an antioxidant and anti-ischemic agent, is able to regulate ROS/NO-dependent mechanisms of cellular signaling, the affinity of central receptors, incl. and GABA. Therefore, it is important to develop a combination drug based on GABA and thiotriazoline, which will have significantly higher efficacy in the correction of cognitive dysfunction after ketamine anesthesia, as well as demonstrate a significant reduction in side effects.

Objectives: to study the pharmacological action of a combination drug based on GABA with thiotriazoline in the correction of cognitive dysfunction after ketamine anesthesia.

Materials and methods: the substance GABA and thiotriazoline was used in the study. The study was carried out on 80 white outbred rats weighing 180-190 grams of both sexes. Ketamine anesthesia was carried out by intraperitoneal administration of 100 mg/kg ketamine. The studied drugs were administered in the following doses: a combination of GABA + thiotriazoline 1:1; 2:1; 3:1; 4:1; 5:1, piracetam 500 mg/kg, intragastrically.

Results. The administration of piracetam (500 mg/kg) did not have a significant effect on such indicators of the cognitive-mnestic functions of animals as errors in the reference memory, as well as on the indicator of general activity when examining the labyrinth on day 10 after ketamine anesthesia. The administration of combinations of GABA with thiotriazoline (1:1; 2:1; 3:1; 4:1 and 5:1) had a significant effect on the cognitive-mnestic functions of the central nervous system after ketamine anesthesia. Thus, the studied combinations of GABA with thiotriazoline (1:1; 2:1; 3:1; 4:1 and 5:1) exhibit pronounced nootropic and antiamnestic effects, increasing the general activity during learning and reducing the number of errors in the reference and working memory during reproducing learning skills. Findings: Thus, after analyzing the results of the conducted studies, it was found that in terms of the efficiency, all combinations of GABA with thiotriazoline significantly exceed the reference drug – piracetam. The most effective combinations were GABA + thiotriazoline in ratios of 3:1 and 4:1, respectively.

Keywords: gamma-aminobutyric acid, thiotriazoline, postoperative cognitive dysfunction, neuroprotection

Introduction

General anesthesia, especially ketamine, can cause damage to the central nervous system (CNS) in the postoperative period, among which a special place is occupied by postoperative cognitive dysfunction (POCD), which can develop in patients of different age groups, including in cases with an unburdened neuropsychiatric history [3, 7, 9, 15]. The frequency of POCD, according to different authors, averages 36.8%, and, for example, after cardiac surgery - up to 47% (in 42% of patients even 3-5 years after surgery). The most vulnerable to the action of general anesthetics are attention function, short-term memory, psychomotor and cognitive response rate [6]. At the same time, only a few works in the literature are devoted to the use of neuroprotective drugs for the prevention and treatment of neurocognitive disorders in the postoperative period [8, 13]. Neuroprotective therapy, along with the choice of an adequate option for anesthesia, timely correction of hemodynamic disturbances, gas exchange and homeostasis, becomes essential for preventing neuronal damage or eliminating already existing cognitive dysfunction in the early postoperative period, when these changes are still potentially reversible [12-14]. Influencing the course of cerebral metabolism, it prevents or interrupts pathological cascades that cause dysfunction or death of neurons.

A special place in neuroprotection is occupied by neurometabolic drugs - nootropics. Nootropics have a direct activating effect on learning, improving memory and mental activity, as well as increasing the brain's resistance to the aggressive effects of hypoxia, trauma, and intoxication [10]. Studies in neurochemistry have established that the use of general anesthetics leads to disruption of the GABA-ergic system of the brain, a decrease in the affinity of GABA receptors. All this directs the search for pharmacologists in the development of nootropics effective in POCD towards structural analogs of gamma-aminobutyric acid (GABA). Thiotriazoline, being an antioxidant and anti-ischemic agent, is able to regulate ROS/NO-dependent mechanisms of cellular signaling, the affinity of central receptors, incl. and GABA. Therefore, the development of a combination drug based on GABA and thiotriazoline, which will have significantly higher efficiency in the correction of cognitive dysfunction after ketamine anesthesia, as well as demonstrate a significant decrease in side effects - agitation, insomnia, reduce the risks of hypertensive crisis, transient ischemic attacks, epileptiform seizures, aggressiveness, aggression, is relevant and timely [1, 5, 11].

Aim of the work: to study the pharmacological action of a combination drug based on GABA with thiotriazoline in the correction of cognitive dysfunction after ketamine anesthesia.

Materials and methods

we used the GABA substance, manufactured by Sigma-Aldrich, USA (series 11-021), and the substance of thiotriazoline (manufacturer: State Enterprise “Chemical Reagents Plant” of the Scientific and Technological Complex of the Institute of Single Crystals of the National Academy of Sciences of Ukraine, series 2451117). The study was carried out on 80 white outbred rats weighing 180-190 grams of both sexes. The animals were kept under standard vivarium conditions (12-hour light cycle, temperature 22°C). For the experiments, the animals were subjected to food deprivation, the features of which are described below. In order to tame the rats, before the start of the experiment, they were held in hands for 2–3 min. within 5 days, that facilitated subsequent experimental studies [2].

Ketamine anesthesia was carried out by intraperitoneal administration of 100 mg/kg ketamine. After the anesthesia recovery, in parallel with training in the labyrinth, the studied drugs were injected for 10 days in the following doses: a combination of GABA+thiotriazoline (1:1) - 250 mg/kg, a combination of GABA+thiotriazoline (2:1) - 250 mg/kg , a combination of GABA+thiotriazoline (3:1) - 250 mg/kg, a combination of GABA+thiotriazoline (4:1) - 250 mg/kg, a combination of GABA+thiotriazoline (5:1) - 250 mg/kg, piracetam - 500 mg/kg, intragastrically. The control and intact groups received intragastrically water with Tween-80 for 10 days. The drugs were injected in the form of an aqueous

suspension with Tween-80 using a metal probe once a day in 60 min. before the training session.

Results

The experimental study was carried out on animals in accordance with Directive 2010/63EU of the European Parliament and of the Council as of September 22, 2010 ‘On the Protection of Animals Used for Scientific Purposes”, as well as the national "General Ethical Principles of Animal Experiments" (Ukraine, 2001) and guidelines described in "Preclinical Study of the Specific Activity of Potential Drugs of Primary and Secondary Neuroprotection" (State Pharmacological Center of Ukraine, K., 2016). The experiment was approved by the Bioethics Commission of Zaporozhye State Medical University.

During the training period, the rats were subjected to food deprivation. Food was available daily for 1 hour. The animals were brought to 85% of the original weight by restricting the food diet with free access to water. Memory studies were performed using the radial labyrinth LE760 (AgnTho's, Sweden). The eight-beam radial labyrinth consists of an octagonal platform (side length 22 cm), from which there are radial tracks numbered from 1 to 8, 70 cm long and 10 cm wide, with recesses for feeders at the end (diameter 2 cm, depth 1.5 cm). Each track can be closed independently with a guillotine mechanism. The entire installation was located at a height of 70 cm from the floor. The study was carried out in complete silence. Starting from the first day, the animals were placed in the central area of the labyrinth with 4 closed beams and 4 open beams, in the feeders of which 200 mg of food granules were placed. The combination of open and closed beams was individual and constant for each animal. Over the next 10 days, the animal was trained to find food using external visual cues. Training was carried out for 10 minutes or until the animals found all four food sources. The experiment was repeated twice daily with each animal. After the experiment, the animal received a daily food ration. On day 10, the animal was placed in a radial labyrinth with eight open beam-tracks, in 4 of which food was placed according to the usual pattern for the animal. We assessed the reference memory (the general long-term idea of the structure of the labyrinth and the location of food, which was formed in the animal during the training process) and the number of errors in the reference memory (the first visit to a previously closed beam in which the animal never found food), as well as working memory (short-term representation of the animal about the location of food in a specific experiment) and the number of errors in working memory (re-visit to the beam in which the animal has already found or did not find food). In addition, we assessed the distance traveled and overall motor activity.

The experiments were carried out in a well-lit room in complete silence. During the experiments, the influence of external and internal visual, olfactory and auditory stimuli was excluded. The assessment of the animal behavior was carried out by a laboratory assistant who was not aware of the animal's belonging to a particular experimental group. Image capture and recording was carried out

using an SSC-DC378P color video camera (Sony, Japan). The video file was analyzed using Smartv 3.0 software (Harvard Apparatus, USA). Statistical processing of the results was carried out using Microsoft Excel 2016 with the AtteStat 12 statistical processing package. To assess the reliability of differences in the study groups, the Kruskal-Wallis test with Dunn's correction was used. Differences were considered significant at $p < 0.05$.

When evaluating specific indicators of training in the radial labyrinth, it was found that the animals showed cognitive dysfunction 10 days after ketamine anesthesia. The total activity of the animals in the control group did not differ significantly compared with the intact group. When reproducing the results of training animals, it was found that on the 10th day after the introduction of ketamine, the number of errors in working memory increased by 4 times, and the number of errors in reference memory increased by 2.5 times, which indicated a violation of mnestic functions in animals after ketamine anesthesia. Our findings fit into the concept of postoperative cognitive dysfunction. Ketamine anesthesia leads to the formation of persistent cognitive deficits, as well as psycho-emotional disorders - block, fear, anxiety, disorientation, aggressiveness, irritability.

The administration of the studied combinations of GABA with thiotriazoline (1:1; 2:1; 3:1; 4:1 and 5:1) and the reference drug, piracetam, immediately after the anesthesia recovery, had different effects on the indicators of the cognitive-mnestic functions of the central nervous system. (Table 1).

Table 1

Effect of the studied combinations of GABA with thiotriazoline (1:1; 2:1; 3:1; 4:1 and 5:1) and piracetam on training and memory in rats after ketamine anesthesia in the radial labyrinth

Experimental groups	**Total activity, cm2/s**	**Number of reference memory errors**	**Number of working memory errors**
Intact (п=10)	24380,98±1242,43	2±0	4±1
Control (ketamine anesthesia) (п=10)	26867,58±1543,58	5±0	16±1
ketamine anesthesia + GABA+thiotriazoline (1:1), 250 mg/kg (п=10)	42877,75±1186,52* +59,6%	3±0 --40%	10±1*[1] -37,5%
ketamine anesthesia + GABA+thiotriazoline (2:1), 250 mg/kg (п=10)	44811,55±1081,15* +66,8%	2±0*[1] -60%	7±1*[1] -53,3%
ketamine anesthesia + GABA+thiotriazoline (3:1), 250 mg/kg (п=10)	34644,12±1271,31 +28,9%	1±0*[1] -80%	5±1*[1] -66,7%

Table 1 (continued)

Effect of the studied combinations of GABA with thiotriazoline (1:1; 2:1; 3:1; 4:1 and 5:1) and piracetam on training and memory in rats after ketamine anesthesia in the radial labyrinth

ketamine anesthesia + GABA+thiotriazoline (4:1), 250 mg/kg (n=10)	30572,72±1422,03 +13,8%	1±0*[1] -80%	4±1*[1] -73,3%
ketamine anesthesia + GABA+thiotriazoline (5:1), 250 mg/kg (n=10)	29412,39±2082,07 +9,47%	2±0*[1] -60%	6±1*[1] -60%
ketamine anesthesia + piracetam, 500 mg/kg (n=10)	35511,10±1412,11 +32,1%	4±0 -20%	13±1* -13,4%

Notice:

[*] *– significant difference (p <0.05) compared with the control group*

[1] *– significant difference (p <0.05) compared with the Piracetam group*

Discussions

The administration of piracetam (500 mg/kg) did not have a significant effect on such indicators of the cognitive-mnestic functions of animals as errors in the reference memory, as well as on the indicator of general activity during the examination of the labyrinth on day 10 after ketamine anesthesia. At the same time, the administration of piracetam significantly reduced the number of working memory errors by 13.4%. All of this demonstrates the ineffectiveness of using piracetam to reduce cognitive dysfunction after ketamine anesthesia. The introduction of combinations of GABA with thiotriazoline (1:1; 2:1; 3:1; 4:1 and 5:1) had a significant effect on the cognitive-mnestic functions of the central nervous system after ketamine anesthesia. Thus, the administration of a combination of GABA+thiotriazoline (1:1) led to a significant increase by 59.6% (which indicates the disinhibition effect of a 1:1 combination) in the overall exploratory activity and a significant decrease in working memory errors by 37.5%, without showing a significant influence on the number of reference memory errors. The administration of GABA+thiotriazoline (2:1) led to a significant 66.8% increase in the total exploratory activity (which indicates an increase in the disinhibition effect of the 2:1 combination) and a significant decrease in working memory errors by 53.3% and 60% reference memory errors. Administration of GABA+thiotriazoline (3:1) did not lead to a significant change in the overall exploratory activity (it decreased compared to the group receiving the combination of GABA+thiotriazoline 1:1 and 2:1 (possibly due to an increase in the inhibitory ingredient - GABA), but more significantly reduced the number of errors in working memory - by 66.7% and the number of errors in reference

memory - by 80%. The introduction of GABA+thiotriazoline (4:1) also did not lead to a significant change in the total exploratory activity (due to an increase in the combination inhibitory ingredient - GABA), but more significantly reduced the number of errors in working memory - by 73.3% and the number of errors in reference memory - by 80%. The introduction of a combination of GABA+thiotriazoline (5:1) also did not lead to a significant change in the overall exploratory activity (due to the increase in the combination of the inhibitory ingredient - GABA), but significantly reduced the number of errors in working and reference memory - by 60%. possible combinations of GABA with thiotriazoline (1:1; 2:1; 3:1; 4:1 and 5:1) exhibit pronounced nootropic and antiamnestic effects, increasing overall activity during training and reducing the number of errors in reference and working memory when reproducing training skills. In terms of the strength of action, all combinations reliably surpass the reference drug - piracetam.

Findings: Thus, after analyzing the results of the studies carried out, it was found that the proposed combination agent provides significantly higher activity in comparison with known agents in relation to the correction of cognitive disorders after ketamine anesthesia. The combination of GABA with thiotriazoline in a 1:1 ratio; 2:1; 3:1; 4:1 and 5:1, respectively, exhibits pronounced nootropic and antiamnesic effects; the most effective were the combinations of GABA+thiotriazoline in ratios of 3:1 and 4:1, respectively.

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