Article

Coexposure of heavy metals and piracetam destroys adaptive behaviour

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Abstract: In experiments on rats, it was found that lead, cadmium and cobalt salts inhibit the acquisition of an avoidance responses. Cases of increased neurotoxic effect of heavy metals on learning and memory in rats with their combined administration with piracetam have been noted. Ascorbic acid counteracts this neurotoxic impact of the heavy metals and piracetam cooexposure on the adaptive behaviour.

Keywords: heavy metals, piracetam, ascorbic acid, learning, memory.

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1. Introduction

The modern ecological situation is characterized by an oversaturation of pollutants of different nature, the most common and dangerous, among which are supertoxicants – heavy metals [1-2]. The unfavorable environmental situation that has been observed recently in industrially developed regions, according to many researchers, is the reason for the growth of neuropsychic disorders and various cognitive disorders [3]. Neurodegenerative disorders, including Alzheimer's and Parkinson's diseases, have been identified in people engaged in the production of heavy metals [4, 5].

Exposure to heavy metals causes delays in neuropsychic development in children living in industrial cities: memory, learning, motor skills, speech, decreased IQ, etc. [6]. Recently, the toxic effect of heavy metals on the immune system has also been shown [7]. This prompted us to investigate the possibility of correcting such a negative effect of heavy metals with the help of the immunomodulatory drug taktivin [8].

The discussed problem of environmental pollution by heavy metal salts is very relevant for the North Caucasian region, in particular, for North Ossetia, where the amount of heave metals in air, water and soil, due to the developed metallurgical industry, is ten times higher than average.

For the treatment of cognitive disorders, nootropic agents are used, in particular, piracetam, the main property of which is the ability to resist various learning and memory disorders, as shown in numerous animal experiments (9-11).

Until recently, the study of the effect of nootropics and heavy metals on cognitive and mnestic processes was carried out independently, although there is reason to believe that there is a certain interdependence between the effects of the factors under consideration. In particular, the effects of nootropic agents may vary in the presence of heavy metals. It has been shown that the presence of heavy metal salts in drinking water as a solvent, even in minimal concentrations, can significantly affect the effectiveness of the drug [12, 13].

Heavy metals initiate lipid peroxidation by changing the activity of membrane enzymes, while the cells of the central nervous system are most vulnerable to free radical processes [14]. The mechanism of LPO in the cells of the central nervous system is similar to the mechanisms in other tissues, but the intensity of the process is much higher here. In addition, the brain is characterized by a low content of the main components of antioxidant protection. In general, it is the deficiency of the antioxidant system in brain tissue that explains its particular sensitivity to the production of free radical compounds [15].

The main active element of the protective system against the damaging effects of free radicals are antioxidant compounds. The antioxidant properties of ascorbic acid, which is part of the redox

system, are the most important element in the mechanisms of the body's resistance to the effects of toxic substances, including the harmful effects of heavy metals [16].

In this scientific work, we investigated the features of the formation of adaptive reactions of rats under the combined effect of various heavy metal compounds, the reference nootropic drug piracetam and ascorbic acid.

2. Materials and methods

The study was carried out on mongrel white male rats weighing 200-250 g, which were kept under normal light conditions and free access to water and food. The following substances were injected intraperitoneally into the animals: reference crystal piracetam (AKRIKHIN, Russia; 300 mg/kg), lead diacetate (0.08 mg/ml), cobalt sulfate (0.08 mg/ml) or cadmium chloride (0.08 mg/ml), ascorbic acid (VIFITECH, Russia; 250 mg/kg).

The development of the conditioned reflex of active avoidance (CRAA) was carried out in a shuttle box divided by a partition with an opening for two identical compartments measuring 19x21x22, with an electrified grating floor. The rat was placed in the box 5 minutes before the start of the experiment. The CRAA production began by determining the threshold of individual sensitivity of a particular animal to electricity. The sensitivity threshold was considered to be the one that caused the animal to move around the box. 10 seconds after the isolated action, an unconditioned stimulus was added to the conditioned stimulus (700 Hz sound) – electricity flow of 0.5-0.6 mA. When the animal passed into the other half of the box through the opening (the escape response), the sound and electricity were turned off. If the animal moved to the other compartment away from the sound signal (the avoidance response), then there was no electricity, and the sound stopped. If the animal did not relocate under the influence of the stimuli, then 10 seconds later the stimuli were turned off automatically. Each experiment consisted of 25 attacks with a 30-second inter-signal period. The experiments were carried out during the period of 5 days until the acquisition of a stable reflex, which was estimated according to the training criterion (no less than 80% of avoidance reactions out of the number of attacks). The number of avoidance reactions, escape reactions and inter-signal reactions (ISR) was recorded.

Two series of experiments were conducted. In the first series, 4 hours before the development of the avoidance reaction in each of the 5 experimental days, rats were intraperitoneally injected with a salt solution of one of the heavy metals, and a solution of piracetam was administered half an hour later. Control animals were injected with an equivalent volume of solvent half an hour before the experiment. In the II series, in addition to these substances, a solution of ascorbic acid was injected either before the introduction of a solution of a heavy metal salt (lead diacetate, cadmium chloride), or after (experiments with cobalt sulfate). Statistical processing of the studies was carried out using parametric (Student's t-test) and nonparametric (Wilcoxon and Mann-Whitney criteria) methods. The differences were considered significant at $p<0.05$. Calculations were performed using Microsoft Excel computer programs; Origin Pro 6.1 and Statistica for Windows 7.0.

3. Results

The presented experimental data of the I series (Fig. 1) show that the average values of avoidance in animals treated with piracetam were comparable with the control ones. Heavy metals inhibited animal learning. The greatest oppression was exerted by cadmium and cobalt salts. Against the background of heavy metals, there was no statistically significant increase in the number of avoidance reactions from experiment to experiment in general, which indicates a deep inhibition of learning; animals avoided exposure to current only in 10-17% of all possible cases of its exposure.

The effect of piracetam with the combined substances exposure was expressed only in a decrease in the inhibition of learning by pre-administered heavy metals. The effect of cobalt sulfate was reduced by piracetam in the last three experimental days, and the number of avoidance reactions when introducing the metal with piracetam was greater than without the drug. At the same time, however, the level of avoidance remained below the control; the latter indicates that the oppression of learning manifested also in these conditions. It should also be noted that cobalt sulfate reduced the activity of piracetam, so that the number of avoidance reactions with joint administration was less than with separate administration of nootropics.

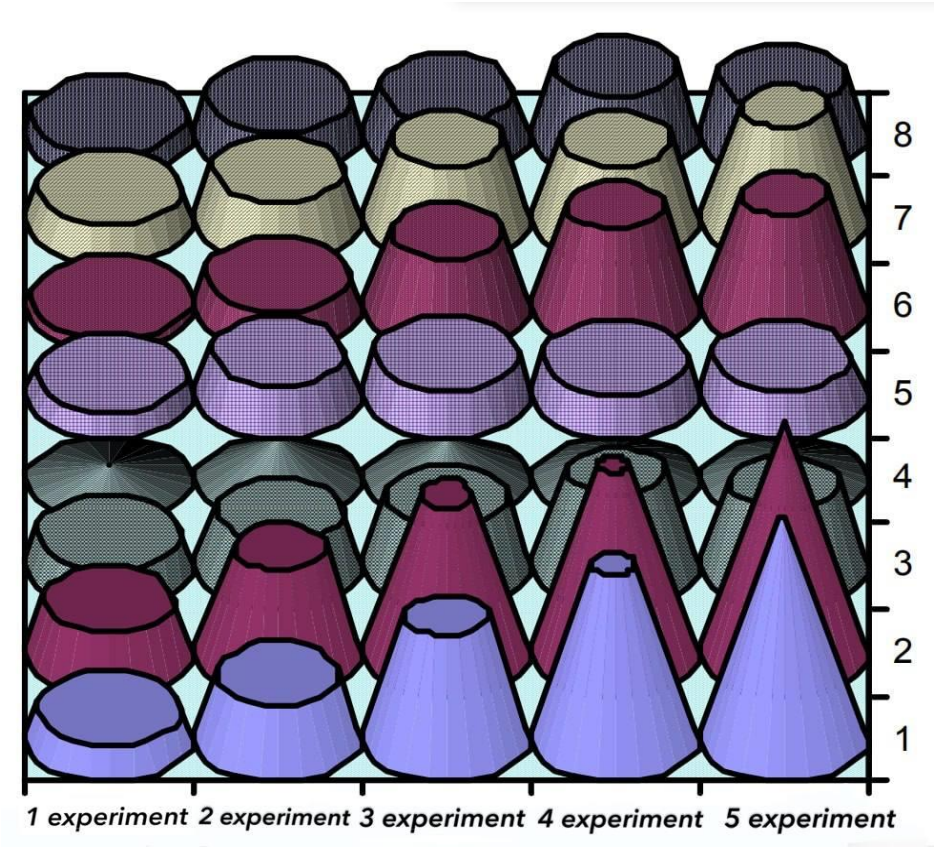

Figure 1. Acquisition of the conditioned reaction of active avoidance in the first series of experiments: on the abscissa axis – the number of experiments; on the ordinate axis – the number of avoidance reactions (% relative to all presentations) against the background of: 1 – saline solution; 2 – piracetam; 3 – lead diacetate; 4 – cobalt sulfate; 5 – cadmium chloride; 6 – piracetam and lead diacetate, 7 – piracetam and sulfate cobalt, 8– piracetam and cadmium chloride.

Of the three days in which learning was inhibited by lead diacetate, piracetam reduced the inhibition only on the last day, in which the level of avoidance with combined exposure to metal and nootropic was higher than with separate administration of metal; however, avoidance remained below the control. In addition, on this day, as on the others, lead diacetate, in turn, reduced the activity of piracetam, and the number of avoidance reactions when nootropic drug was administered against a metal background was less than when it was administered separately. Moreover, the combined administration of piracetam and lead salt led to additional inhibition – a decrease in the number of avoidance reactions relative to the control in the first two days, in which it was absent with separate metal exposure, so that the level of avoidance became lower than the control for all 5 experimental days.

The number of avoidance reactions with the combined administration of cadmium chloride and piracetam was greater than with the separate administration of metal salt, remaining, however, 2.5 times less than the control value. At the same time, the effect of piracetam was weakened by the preliminary injection of cadmium chloride.

The obtained experimental results indicate that along with the positive effect of piracetam, expressed in a decrease in the depressing effect on learning and memory of rats from heavy metal salts, a significant number of cases have been noted in which the nootropic effect was weakened by the preliminary injection of metals.

In the II series of experiments, it was shown that piracetam accelerates the formation of CRAA on all experimental days (Fig. 2, 3, 4), which is different from the effect of the drug in the 1st series. This ambiguous effect of nootropics on learning and memory was noted earlier by other researchers [17], which indicates the sufficient prevalence and significance of this fact. The ambiguity of the piracetam effect can be explained by the influence of several factors, among which we note the following:

- features of the crystal structure of piracetam, namely, the existence of several polymorphic forms of the drug with different physico-chemical properties and, as a consequence, with different pharmacological activity [18];

- environmental factor (interaction of the drug with xenobiotics that have a negative impact on the functioning of biological systems, in particular, on the central nervous system);
- the chemical composition of the water used for solutions, in particular, the presence of certain amounts of heavy metal compounds in it.

This can be confirmed by the results of a UV spectrometric study of the kinetics of dissolution of crystalline piracetam in aqueous solutions of heavy metal salts at concentrations of lead diacetate, cadmium chloride and cobalt sulfite equal to and below the established MPC for drinking water [12, 13, 19]. The revealed features of the absorption spectra of piracetam solution in the presence of these active salts of heavy metals allowed us to conclude about their catalytic effect on the structure of the drug, which, in turn, is the cause of the unstable nootropic effect of piracetam.

The experiments carried out showed a deterioration in the acquisition of conditioned reflex in the shuttle chamber in rats against the background of the combined effect of piracetam and lead diacetate (Fig. 2). It should be emphasized that the level of CRAA in rats receiving injections of piracetam and lead diacetate was less than the indicators of rat teaching with separate exposure to metal, which is consistent with the data of experiments I series. Consequently, with the combined action of these substances, there is a stronger inhibition of learning and memory.

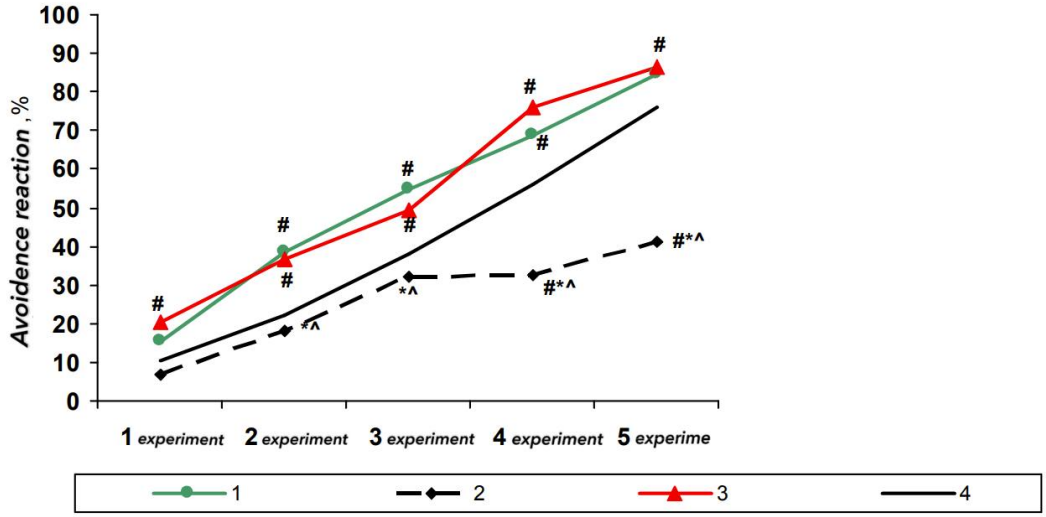

Figure 2. Acquisition of the conditioned reaction of active avoidance under the influence of ascorbic acid, piracetam and lead diacetate:
on the abscissa axis – the number of experiments; on the ordinate axis – the number of avoidance reactions, % relative to all presentations. 1 – piracetam; 2 – piracetam + lead salt; 3 – ascorbic acid+ piracetam + lead salt; 4 – control.
– $p<0.05$ relative to the control; * – $p<0.05$ relative to the experimental group of animals that were injected with piracetam solution; ^ – $p<0.05$ relative to the experimental group of animals that a solution of ascorbic acid was injected, followed by a solution of piracetam and lead diacetate.

Ascorbic acid eliminated the suppression of avoidance under the influence of heavy metals. From the first experiment, the learning indicators in animals with the combined administration of lead diacetate, piracetam and ascorbic acid were higher than the control and statistically significantly exceeded the indicators of rats that did not receive a vitamin solution (Fig. 2).

When CRAA was formed against the background of the combined action of cadmium chloride and piracetam, as in series I, there was a significant decrease in indicators relative to the control and the group receiving piracetam injection. Cadmium weakened the effect of nootropics. As follows from the presented data (Fig. 3), previtamination of animals significantly facilitated the CRAA acquisition in rats injected with a solution of piracetam and cadmium salts. From the first experiment, the percentage of avoidance reactions against the background of the combined action of cadmium chloride, piracetam and ascorbic acid was greater than in the group of animals that were injected with a metal salt solution and a nootrope.

Cobalt sulfate, as well as the compounds of other heavy metals studied by us, had a negative impact on the CRAA acquisition in both series of experiments conducted by us (Fig. 1, 4). Moreover, when a nootropic drug was administered against the background of cobalt salts, as well as in experiments with lead and cadmium compounds, there was a greater inhibition of the avoidance reaction than with a separate introduction of metal.

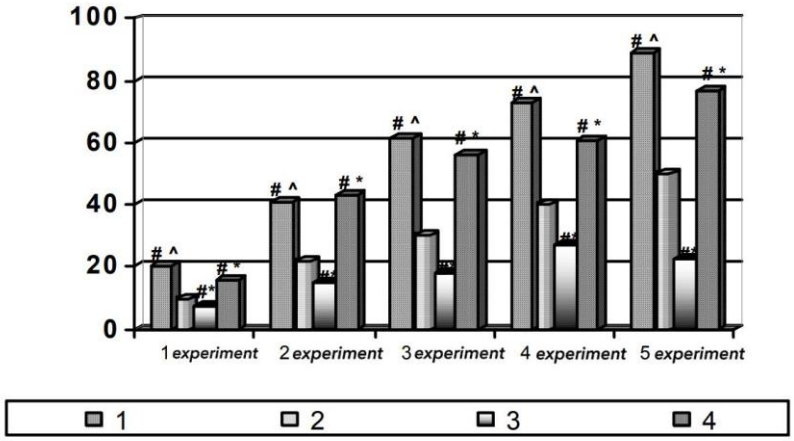

Figure 3. Acquisition of the conditioned active avoidance reaction against the background of the combined action of ascorbic acid, piracetam and cadmium chloride:
on the abscissa axis – the number of experiments; on the ordinate axis – the number of avoidance reactions, % relative to all presentations 1 – piracetam; 2 – control; 3 – piracetam + cadmium chloride;4 – ascorbic acid + piracetam + cadmium chloride.
- p<0.05 relative to the control (physical solution); * – p<0.05 relative to the experimental group of animals injected with piracetam; ^ – p<0.05 relative to the experimental group of animals injected with cadmium chloride and piracetam.

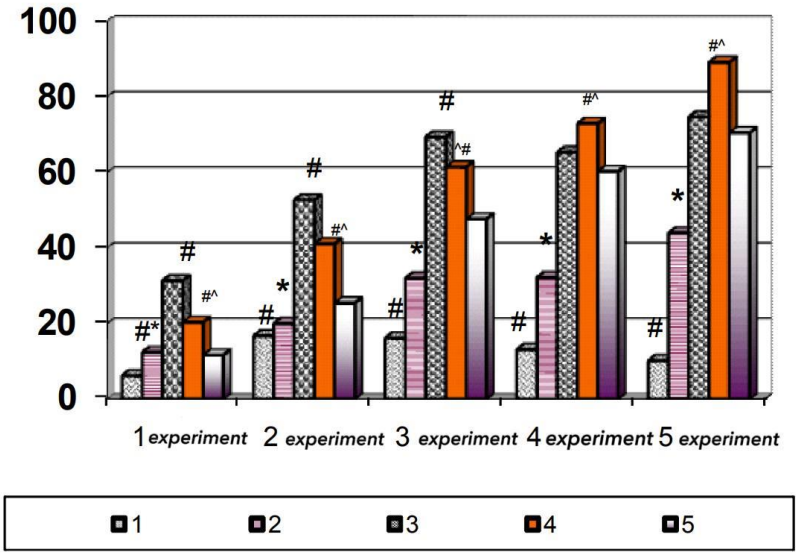

Figure 4. Acquisition of the conditioned active avoidance reaction against the background of the combined action of piracetam, cobalt sulfate and ascorbic acid:
on the abscissa axis – the number of experiments; on the ordinate axis – the number of avoidance reactions, % relative to all presentations 1 – cobalt sulfate; 2 – cobalt sulfate + piracetam; 3 –cobalt sulfate + piracetam + ascorbic acid; 4 – piracetam; 5 – control.
– p ‹ 0.05 relative to control; * – p ‹ 0.05 relative to the experimental group of animals injected with piracetam solution; ^ – p ‹ 0.05 relative to the experimental group of animals that were injected with a solution of cobalt sulfate and piracetam.

The administration of the antioxidant significantly increased the learning rate in the experimental group compared with the results in rats trained in avoidance against the background of the action of cobalt salt and piracetam (Fig. 4). This can be explained by the fact that when ascorbic acid is added to piracetam solutions containing heavy metal salts, significant changes in the UV spectra of the corresponding solutions are noted. Ascorbic acid, chemically acting as the strongest reducing agent, is capable of being oxidized into dehydroascorbic acid and, thus, together with it represents a redox system. Reacting with a heavy metal salt, ascorbic acid thereby neutralizes its catalytic effect on the structure of piracetam, thereby preventing the appearance of structural forms of piracetam in the solution, which worsen the formation of CRAA [20, 21].

Thus, analyzing the combined effects of heavy metals, piracetam and ascorbic acid on the learning and memory of rats, we found an aggravation of the neurotoxic effect of metals in the

presence of nootropics. Ascorbic acid performs a protective function, reducing or completely neutralizing the negative neurotoxic effects of heavy metal. The obtained data revealed the theoretical and practical need for further study of the combined effect of neurotropic drugs and heavy metal salts on the central nervous system.

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

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