Article

The research of the effect of the interaction of molybdenum, cobalt, piracetam and ascorbic acid on cognitive processes in rats

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Abstract: The problem of combined effects on animals of a combination of drugs with heavy metals (HM) is considered. The study was carried out at the K.L.Khetagurov North Ossetian State University (Vladikavkaz, RSO-Alania) and the Lomonosov Moscow State University (Moscow). The effect of lead diacetate and ammonium molybdate on the training of rats was studied separately, as well as in combination with ascorbic acid and piracetam. It has been found that lead and molybdenum salts inhibit avoidance reactions and inter-signal reactions. The combined effect of these salts with ascorbic acid and piracetam led to mixed results. The nootrope counteracted the inhibition of avoidance reactions by metals, but increased the inhibition of inter-signal reactions, which serve as an important characteristic of learning. In contrast, ascorbic acid reduced the inhibition of both avoidance reactions and inter-signal reactions caused by HM. The use of 2 drugs with HM led not only to an increase in the positive effect exerted by each of the drugs on training, but also to its weakening. The results obtained indicate that the interaction of drugs and HM, taking place in real conditions, is fraught with unpredictable consequences and may pose a danger to human and animal health. This justifies the need for further analysis of the combined effects of several drugs with HM.

Keywords: molybdenum, cobalt, ascorbic acid, piracetam, training, rats.

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1. Introduction

In recent years, the anthropogenic load on the environment has increased catastrophically. Among the wide variety of pollutants, heavy metals (HMS) play a special role, representing a serious danger in industrialized regions [1]. For a number of reasons, HM is particularly dangerous for the central nervous system (CNS), causing severe neurodegenerative diseases, including Alzheimer's and Parkinson's diseases [2? 3].

Until recently, the effect of HM was studied independently of other factors. However, in order to fully understand the meaning of HM for living organisms, it is necessary to take into account that in real life they do not affect the body in isolation, but simultaneously or sequentially with other factors. As a result, the importance of the influence of HM with each other gradually began to be emphasized [4, 5], with drugs [5], with stress [6], etc. It was shown that the effects of joint and separate exposure to agents differ. In particular, it was found that nootroppiracetam used to protect the central nervous system from various adverse effects, instead of countering the neurotoxic effect of heavy metals, can lead to its aggravation [6, 7]. This is an additional danger for industrialized areas with a high content of HM in the environment.

The main mechanism of the negative action of HM consists in the oxidative stress caused by them [8, 9]. This opens up prospects for the use of antioxidants as a means of countering the neurotoxic effects of HM. To date, separate studies have been carried out showing such a potential of antioxidants [7, 8].

In contrast to the study of the interaction of HM with each other, the joint use of drugs in the presence of heavy metals has not yet been analyzed sufficiently to date. In this paper, the features of the effect on learning and memory of rats of the combined use of ascorbic acid and piracetam with lead and molyb-denum salts are analyzed.

2. Materials and methods of research

Experiments were conducted on 11 groups of male white mongrel rats (20 animals per group) weighing 180-200 g by the beginning of the experiment. The first group was injected with piracetam at a dose of 300 mg/kg; the 2nd and 3rd groups were injected with aqueous solutions of lead diacetate (10-7 mol/l) and ammonium molybdate (10-5 mol/l), respectively; the 4th group was injected with ascorbic acid (250 mg/kg), which serves as a comparison drug in as an antioxidant; 5th and 6th - ascorbic acid in combination with lead and molybdenum salts, respectively; 7th served as a control; The 8th and 9th groups were injected with piracetam in combination with lead and molybdenum salts, respectively; the 10th and 11th groups in

combination with piracetam and ascorbic acid were injected with lead and molybdenum salts, respectively. HM salts were administered intraperitoneally 5 hours before the experiments, ascorbic acid was administered 3 hours later, and piracetam was administered 1 hour later. Control animals were injected with an equivalent volume of distilled water an hour before the experiment.

The experiments were carried out in a chamber divided by a partition with an opening into 2 equal halves. In animals, for 5 days (25 stimuli presented daily), a conditional reaction of active avoidance 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 during the isolated action of the sound led to its shutdown and avoidance of current exposure, and during the current – to the shutdown of both stimuli.

The dynamics of learning in groups was assessed using a one-factor nonparametric Kruskall–Wallis analysis of variance; the difference between groups was assessed using the Wilcoxon criterion.

3. Results

The results shown in Fig. 1 show that lead diacetate caused inhibition of the development of the avoidance reaction. The maximum value of avoidance was only 19.2 ± 2.9% of the number of presentations, that is, the animals "missed" more than 80% of the shocks during the experiment. The use of the Kraskel-Wallis analysis of variance showed that there was no statistically significant increase in the number of avoidance reactions against the background of lead salt, starting from the 2nd experiment, which indicates a deep inhibition of learning. Ammonium molybdate reduced the number of avoidance reactions relative to control only in the last three experimental days; on the 1st day, their growth was observed. Piracetam and ascorbic acid accelerate the production of the avoidance reaction, and in the first 2 days to the same extent.

The acqisition of an avoidance reaction with the combined administration of both ascorbic acid and piracetam with HM salts throughout the experiment occurred statistically significantly faster than with the introduction of metals separately. At the same time, the combination of piracetam with lead diacetate turned out to be more effective than the combination of acid with this salt (Fig. 2). The positive effect of ascorbic acid in these conditions is consistent with the previously described data [10, 11], which confirms the role of antioxidants in overcoming the negative effects of HM. The positive effect of piracetam in these conditions differs from the previously described effect [6, 10, 14].

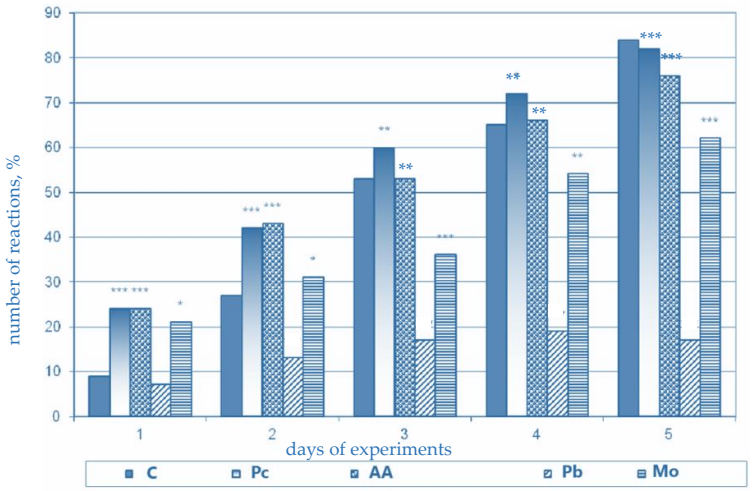

Figure 1. The effect of piracetam, ascorbic acid, lead diacetate and ammonium molybdate on the acqisition of the avoidance reaction. Abscissa – days of experiments; ordinate – the number of reactions as a percentage relative to all presentations. Designations: *,**,*** – p<0.05, p<0.01, p<0.001 relative to the control values. C – control, Pc – piracetam, AA – ascorbic acid, Pb – lead, Mo – molybdenum.

This can be explained by the fact that this salt, when interacting with piracetam, may, under certain conditions uncontrolled by the experimenter, undergo changes with the formation of intermediates that significantly affect the functional properties of the nootrope and impair animal learning [14].

Evaluating these two results in general, it is necessary to agree that the data obtained in this work do not deny the possibility of aggravating the neurotoxic effect of lead when combined with piracetam.

Moreover, they are consistent with the results presented below on the aggravation of the inhibition of inter-signal reactions (ISR) by the combined use of these agents.

As the results shown in Table 1 show, both HM salts inhibit ISR, which reflect the essential role of the avoidance development process [15], which emphasizes the negative role of these agents in learning. At the same time, as in the case of avoidance reactions, the negative effect of molybdenum salt is less pronounced than lead salts. Combined with molybdenum salt, the use of piracetam led to increased inhibition of ISR, so that their number throughout the experiment was less than with a separate introduction of the metal. This

distinguishes the effect of the combination of these agents from their combined positive effect on the avoidance response.

The combined use of piracetam with lead salt also differs from the positive effect of this combination on the avoidance reaction. In the first 3 days, there is no effect, and on the 4th day, the number of ISR under the influence of agents was even less than with a separate exposure to lead. Consequently, nootropics not only do not counteract the depressing influence of metals on the specified characteristic of learning, but also aggravate this oppression. The effect of the combination of ascorbic acid with the HM used is of a different nature. The number of ISR when exposed to this combination of agents exceeds the value that is observed when exposed to each of the metals separately.

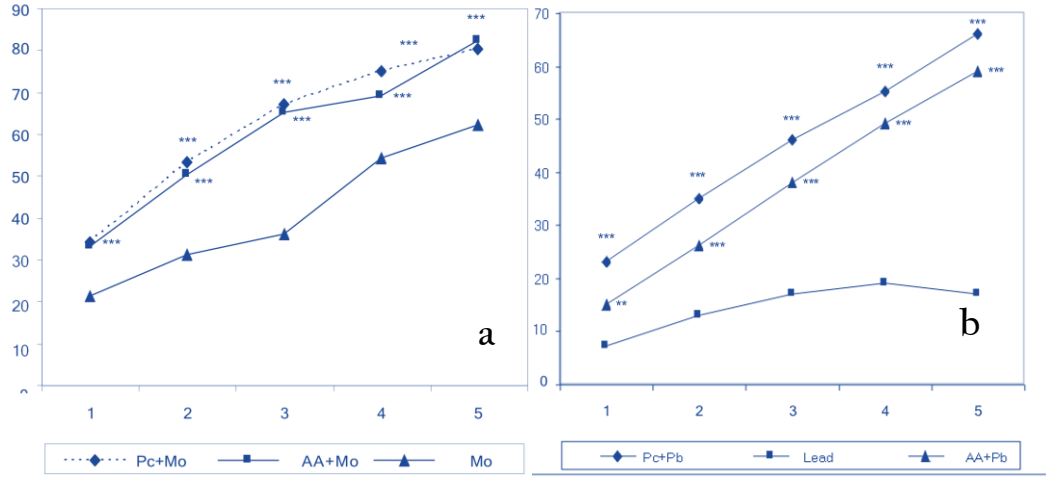

Figure 2. The combined effect of piracetam and ascorbic acid with lead diacetate (a) and ammonium molybdate (b) on the acqisition of the avoidance reaction. Notation – as in Fig. 1.

Since in real life HM works together not only with one, but also with several medicines, we investigated the combined effect of two medicines with molybdenum and lead. As shown above, the combined effect of both piracetam and ascorbic acid with each of the HM leads to the fact that rats develop an avoidance reaction more successfully than it occurs against the background of metal salts, which indicates the positive role of the drugs used. The combined effect of piracetam and ascorbic acid with lead diacetate provided a further increase in the positive effect of the combination of acid with lead, which reached a statistically significant level in the last 2 experimental days (Table 2). At the same time, the effect of the combined effect of two drugs with lead did not exceed the effect of the effect of piracetam with metal.

Table 1. Effect of heavy metal preparations and salts on inter-signal reactions. Note: *,**,*** - p<0.05, p<0.01, p<0.001 relative to control animals; +, ++, +++ - p<0.05, p<0.01, p<0.001 relative to animals receiving the corresponding drug;*, **, *** - p<0.05, p<0.01, p<0.001 relative to animals receiving the corresponding metal.

Agent	Days of experience				
	1	2	3	4	5
Solvent	8,8±1,8	19,2±2,1	29,2±2,8	19,8±1,5	10,4±1,2
Pyracetam	16,6±2,1**	30,6±2,9**	35,8±5,3*	35,8±5,3**	21,4±2,7***
Lead diacetate	2,4±0,9**	3,8±1,2***	4,2±1,7***	3,2±1,0***	2,8±1,4***
Ascorbic acid	3,2±0,9*	4,0±1,1***	2,4±0,9***	2,2±0,7***	3,2±1,0***
Ammonium molybdate	10,0±1,6	7,8±1,0***	6,8±1,2***	4,6±1,1***	5,6±1,0**
Lead diacetate + piracetam	1,8±0,5**, ++	4,4±1,5*,+++	5,2±1,3***,+++	0,6±0,3***, +++, #	4,8±1,1**, +++ ,#
Lead diacetate + Ascorbic acid	3,8±1,1*	8,6±1,7**, +, #	8,4±2,6***, +, #	5,2±2,1***	4,8±1,1***
Ammonium molybdate + piracetam	4,0±0,6*, +++, ##	3,8±0,7***, +++,##	0,6±0,3***, +++, ###	0,6±0,3*****, +++,###	1,0±0,5***, ###, +++
Ammonium molybdate	7,8±1,7+	11,4±2,9*, +	12,6±4,5**, +	10,6±3,3***, ++	9,8±2,4+

+ ascorbic acid					

Table 2. Combined effect of two drugs with heavy metal salts on avoidance reactions and inter-signal reactions. Note: *,**,*** - p<0.05, p<0.01, p<0.001 relative to control animals; #, ##, ### - p<0.05, p<0.01, p<0.001 relative to the combination of the corresponding metal with ascorbic acid; ^,^^,^ - p<0.05, p<0.01, p<0.001 relative to the combination of the corresponding metal with piracetam.

Agent	Days of experience				
	1	2	3	4	5
	avoidance reactions				
Lead diacetate + piracetam	23,0±2,6***	35,2±2,9*	46,2±2,2*	55,4±2,0**	65,8±3,1***
Lead diacetate + ascorbic acid	15,4±2,6*	26,4±2,9	37,8±4,3**	48,5±4,3**	56,2±3,4***
Ammonium molybdate + piracetam	34,0±3,4***	56,2±2,1***	66,6±2,9***	75,2±2,0***	79,6±2,4
Ammonium molybdate + ascorbic acid	33,0±4,5***	50,4±3,4***	64,6±3,1**	88,8±3,1	82,2±1,9
Lead diacetate + ascorbic acid + piracetam	16,8±1,9**	33,0±3,3	46,4±4,7	59,8±3,4#	69,8±3,5***,#
Ammonium molybdate + ascorbic acid + piracetam	16,4±3,0##, ^^^	34,8±3,4*, ##, ^^^	48,0±3,5##, ^^^	60,6±4,1#	75,4±2,0**, #, ^
Inter - signal reactions					
Lead diacetate + piracetam	1,8±0,5***	4,4±1,5***	5,2±1,3***	0,6±0,3***	4,8±1,1***
Lead diacetate + Ascorbic acid	3,8±1,1*	8,6±1,7**	8,4±2,6***	5,2±2,1***	4,8±1,1***
Ammonium molybdate + piracetam	4,0±0,6***	3,8±0,7***	0,6±0,3***	0,6±0,3***	1,0±0,5***
Ammonium molybdate + ascorbic acid	7,8±1,7	11,4±2,9*	12,6±4,5**	10,6±3,3*	9,8±2,4+
Lead diacetate + ascorbic acid + piracetam	4,2±1,2*,##	6,0±1,8***	7,6±2,4***, ##	5,0±2,0***, #,^^	3,2±0,9***
Ammonium molybdate + ascorbic acid + piracetam	5,2±1,6#,^	8,6±1,6***, ^	11,4±3,5***, ^^^	7,2±1,9***, ^^^	3,4±0,8***, #, ^^^

With the combined use of drugs with ammonium molybdate, instead of enhancing the positive effect, the opposite result was observed. In fact, the use of piracetam, ascorbic acid and HM salt combined led to the suppression of avoidance regarding the use of both piracetam and ascorbic acid with this salt.

As shown above, the number of ISR when exposed to piracetam with both ammonium molybdate and lead acetate decreased relative to the effects of each of these salts. The addition of ascorbic acid to this combination changed the situation, and the number of ISR increased.

On the contrary, the combined effect of the two drugs was less than the effect of one ascorbic acid with molybdenum. Consequently, an increase in the number of drugs, in addition to strengthening the counteraction to the negative effects of HM, can lead to a weakening of the influence of each of them on both avoidance reactions and ISR.

5. Conclusions

It was found that lead and molybdenum salts inhibited avoidance and ISR reactions. The combined effect of these salts with ascorbic acid and piracetam led to mixed results. The nootrope counteracted the inhibition of avoidance reactions by metals, but increased the inhibition of ISR, which serve as an important characteristic of learning. In contrast, ascorbic acid reduced the HM-induced inhibition of both avoidance reactions and ISR. The use of 2 drugs with HM led not only to an increase in the positive effect provided by each of the drugs, but also to its weakening. The results obtained indicate that the interaction of drugs and HM, which takes place in real conditions, is fraught with unpredictable and poorly studied consequences and may pose a danger to human and animal health.

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

References

1. Järup L. Hazards of heavy metal contamination. Br Med Bull. 2003;68:167-82.
2. Bakulski KM, Seo YA, Hickman RC, Brandt D, Vadari HS, Hu H, Park SK. Heavy Metals Exposure and Alzheimer's Disease and Related Dementias. J Alzheimers Dis. 2020;76(4):1215-1242.
3. Vellingiri B, Suriyanarayanan A, Selvaraj P, Abraham KS, Pasha MY, Winster H, Gopalakrishnan AV, G S, Reddy JK, Ayyadurai N, Kumar N, Giridharan B, P S, Rao KRSS, Nachimuthu SK, Narayanasamy A, Mahalaxmi I, Venkatesan D. Role of heavy metals (copper (Cu), arsenic (As), cadmium (Cd), iron (Fe) and lithium (Li)) induced neurotoxicity. Chemosphere. 2022; 301: 134625.
4. Inozemtsev AN, Karpukhina OV, Bokieva SB, Gumargalieva KZ. Heavy metals: joint effect with other chemical agents on the central nervous system. Trace elements in medicine 2015, 16(3): 20-28.
5. Cory-Slechta D.A. Studying toxicants as single chemicals: does this strategy adequately identify neurotoxic risk? Neurotoxicology 2005, 26(4): 491-510.
6. Inozemtsev A, Bokieva S, Karpukhina O, Gumargalieva K. Effects of combined treatment with heavy metals and piracetam on learning and memory in rats. Doklady Biological Sciences 2008, 442: 301–304.
7. Jomova K, Vondrakova D, Lawson M, Valko M. Metals, oxidative stress and neurodegenerative disorders. Mol. Cell.Biochem 2010, 345(1-2): 91-104.
8. Paithankar JG, Saini S, Dwivedi S, Sharma A, Chowdhuri DK. Heavy metal associated health hazards: An interplay of oxidative stress and signal transduction. Chemosphere. 2021; 262: 128350.
9. Karpukhina OV. Search for an effective means of protection of cognitive disorders caused by the combined effect of heavy metals and the neurotropic drug piracetam. Karpukhina OV., Gumargalieva KZ., Kostikova NP., Bokieva SB., Inozemtsev AN. Dynamics of chemical and biological processes, XXI century. Publishing House of the Russian Academy of Sciences 2012: 303-312.
10. Karpukhina O, Gumargalieva K, Inozemtsev A. A lecture note on the effect of antioxidant compounds on oxidative stress. Physics and Chemistry of Classical Materials. Applied Research and Concepts 2014, 3: 21-26.
11. Inozemtsev A, Bokieva S, Karpukhina O, Gumargalieva K, Kamenskii A, Myasoedov N. Paradoxical influence of combined effect of semax and ammonium molybdate on learning and memory in rats. Moscow University Biological Sciences Bulletin 2017; 72 (3): 151–154.
12. Kulikova O, Fedorova T, Stvolinsky S, Orlova V, Inozemtsev A. Carnosine prevents the development of oxidative stress under the conditions of toxic action of cadmium. Moscow University Biological Sciences Bulletin. — 2016. — Vol. 71, no. 4. — P. 240–244
13. Karpukhina O, Gumargalieva K, Soloviev A, Inozemtsev A. Effects of lead diacetate on structure transformation and functional properties of piracetam. Journal of Environmental Protection and Ecology 2004, 5(3): 577–582.
14. Inozemtsev AN. Features of the instrumental defensive reaction at the initial stage of its formation. Bulletin of the Moscow University. Biology 1988, 16(4): 60-66.
15. Zaborova V, Zolnikov O, Dzhakhaya N, Bueverova E, Sedova A, Kurbatova A, Putilo V, Yakovleva M, Shantyr I, Kastyro I, Ozimek M, Korolev D, Krikheli N, Gurevich K, Heinrich KM. The study of the relevance of macro- and microelements in the hair of young wrestlers depending on the style of wrestling. Front Endocrinol (Lausanne). 2022; 13: 985297.