ISSN 0012-4966, Doklady Biological Sciences, 2008, Vol. 422, pp. 301–304. © Pleiades Publishing, Ltd., 2008.
Original Russian Text © A.N. Inozemtsev, S.B. Bokieva, O.V. Karpukhina, K.Z. Gumargalieva, 2008, published in Doklady Akademii Nauk, 2008, Vol. 422, No. 5, pp. 700–703.

PHYSIOLOGY

Effects of Combined Treatment with Heavy Metals and Piracetam on Learning and Memory in Rats

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Presented by Academician L.A. Piruzyan March 6, 2008

Received March 6, 2008

DOI: 10.1134/S0012496608050062

A negative influence of heavy metals on many systems of the body including CNS is well known. Employees in the industry of heavy metals suffer from neurodegenerative disorders, including Alzheimer's and Parkinson's diseases [1]. Children who live in industrial cities exhibit signs of retardation of neuropsychic development, including memory, learning, motor function, and speech, as well as a decrease in IQ and other alterations [2–4]. Neuropsychotropic drugs, particularly, nootropics, which enhance learning and memory, are widely used [5].

Studies on the effects of heavy metals and nootropic drugs on the CNS have been performed independently from one another, though combined effects of these substances could be supposed. Specifically, the antioxidant system of the body may be a target for combined action of heavy metals and nootropics. The antioxidant system plays an important role in the response of the CNS to stress, because it prevents excessive activation of free radical oxidation processes, which damage cell membranes and contribute to progression of pathologies that influence many functions. Cells of the CNS are the most vulnerable to free radical processes [6–10].

It has been reported recently that neurotoxic effects of heavy metals are related to their ability to induce lipid peroxidation and change the activity of membrane-bound enzymes [8, 11]. On the other hand, the mechanism of nootropic action is determined by their antioxidant and membrane-protective effects [5, 7]. Melatonin, a hormone of the pineal gland with nootropic properties [12], decreases free radical damage of neurons induced by lead [13]. The above data suggest combined effects of nootropics and heavy metals on the antioxidant system of the brain.

The purpose of this study was to investigate the effects of the reference nootropic drug piracetam and salts of heavy metals on the conditioning of active avoidance response based on electric footshock reinforcement. This response was used as an animal model of learning and memory.

Fourteen groups of male white nonlinear rats weighing 160–200 g at the beginning of the experiment were used for the study. Group 1 was used as a control for groups 2–10, and group 11 was used as a control for groups 12–14 (table). Groups 1–10 consisted of 11 rats each and groups 11–14 consisted of 12 rats each. The experimental chamber was divided into two equal compartments by a wall with a hole. Avoidance conditioning was performed using the following protocol. Each training trial began with 10-s presentation of an acoustic conditioned stimulus followed by electric footshock (0.5–0.7 mA) through the electrified floor of the chamber. The stimuli were presented in the compartment where the rat was located. If the rat did not enter the safe compartment of the chamber during 10 s, both stimuli were turned off. The stimuli were presented again after a 30-s interval. If the rat entered the adjacent compartment during the sound presentation, it avoided the footshock, and the sound was turned off. If the rat entered the adjacent compartment during the footshock, both stimuli were turned off. Each training session consisted of 25 presentations of the stimuli.

During five experimental days, the rats were intraperitoneally injected with one of the solutions of heavy metals and a solution of piracetam at a dose of 300 mg/kg 4 h and 0.5 h prior to avoidance conditioning, respectively. Water solutions of heavy metal salts were used at the following concentrations: 10^{-7} M lead diacetate, 10^{-4} M cobalt sulfate, 10^{-7} M cadmium chloride, and 10^{-5} M ammonium molybdate. Control animals were injected with an equal volume of the solvent 0.5 h prior to training. The results are presented as the mean numbers of avoidance responses and standard errors of the means. The time courses of avoidance learning in groups were analyzed using the one-way nonparametric Kruskal–Wallis test. The differences between groups were estimated using Wilcoxon's test.

The data presented in the table demonstrate that the mean numbers of avoidances in the animals treated

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Effects of piracetam and salts of heavy metals on avoidance response formation

Group	Substance	Experimental days				
		1	2	3	4	5
1	Solvent	11.6 ± 3.6	25.5 ± 4.8	47.6 ± 3.3	67.3 ± 4.3	85.5 ± 1.8
2	Piracetam	20.4 ± 4.5	41.1 ± 4.4 *	61.5 ± 1.8 **	72.9 ± 2.4	89.1 ± 1.8
3	Lead diacetate	13.5 ± 5.6	21.8 ± 6.1	27.3 ± 6.6 **	36.7 ± 5.7 **	32.4 ± 4.5 **
4	Cobalt sulfate	6.2 ± 1.7	16.7 ± 3.0	16.2 ± 3.6 ***	13.1 ± 3.7 ***	10.2 ± 2.9 **
5	Cadmium chloride	8.4 ± 4.5	17.1 ± 5.4	15.6 ± 5.5 ***	14.2 ± 5.9 ***	14.9 ± 4.2 ***
6	Ammonium molybdate	25.8 ± 6.9	36.7 ± 6.3	42.2 ± 7.5	60.7 ± 7.3	64.4 ± 9.7 *
7	Lead diacetate + piracetam	3.6 ± 1.7 *, +	12.7 ± 2.6 *, +++	29.1 ± 5.3 **, +++	39.6 ± 7.3 **, +++	43.3 ± 7.0 ***, #, +++
8	Cobalt sulfate + piracetam	12.4 ± 1.7	20.0 ± 2.2 +	32.0 ± 3.8 *, ##, +++	32.2 ± 5.4 ***, ##, +++	44.0 ± 4.7 ***, ###, +++
9	Cadmium chloride + piracetam	7.3 ± 2.1 +++	15.0 ± 3.9 +++	17.6 ± 3.1 ***, +++	26.9 ± 2.0 **, ##, +++	22.6 ± 4.3 ** , +++
10	Ammonium molybdate + piracetam	22.6 ± 4.5	34.6 ± 5.2	52.7 ± 5.4	58.6 ± 5.8 +	71.1 ± 5.2 +
11	Solvent	12.0 ± 3.2	36.7 ± 2.9	61.3 ± 2.9	79.0 ± 1.7	92.6 ± 0.9
12	Piracetam	21.7 ± 5.1	40.0 ± 4.6	59.0 ± 1.9	73.0 ± 3.4	89.0 ± 1.6
13	Cadmium chloride	10.7 ± 4.3	15.3 ± 5.1 **	14.0 ± 5.2 ***	17.3 ± 6.1 ***	15.7 ± 5.1 ***
14	Cadmium chloride + piracetam	3.7 ± 1.6 **, ++	12.0 ± 3.6 ***, +++	12.7 ± 2.7 ***, +++	18.3 ± 2.6 ***, +++	15.3 ± 3.3 ***, +++

Note: Significant differences between the mean values in each experimental group treated with the drug or metal and the respective control group are indicated by *, +, or #. One, two, or three signs mean the first, second, or third level of significance.

with piracetam were equal to, or even significantly higher than, those in the control animals. These changes were observed in animals of the second group on the second and third experimental days. This is in accordance with the known data on the influence of piracetam on learning and memory under normal conditions [5, 14, 15].

Heavy metals inhibited learning in the animals. Salts of cadmium and cobalt had more pronounced effects. Administration of these compounds led to a lower number of footshock avoidances. The increase in the number of avoidance responses was found on the second experimental day only. Later on, the level of electric footshock avoidances did not elevate, possibly due to the cumulative effect of treatment with these metals. The animals avoided the footshock in 10–17% of all trials. The Kruskal–Wallis test showed the absence of significant increase in the number of avoidances from session to session, which confirmed a strong inhibition of learning by these metals. The inhibiting effect of ammonium molybdate on learning was the weakest, and a significant decrease in the number of avoidances compared to the control was observed on the last experimental day only.

In accordance with the nootropic hypothesis, the effects of piracetam are more expressed when gnostic and mnestic processes occur under adverse conditions. It has been demonstrated in experiments with animals and in clinical practice that nootropics, including piracetam, are more effective during aging; in amnesia induced by maximal electroshock, scopolamine, or hypoxia; in the cases of functional impairments of avoidance conditioning; etc. [5, 14].

The expected protective properties of piracetam could be estimated by its capacity for preventing or attenuating the inhibition of learning induced by heavy metals. The ability of piracetam to prevent inhibition of avoidance was observed only in rats treated with the

molybdenum salt, which had the weakest effect on learning. The number of avoidances in group 10, where the rats were injected with this metal and piracetam, did not significantly differ from the control level on the fifth day only. During the last two days of training, the rats treated with ammonium molybdate prior to piracetam exhibited fewer avoidances than the animals injected with the nootropic alone. In experiments with other groups of animals treated with combinations of substances, piracetam only attenuated the inhibiting influence of heavy metals.

Piracetam decreased the effect of cobalt sulfate during the last three days, and the number of avoidance responses in the rats treated with this metal and piracetam was greater than in the animals injected with the metal salt only. However, avoidances were fewer as compared to the control group. These data show inhibition of learning under these conditions. The number of avoidance responses in the rats treated with cobalt sulfate and piracetam was smaller than in the animals injected with the nootropic only.

Lead diacetate, which was injected to rats of group 3, inhibited learning during three days. Piracetam decreased this effect on the last day only, when the level of avoidance responses in the rats treated with this metal and the nootropic was greater as compared to the animals injected with the metal alone. However, this level was lower than in the control animals. On this day, as well as on others, lead diacetate attenuated the protective action of piracetam, and the level of avoidances was lower in the animals injected with the combination of substances, in comparison with the rats treated with the nootropic alone. The above-mentioned improvement of learning after piracetam treatment, which was observed on the second and third experimental days, was absent after simultaneous administration of lead diacetate and piracetam. Moreover, administration of both substances additionally decreased the number of avoidances during the first two days of training, and the level of these responses remained lower than that in the control group during all five days of training. This effect was not found in the group treated with the metal alone.

Piracetam decreased the inhibition of avoidance learning in rats of group 9, which were injected with cadmium chloride, on the fourth day only. Though the number of avoidances was higher after treatment with both substances as compared to treatment with the metal alone, it was 2.5 times smaller than in the control group. Throughout the period of training, the effect of piracetam was attenuated by preliminary administration of cadmium chloride, so that the numbers of avoidances in groups 9 and 14 were lower compared to groups 2 and 12, which were injected with the nootropic alone. Moreover, simultaneous administration of cadmium salt and piracetam to the rats of group 14 decreased the number of avoidances compared to the control on the first training day. This effect was not found in group 13, which was treated with the metal alone.

These data demonstrate that piracetam protected from the inhibitory effect of heavy metals on learning and memory. However, the protective effect of piracetam was attenuated by heavy metals in many cases. This inability of piracetam to prevent the inhibitory effect of heavy metals contradicts the results of many studies that demonstrated the capacity of nootropics for recovering impairments of learning and memory. Moreover, the inhibition of avoidance was even more expressed after administration of a nootropic drug together with salts of lead and cadmium as compared to the treatment with these metals without piracetam. Our data show that the use of nootropics is dangerous in the regions with high levels of heavy metals, because it may aggravate the neurotoxic effects of the metals on the central nervous system of humans.

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