Article

Paradoxical Effect of Combined Exposure of Semax and Ammonium Molybdate on Learning and Memory of Rats

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Abstract: The combined effect of semax with aqueous solutions of lead diacetate (10-7 M) and ammonium molybdate (10-5 M) on the formation of a conditional two-way avoidance reaction in rats in a shuttle chamber was studied. It was found that both heavy metal salts inhibit learning and memory; lead diacetate caused greater depression. Semax slowed down the production of the conditional reaction, but counteracted the negative influence of both metals on this process. At the same time, the influence of semax on the formation of the avoidance reaction in the presence of ammonium molybdate, which in itself inhibited avoidance, paradoxically intensified. With the combined effect of the peptide and ammonium molybdate, the formation of a conditional reaction occurred much faster than against the background of semax without combination with molybdenum. In general, the data obtained indicate that semax counteracts the neurotoxic effect of lead and molybdenum salts. Since the main mechanism of the neurotoxic effect of heavy metals is oxidative stress, the indicated positive effect of semax can, in our opinion, serve as confirmation of the presence of antioxidant properties in the spectrum of pharmacological activity of the peptide.

Keywords: oxidative stress, semax, ammonium molybdate, lead acetate, learning, memory.

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1. Introduction

Endogenous peptide regulators, similar to adrenocorticotropic and melanocyte-stimulating hormones (melanocortins), play an important role in regulating the functions of the central nervous system. Currently, there is a lot of evidence of the positive effect of melanocortins on the development of conditioned reflexes. However, the nootropic effects of natural fragments of adrenocorticotropic hormone (ACTH) are short-lived [1]. This prompted the search for ways to increase the effectiveness of natural melanocortins by modifying their structure, which led to the creation of synthetic analogues with pronounced activity, but devoid of hormonal effects [2]. Among them, an important place is occupied by semax – a nootropic analogue of the ACTH fragment (4-10) of prolonged action, the structure of which includes the ACTH fragment (4-7) and the tripeptide Pro-Gly-Pro. Animal experiments have shown that this peptide, like natural melanocortins, has a wide spectrum of neurotropic activity [3]. Currently, it is used in the clinic as a nootropic and neuroprotective drug. Despite the fact that drugs developed on the basis of semax have been used in clinical practice for a long time, studies of its physiological effects continue. The relevance of such studies is determined by the need to clarify the mechanisms of action and the possibility of expanding the spectrum of clinical use of the drug.

Recently, the antioxidant activity of the peptide has been established, which significantly expands the spectrum of its action [4]. In particular, the ability of the peptide to resist the neurotoxic effect of heavy metals, the main mechanism of which is the induction of oxidative stress, is of great interest [5]. It is known that oxidative stress caused by heavy metals leads to neurodegenerative diseases, including Alzheimer's and Parkinson's diseases [6-9]. To date, separate studies have been carried out, which show the prospects of using antioxidants to counteract the neurotoxic effects of heavy metals [10-12]. This paper analyzes the possibility of using semax as a drug with antioxidant properties to counteract the inhibition of learning and memory in rats by molybdenum and lead salts.

2. Materials and methods

The experiments were conducted on six groups of male white mongrel rats (20 animals per group) at the age of 7-8 weeks. by the beginning of the experiment. The first group was injected with semax at a dose of 0.05 mg/kg; the 2nd and 3rd groups were injected with aqueous solutions of lead diacetate (10-7 M) and ammonium molybdate (10-5 M), respectively; the 4th and 5th groups

were injected with semax in combination with lead and molybdenum salts, respectively; 6-I group served as a control. Aqueous solutions of heavy metal salts were administered intraperitoneally by 2 ml 5 hours before the experiments, semax – 4 hours after the introduction of salt solutions. Control animals were injected with 2 ml of distilled water an hour before the experiment.

The experiments were carried out in a chamber divided into 2 equal halves by a partition with an opening. In animals, for 5 days (25 stimuli were presented daily), a conditional two-way avoidance reaction was developed, which serves as an experimental model of learning and memory. A sound conditional stimulus was turned on and after 10 seconds a current (0.5–0.7 mA) was applied to the floor wiring of the half of the chamber in which the rat was located. If the rat did not move to the safe half of the chamber, then after 10 seconds both stimuli were turned off; after 30 seconds, the stimuli were presented again. The transition of the animal to the safe half of the chamber, during the action of stimuli, led to their shutdown.

The study was conducted in accordance with the rules adopted by the European Convention for the Protection of Vertebrate Animals (Strasbourg, 1986) and Directive 2010/63/EU of the European Parliament and of the Council of the European Union on the protection of animals used for scientific purposes.

The dynamics of learning in groups was assessed using a one–factor nonparametric Kraskel–Wallis analysis of variance; the difference between groups was assessed using the Wilcoxon criterion. The differences were considered statistically significant at $p<0.05$.

3. Results and discussion

The analysis of the graphs shown in Fig. 1 shows that both heavy metal salts inhibited the production of the avoidance reaction. Lead salt had a stronger depressing effect on learning, against the background of which animals, even at the final stage of training, received on average more than 20 shocks of current per experience out of 25 possible. The use of the Kraskel–Wallis analysis of variance showed that when exposed to lead diacetate, there was no statistically significant increase in the number of avoidance reactions, starting from the 2nd experiment, which indicates a deep inhibition of learning and memory. These data are consistent with those previously obtained by us [13]. The use of molybdenum caused a statistically significant decrease in the number of avoidance reactions relative to control only in the last three days of the experiment.

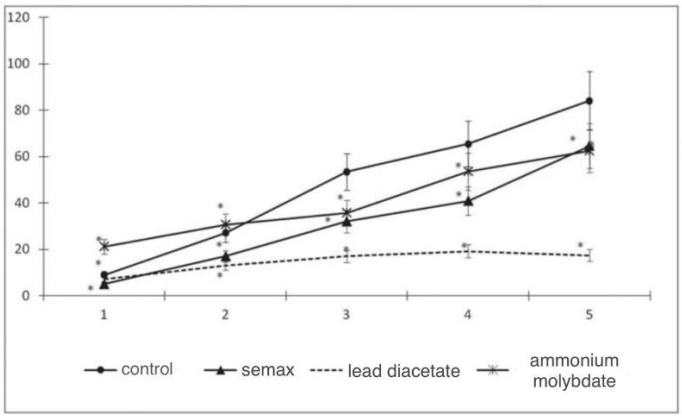

Figure 1. The influence of semax, lead diacetate and ammonium molybdate on the dynamics of the avoidance reaction. On the abscissa axis – the time of experiments, days; on the ordinate axis – the number of correct reactions as a percentage relative to all presentations. The data here and in Figures 2 and 3 are represented as the mean and the error of the mean. * – $p<0.05$ relative to control values.

Semax slowed down the development of the avoidance reaction. This is consistent with the data that the effect of the peptide depends on the methodological features of the experiments. In particular, it was shown that semax increases the number of conditional reactions of unilateral avoidance, but reduces the number of reactions of bilateral avoidance, while increasing, in comparison with control data, the number of these reactions in conditions of emotional stress and the need to learn a modified skill after functional disorders [14]. According to the results obtained in the present experiment, semax counteracts the neurotoxic effect of salts of both heavy metals on the formation of an avoidance reaction in animals, which confirms the above-mentioned ability of the peptide to have a positive effect in conditions that make learning difficult. The experimental results presented in Fig. 2 show that the levels of avoidance reactions with combined exposure to semax with salts of both lead and molybdenum exceed the corresponding indicators recorded when exposed to salts without a peptide. At the same time, the peptide's resistance to the inhibition of learning by lead and molybdenum salts manifests itself to varying degrees. The combined

effect of semax with lead accelerated the formation of an avoidance reaction with respect to animals that were injected only with metal, starting from the third day. The neuroprotective effect of the peptide increased in the course of the experiment. A statistically significant excess of the avoidance level in the combination of semax with molybdenum relative to the values observed when exposed to a salt of this metal without a peptide was noted throughout the experiment.

As mentioned above, both semax and molybdenum, introduced separately, slow down the learning of animals. In this regard, it is interesting to note that with combined exposure to peptide and ammonium molybdate, the formation of an avoidance reaction occurs statistically significantly faster than against the background of semax without combination with molybdenum salt (Fig. 3). On the first day of the experiment, the number of avoidance reactions with combined exposure was almost 7 times higher than the magnitude of the avoidance reaction in rats under by the influence of a single peptide. To a certain extent, this result looks paradoxical and requires explanation. It is known that the metal molybdenum is a part of the enzyme xanthine oxidase and can increase the activity of antioxidants [15]. In view of this, the specified feature of the combined effect of molybdenum and peptide can be explained by an increase in the antioxidant activity of the latter. In contrast, the combined effect of the peptide with lead diacetate leads to a decrease in the level of the avoidance reaction relative to this indicator when exposed only to semax. The latter means that exposure to lead salt weakens the activity of the peptide. A comparison of the combined effects on learning of semax with lead diacetate and semax with ammonium molybdate shows that the enhancement of the neuroprotective activity of the peptide is selective, since it occurs only in combination with molybdenum.

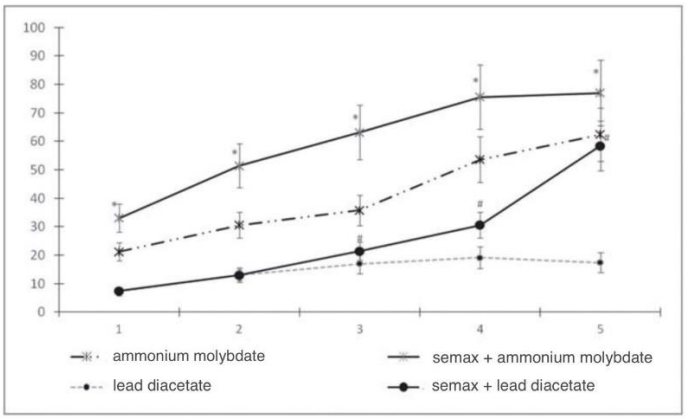

Figure 2. Semax's counteraction to the negative influence of lead diacetate and ammonium molybdate on the production of the reaction of the solution. The axis designations are as in Figure 1. * – p<0.05 relative to the values with a separate introduction of ammonium molybdate; # – p<0.05 relative to the values with a separate introduction of lead diacetate.

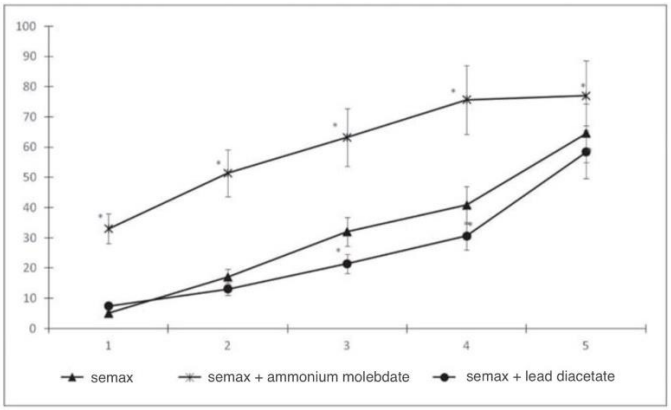

Figure 3. Comparison of the combined effect of semax and metal salts with the effect of one peptide on avoidance reactions. About the axis values – as in Figure 1. * – p<0.05 relative to the values with a separate introduction of semax.

Thus, it follows from the data obtained that the effect of semax on the formation of the avoidance reaction in rats in the presence of ammonium molybdate, which in itself inhibits learning,

paradoxically increases. In general, the data obtained indicate that semax counteracts the neurotoxic effect of lead and molybdenum salts. Since the main mechanism of the neurotoxic effect of heavy metals is oxidative stress, the positive effect of semax on the background of ammonium molybdate and lead diacetate can, in our opinion, serve as confirmation of the presence of antioxidant properties in the spectrum of pharmacological activity of the peptide.

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Conflicts of Interest: The authors declare no conflict of interest.

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