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SEMAX INTRANASAL IMPROVE MEMORY DEFICIENCY IN CONDITIONS OF EXPERIMENTAL CHRONIC BRAIN ISCHEMIA

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Abstract

Statistical indexes of the incidence of acute cerebrovascular accidents in our country highlight the negative dynamics of the progressive increase in the number of new cases of strokes. It is interesting, promising and expedient to clarify the effectiveness of endonasal administration of the peptide drug Semax in the dynamics of the post-ischemic period from the point of view of the potential restoration of impaired mnestic functions. The purpose of the work was the determination of the effectiveness of endonasal administration of Semax in restoring memory impairment in rats in the dynamics of the post-ischemic period. A model of chronic brain ischemia was reproduced by isolating and bilaterally ligating the carotid arteries. Rats with chronic brain ischemia were administered Semax endonasally. The rats were observed for 7 days, during which the animals were examined for mnestic functions under the conditions of formation of a conditioned active avoidance reflex and a conditioned food reflex. The obtained data indicate the formation of pronounced mnemic dysfunctions in the dynamics of the postischemic period, which

are characterized by a deterioration in the learning process, as well as a pronounced suppression of short- and long-term memory under the conditions of the conditioned reflex of active avoidance, which is combined with violations of the processes of learning, preservation and extinction of the food conditioned reflex. The noted amnestic effects are persistent, are registered in rats already 24 hrs after bilateral occlusion of the carotid arteries and continue throughout the experiment. Considering the mechanisms of implementation of the neuroprotective effect of Semax and the endonasal route of its administration, the authors consider it appropriate and pathogenetically justified to include Semax in a comprehensive scheme of pharmacological correction of cognitive disorders or prevention of their development in cerebrovascular pathology.

Key words: chronic brain ischemia; cognitive disorders; semax; conditioned reflexes; short-term memory; long-term memory; pathogenetic mechanisms; pharmacological correction

Statistical indexes of the incidence of acute cerebrovascular accidents in our country highlight the negative dynamics of the progressive increase in the number of new cases of strokes [1, 9]. According to such indexes, our country ranks among the first in Europe, which generally highlights the global trend of "cerebrovascular catastrophe", which was declared by WHO at the end of the last century. Today, patients with strokes that occurred as a result of acute cerebrovascular accidents constitute the vast majority of all patients with cerebrovascular pathology [10]. Therefore, from a social, economic and medical point of view, the tasks of treating this contingent of patients, their full rehabilitation with the restoration of adequate physiological needs of motor, sensory, cognitive and other functions of the body and preventing the development of complications of strokes [6, 10].

We conducted certain experimental studies aimed at helping to restore lost or distorted behavioral reactions during the postischemic period [3]. It is important when organizing such studies to imagine the complexity of pharmacological drugs penetration through the blood-brain barrier to provide a direct effect, which forced us to investigate the possibility of alternative routes of access of drugs to the site of damage. The existing positive experimental and clinical results of the endonasal route of drug administration [4, 7] interested us from the point of view of their promising effectiveness under the conditions of chronic brain ischemia (CBI). Therefore, taking into account the above, for the probable activation of sanogenetic mechanisms in the CBI

complex pathogenetic manifestation, we consider it interesting, promising and expedient to clarify the effectiveness of endonasal administration of the peptide drug Semax in the dynamics of the postischemic period from the point of view of potential restoration of impaired mnestic functions.

The aim of the work is to determine the effectiveness of Semax endonasal administration in memory impairment restoring in rats throughout the post-ischemic period.

Material and methods

The experiments were conducted under chronic experimental conditions on 62 male Wistar rats weighing 180-250 g, which were kept in vivarium conditions. The maintenance, handling and manipulation of animals were carried out in accordance with the "General Ethical Principles of Animal Experiments" approved by the Fifth National Congress on Bioethics (Kyiv, 2013), while being guided by the recommendations of the European Convention for the Protection of Vertebrate Animals for Experimental and Other Scientific Purposes (Strasbourg, 1985), the methodological recommendations of the State Research Center of the Ministry of Health of Ukraine "Preclinical Studies of Drugs" (2001) and the rules for the humane treatment of experimental animals.

Chronic brain ischemia was reproduced by skin incision, isolation and bilateral ligation of the carotid arteries [3]. The following groups of animals were distinguished: group 1 - control (intact rats, in which only the skin was dissected, and the carotid arteries were not ligated, n=7). group 2 - experimental (rats with carotid artery ligation and with reproduction of CBI, n=12). Rats of the 3rd group with CBI were administered Semax (0.1%, endonasally, 10 μl, n=12).

Rats were observed for 7 days after carotid artery ligation. During the specified postischemic period, the animals' mnestic functions were investigated in two tests – in conditioned active avoidance (CAAR) [16] and in conditioned food reflexes [12].

The obtained results were statistically calculated using the parametric one-way ANOVA criterion. The minimum statistical probability was determined at $p<0.05$.

Results

A pronounced deterioration of learning processes was recorded in rats, in the dynamics of the post-ischemic period, as well as the severity of short-term and long-term memory, which was manifested by a significant increase in the number of combinations of conditioned and

unconditioned stimuli necessary for the occurrence of URAU (Fig. 1), its reproduction after 1 day (Fig. 2) and after 7 days (Fig. 3) from the moment of formation.

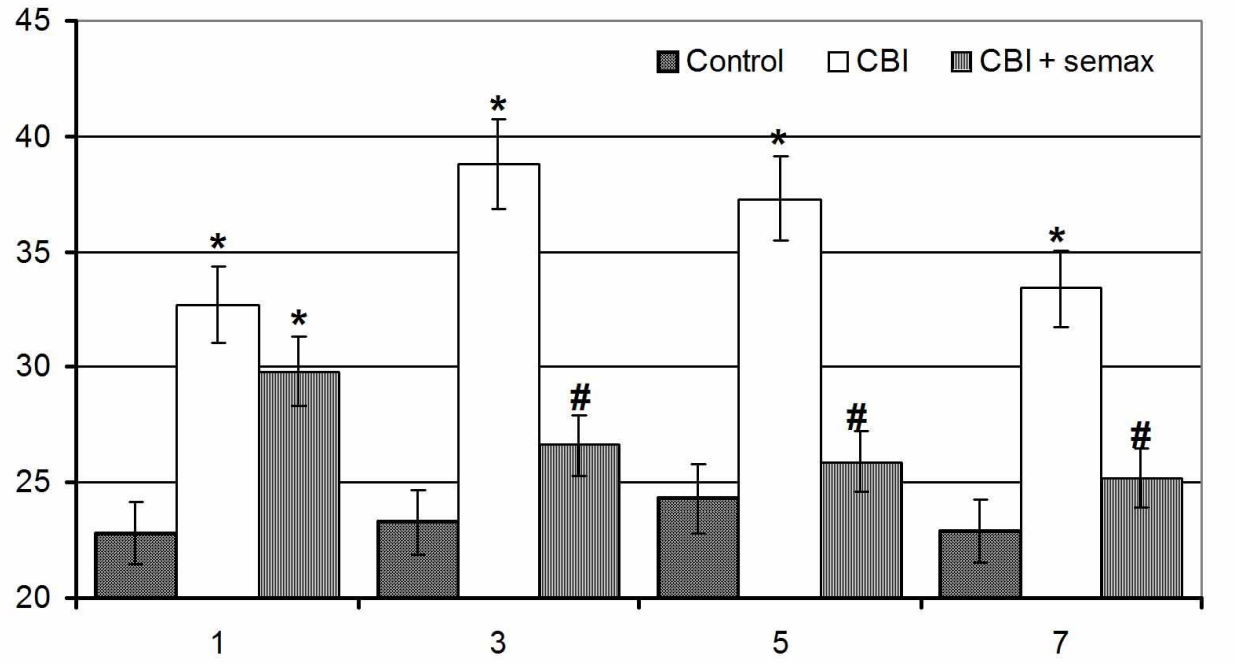

Fig. 1. The influence of Semax endonasal administration on the expression of learning processes in rats in the dynamics of the post-ischemic period

Designations: along the abscissa axis - observation days (duration of the experiment).

Along the ordinate axis - the number of conditioned and unconditioned stimuli combinations.

Notes: * - $p<0.05$ - significant differences of investigated indexes vs the same data in control observations;

- $p<0.05$ - significant differences of investigated indexes vs the same data in rats with chronic brain ischemia without pharmacological correction.

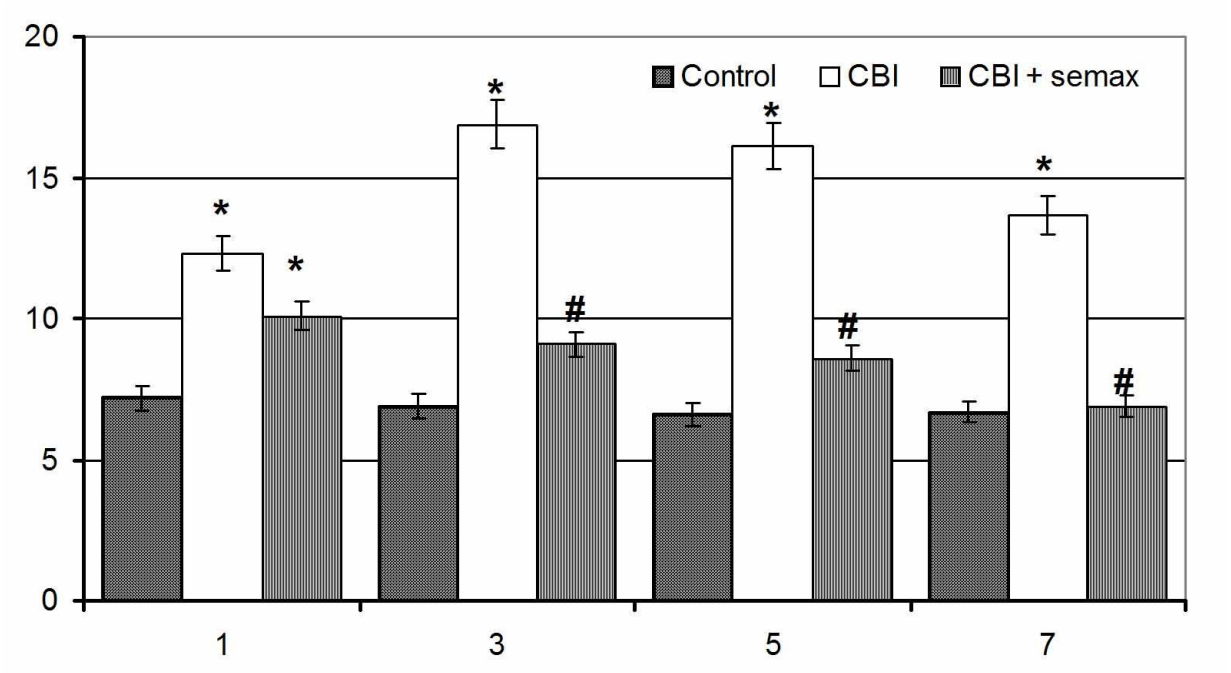

Fig. 2. The influence of Semax endonasal administration on short-term memory characteristics in rats in the dynamics of the post-ischemic period

Designations: along the abscissa axis - observation days (duration of the experiment).

Along the ordinate axis - the number of conditioned and unconditioned stimuli combinations.

Notes: * - $p<0.05$ - significant differences of investigated indexes vs the same data in control observations;

- $p<0.05$ - significant differences of investigated indexes vs the same data in rats with chronic brain ischemia without pharmacological correction.

Thus, on the 1st day of CBI, the value of the studied index necessary for conditioned reflex formation (learning process) exceeded the corresponding index in control group by 43.2% ($p<0.05$; Fig. 1). The number of both conditioned and unconditioned stimuli combinations necessary for CAAR occurrence after 1 day and 7 days from the moment of this conditioned reflex combinations was 70.8% and twice as high, respectively, as in the control (in both cases $p<0.05$; Fig. 2, 3).

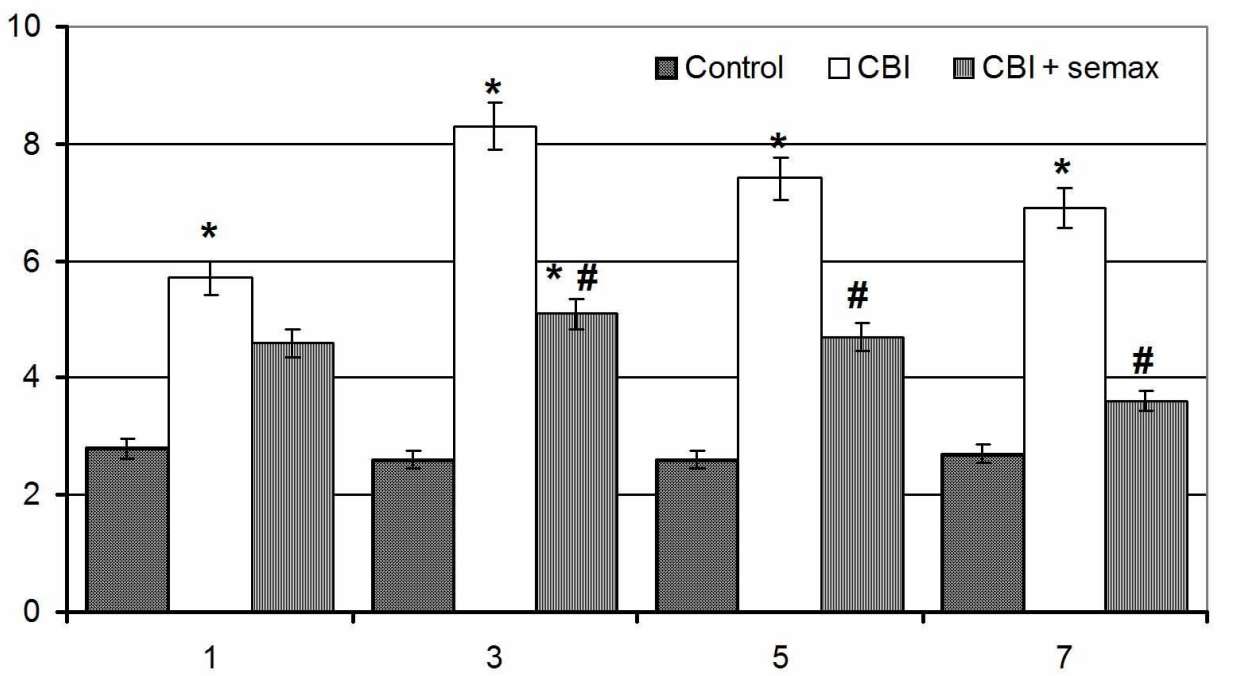

Fig. 3. The influence of Semax endonasal administration on longt-term memory characteristics in rats in the dynamics of the post-ischemic period

Designations: along the abscissa axis - observation days (duration of the experiment).

Along the ordinate axis - the number of conditioned and unconditioned stimuli combinations.

Notes: * - $p<0.05$ - significant differences of investigated indexes vs the same data in control observations;

- $p<0.05$ - significant differences of investigated indexes vs the same data in rats with chronic brain ischemia without pharmacological correction.

Starting from the 3rd day of the trial, the value of the studied index of learning, short- and long-term memory in rats with CBI, which underwent Semax endonasal administration, was comparable to such data in the control, and was also significantly less when compared with similar indexes in rats with CBI without pharmacological correction ($p<0.05$; Fig. 1).

When studying cognitive dysfunctions in rats with CBI in the food conditioned reflex test, it was found that attempts to enter the rays of the maze and successfully localize food during training were effective only on the 3rd day of the postischemic period (Fig. 4), and when studying the conditioned reflex both preservation and extinction – on the 5th day of the trial (Fig. 5 and 6,

respectively), which we explain by the adynamia of the animals in the first days of the postischemic period.

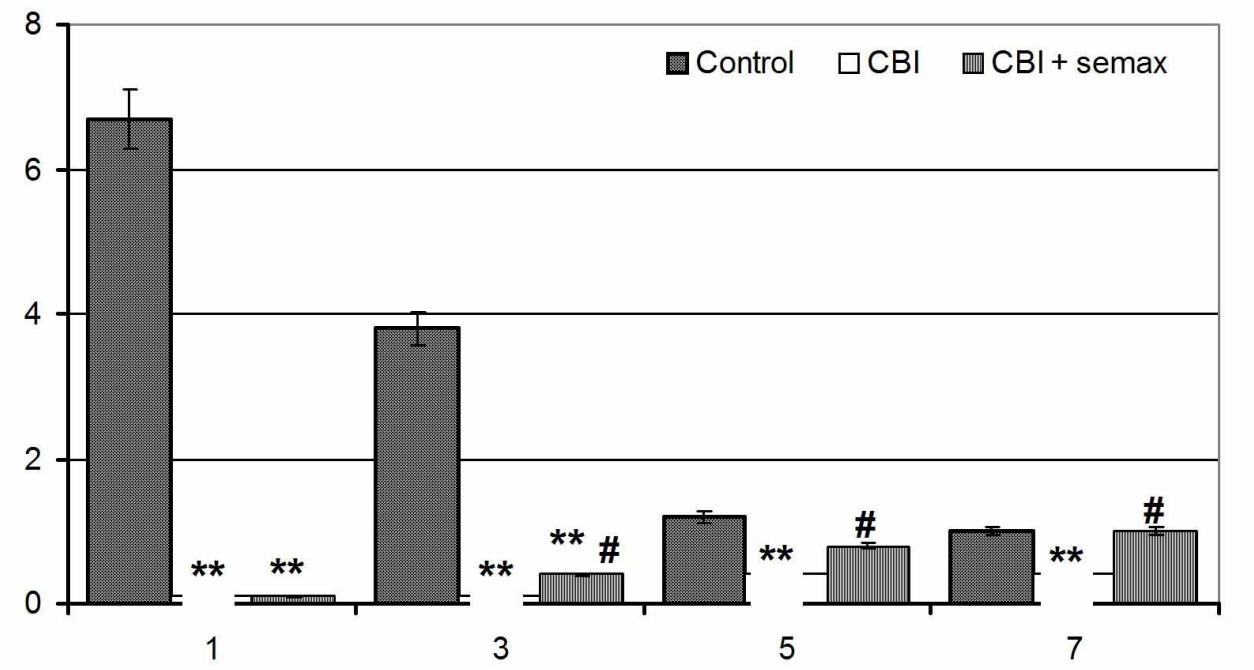

Fig. 4. The influence of Semax endonasal administration on processes of learning expression in food conditioned reflex in the 8-beam radial maze test in rats throughout the postischemic period.

Designations: along the abscissa axis - observation days (duration of the experiment).

Along the ordinate axis - the number of attempts to enter the maze beam until the food is successfully localized.

Notes: ** - $p<0.01$ - significant differences of investigated indexes vs the same data in control observations;

- $p<0.05$ - significant differences of investigated indexes vs the same data in rats with chronic brain ischemia without pharmacological correction.

During the training period, the number of attempts to enter the maze beam until the moment of food successful localization on the 3rd day of the trial in rats after the Semax administration in the post-ischemic period. This index in rats with CBI with Semax administration was 0.4±0.2, which was higher compared to the similar indicator in rats with CBI

without pharmacological correction ($p<0.05$; Fig. 4). Similar Semax endonasal administration efficacy in terms of learning process improving was recorded until the end of the experiment.

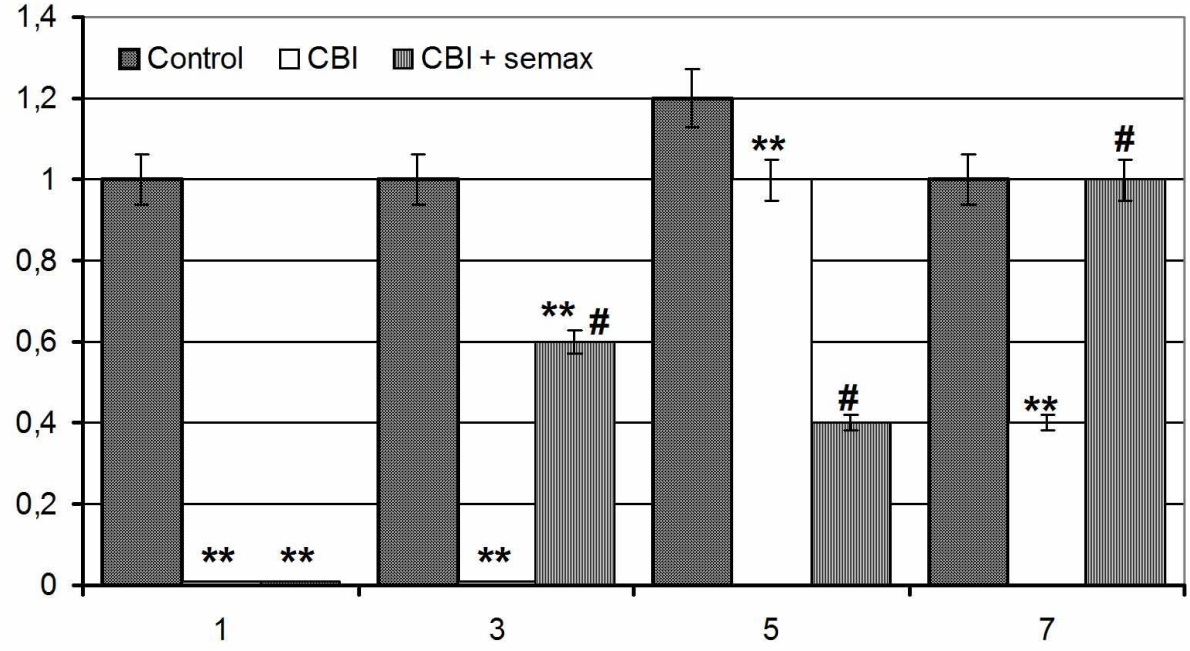

Fig. 5. The influence of Semax endonasal administration on processes of food conditioned reflex retention in the 8-beam radial maze test in rats throughout the postischemic period.

Designations: along the abscissa axis - observation days (duration of the experiment).

Along the ordinate axis - the number of attempts to enter the maze beam until the food is successfully localized.

Notes: ** - $p<0.01$ - significant differences of investigated indexes vs the same data in control observations;

- $p<0.05$ - significant differences of investigated indexes vs the same data in rats with chronic brain ischemia without pharmacological correction.

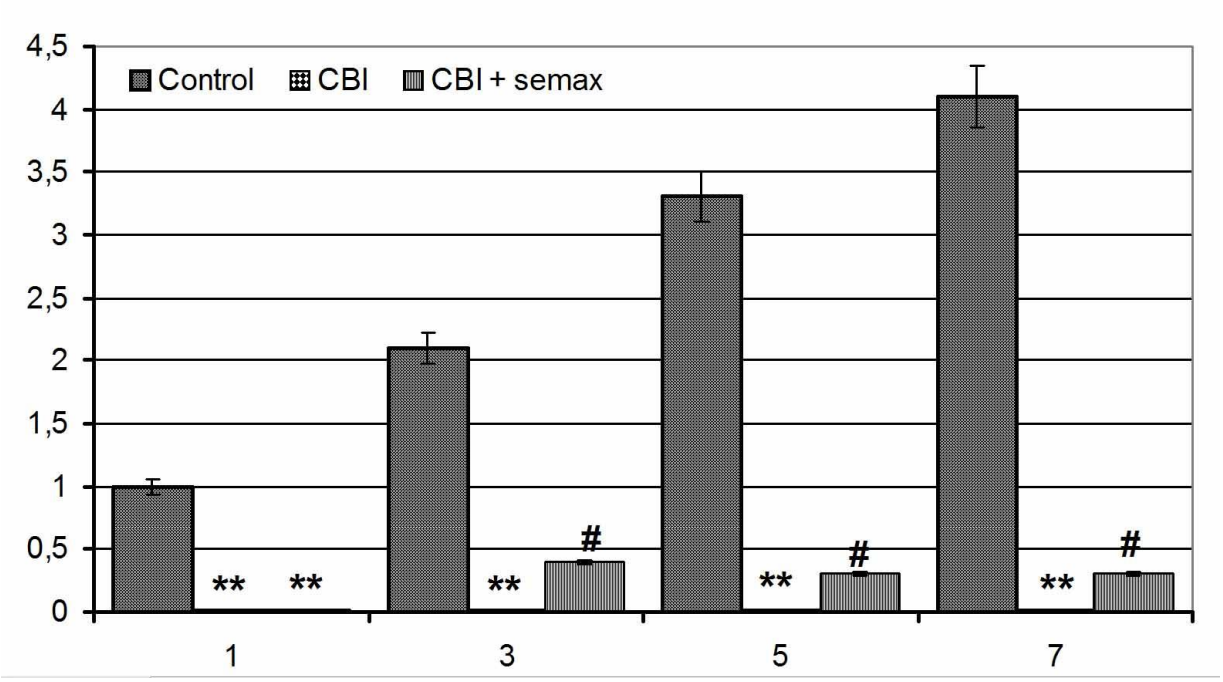

Fig. 6. The influence of Semax endonasal administration on processes of food conditioned reflex extinction in the 8-beam radial maze test in rats throughout the postischemic period.

Designations: along the abscissa axis - observation days (duration of the experiment).

Along the ordinate axis - the number of attempts to enter the maze beam until the food is successfully localized.

Notes: ** - $p<0.01$ - significant differences of investigated indexes vs the same data in control observations;

- $p<0.05$ - significant differences of investigated indexes vs the same data in rats with chronic brain ischemia without pharmacological correction.

Endonasal Semax administration to rats with CBI contributed to food conditioned reflex both preservation (Fig. 5) and extinction (Fig. 6) significant improvement when comparing with similar indexes in rats with CBI without pharmacological correction (in all cases $p<0.05$). A similar situation with mnestic functions restoration using the studied criteria of food conditioned reflex under the influence of Semax endonasal administration was recorded till the end of the trial.

Discussion

Thus, the obtained data characterized a sufficiently long postischemic period of time, when mnestic dysfunctions are registered in rats. The cognitive disorders is confirmed by the

formation and further existence of CAAR and food conditioned reflexes. The learning process disturbance is clearly observed, as well as a pronounced suppression of short- and long-term memory in conditions of CAAR which is combined with violations of the processes of learning, preservation and extinction of the food conditioned reflex. The noted mnestic effects are persistent, are registered in rats 24 hrs after carotid arteries bilateral occlusion and continue throughout the trial.

Firstly, we note that the cognitive disorders formed in rats clearly reflect the neuropathophysiological mechanisms that continue in the body during CBI with almost complete disorganized and/or dysregulatory effects [1, 11]. A similar dysregulatory effect, in our opinion, also contributes to learning process disruption during the food conditioned reflex formation due to the additional mechanism of motor deficit to ischemic neuronal damage which combination does not allow rats to form a memory engram in the 8-beam radial maze test in the post-ischemic period dynamics. This correlates with similar data on motor activity reduction, the neurological deficits and emotional disorders development in conditions of experimental CBI with insufficient vertebral blood supply [6]. We believe that CBI animals adynamia determines the learning processes expression and memory engram preservation and its the resistance to disappear.

Secondly, we consider the cognitive disorders restoration in conditioned reflexes development and preservation during the post-ischemic period due to Semax administration to be an important result. It is interesting that Semax anti-ischemic efficacy was achieved in case of its endonasal administration, as well as the fact that the process of restoration of mnestic disorders was stable, starting from the 3rd day of the experiment and continuing until its completion. The obtained data on Semax endonasal route of administration efficacy in CBI are consistent with the corresponding results that highlight the effectiveness of a similar route of pharmacological compounds administration in ischemic and traumatic brain injury [1, 4, 14, 15]. With pharmacological drugs endonasal administration, the latter penetrate the brain as quickly as possible, while the blood-brain barrier should not be crossed, which generally contributes to an increase in the effective therapeutic concentration and a rapid and effective effect implementation [4, 7, 13].

It is known that in clinical conditions of vascular catastrophe, it is the speed of neuroprotective effect implementation and its efficacy that determine the secondary neuroprotection effectiveness. Therefore, we consider the obtained data to be an experimental

justification for the feasibility of endonasal administration of pharmacological drugs with a neuroprotective mechanism of action in the clinic for acute cerebrovascular accidents.

Finally, we consider it necessary to trace the mechanism of implementation of the protective effects of the applied drug under the conditions of the applied CBI model. Semax has a peptide nature, this compound is characterized by neuroprotective, neurometabolic, nootropic and antiasthenic activity. The therapeutic efficacy of the drug in the treatment of patients with cerebrovascular diseases has been proven, and attention has been focused on the preventive ability of the drug in terms of preventing cognitive disorders [2]. The neuroprotective efficacy of Semax is probably also explained by the acceleration of the synthesis under its influence of nerve growth factor, brain-derived neurotrophic factor and other cytokines and neurotrophic factors [8].

Therefore, taking into account the mechanisms of Semax neuroprotective effect realization and the route of its administration, we consider it appropriate and pathogenetically justified to include Semax in a comprehensive scheme of pharmacological correction of cognitive disorders or prevention of their development in cerebrovascular pathology.

Thus, the proven effectiveness of the pathogenetically justified method of restoring mnestic disorders during the postischemic period indicates the development of a neuroprotective effect, as well as the possibility of increasing the effectiveness of the treatment of patients with CBI with Semax endonasal administration. Therefore, we consider the obtained data to be an experimental basis for the feasibility of clinical testing of Semax effects in the complex of secondary neuroprotection, which is capable of ensuring the restoration of cognitive dysfunctions in patients with acute cerebral circulation disorders.

Conclusions

The obtained data indicate the formation of expressed mnestic dysfunctions in the dynamics of the postischemic period, which are characterized by learning process deterioration, as well as short- and long-term memory suppression in conditions of conditioned reflex of active avoidance, which is combined with food conditioned reflex violations of processes of learning, preservation and extinction of the.

The noted amnestic effects are persistent, recorded in rats already 24 hrs after carotid arteries bilateral occlusion and continue throughout the experiment.

The anti-ischemic efficacy of Semax was achieved after its endonasal administration, and the process of restoring mnemic disorders was persistent, starting from the 3rd day of the experiment and until its completion.

Considering the mechanisms of implementation of the neuroprotective effect of Semax and the endonasal route of its administration, we consider it appropriate and pathogenetically justified to include Semax in a comprehensive scheme of pharmacological correction of cognitive disorders or prevention of their development in cerebrovascular pathology.

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Institutional Review Board Statement

The experimental studies were carried out in the conditions of a chronic experiment in accordance with international standards of humane treatment of vertebrate animals and approved by the Ethics Committee of Odesa National Medical University (N7/21, 11 October 2021)

Informed Consent Statement

The data of experimental studies are given. Written informed consent from the patients was not necessary to publish this paper.

Data Availability Statement

The data presented in this study are available on request from the author.