Communication

Serum Neuropeptide Content in Wistar Rats After Subchronic Low-Dose Exposure to Mercury Acetate

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Serum Neuropeptide Content in Wistar Rats After Subchronic Low-Dose Exposure to Mercury Acetate

Kristina M. Shchepetkova [1,*], Ekaterina G. Batotsyrenova [1,2], Vadim A. Kashuro [1,3], Tatyana Yu. Kretser [1], Dmitry O. Ivanov [1] and Ruslan A. Nasyrov [1]

[1] Federal State Budgetary Institution of Higher Professional Education «St. Petersburg State Pediatric Medical University» of the Ministry of Healthcare of the Russian Federation, Litovskaya str., 2, St. Petersburg, 194100
[2] The Federal State-Financed Institution Golikov Research Clinical Center of Toxicology, Federal Medical Biological Agency, St. Petersburg
[3] The Herzen State Pedagogical University of Russia, St. Petersburg
* Correspondence: tesh_07@inbox.ru

Abstract: Mercury poisoning has a cumulative effect. Mercury gradually accumulates in tissues and organs, which negatively affects the functions of all body systems, especially the brain. The aim of this study was to investigate the neuropeptides content in the blood serum of Wistar rats after subchronic low-dose mercury acetate poisoning. During the study, rats was intragastrically administered mercury acetate at a dose of 4 mg/kg for 30 days. On days 30 and 44, the blood serum of rats was tested for the levels of calcium-binding protein (S-100), glial fibrillary acidic protein (GFAP), neuron-specific enolase (NSE), pigment epithelial-derived factor (PEDF), myelin basic protein (MBP). The concentration of S-100 protein in the blood serum of the experimental group of rats decreased by 43.9%, and the concentration of MBP increased by 172.6%. Fourteen days after the end of the toxicant administration, the concentration of protein S-100 in the blood serum of poisoned animals was significantly lower by 132.7% compared to the control group. The content of MBP increased by 59.4% and neuron-specific enolase increased by 44.6%, the concentration of neuropeptides PEDF and GFAP changed insignificantly. The results obtained demonstrate that even low concentrations of mercury acetate, entering the body for a long time, affect the concentration of neuropeptides in blood serum, which indicates negative changes in the central nervous system of rats.

Keywords: brain; neuropeptides; mercury acetate; subchronic poisoning; neurotoxicity

1. Introduction

Environmental pollution with heavy metals is a serious public health concern. Mercury is one of the most common heavy metals, and its amount in the environment is increasing not only due to natural sources but also as a result of human economic activities. The main anthropogenic source of mercury is chemical and metallurgical industry [1]. Together with groundwater and dust, mercury spreads over long distances and can be found even in remote regions where there are no direct atmospheric emissions. The problem of mercury contamination of residential buildings, including schools and preschools, is particularly relevant. Acute mercury poisoning is quite rare. However, prolonged exposure to mercury is very dangerous for humans due to its cumulative properties. Consuming contaminated water, food and seafood leads to mercury accumulation in the human body and can damage many body systems, including the central nervous system [2–4]. Exposure to mercury can cause neurodegenerative diseases such as Parkinson's and Alzheimer's [5–7]. There is evidence of long-term effects in children who have had mercury poisoning in the perinatal period [8]. The result of such exposure is a decrease in intelligence, the development of autism spectrum disorders, attention deficit hyperactivity disorder and other impairments [9].

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The toxic effects of mercury are mainly due to the formation of stable complexes of mercury with thiol and selenium groups of proteins, as well as low-molecular weight molecules that are of great biological importance for the cell [10]. An important mechanism of toxicity is also the accumulation of reactive oxygen species and the development of mercury-induced oxidative stress caused by a decrease in the antioxidant protection of the cell [11].

It has been shown that mercury has a high capacity for bioaccumulation in the long-term period after poisoning. Inhibition of the antioxidant defense system, accompanied by an increased concentration of lipid peroxidation products, was detected in the long-term period after acute single mercury nitrate poisoning of experimental animals [12]. This form of intoxication was accompanied by a decreased concentration of reduced glutathione, a decrease in superoxide dismutase and glutathione peroxidase activity, and an increase in malondialdehyde concentration.

Chronic exposure to mercury acetate also led to changes in the activity of the antioxidant system enzymes in red blood cells: significantly increased activity of superoxide dismutase and glutathione peroxidase, and decreased activity of glutathione transferase [13]. The accumulation of diene conjugates indicated an enhanced processes of lipid peroxidation in rat erythrocyte membranes. Alterations in the antioxidant status of red blood cells can lead to endothelial dysfunction. In turn, the integrity and functionality of the endothelium are important for the normal functioning of the brain, since capillary endothelium is the basis of the blood–brain barrier [14].

Assessment of the rats' cognitive skills in the conditioned active avoidance response swimming test (CAR) showed a decrease in memory trace consolidation in the group of animals exposed to mercury acetate poisoning [15].

Endogenous regulatory proteins in the brain that provide neuroprotective effects also play a significant role in higher nervous activity, including processes of short-term and long-term memory formation. Studying neuropeptides in the brain can help to understand disorders in CNS functions after prolonged low-dose exposure to mercury.

The aim of this study was to investigate the content of neuropeptides in the blood serum of laboratory animals after subchronic low-dose mercury acetate poisoning.

2. Results

The results of determination of the neuropeptides content in the blood serum of rats after subchronic administration of mercury acetate for 30 days are summarized in Table 1.

Table 1. Concentration of neuron-specific markers 30 days after subchronic administration of mercury acetate.

Parameters investigated	Animal groups	
	Control group	Experimental group
S-100, ng/ml	7,95±1,28	4,46±0,51*
NSE, mcg/ml	11,7±1,1	13,9±1,2
GFAP, pg/ml	22,6±3,6	23,0±5,4
MBP, ng/ml	12,4±4,0	33,8±10,1*
PEDF, ng/ml	141,8±19,6	125,0±23,1

* – significantly compared to the control group (at $p \leq 0.05$; Mann–Whitney U test).

It was found that 30 days after subchronic exposure to mercury acetate, the concentration of neurospecific proteins in the blood serum of rats from the experimental group differed significantly from the control group in a number of indicators. Thus, the concentration of the calcium-binding protein (S-100) in the blood serum of experimental animals was reliably lower by 1.8 times compared to the control group. The content of myelin basic protein (MBP) increased by 2.7 times, indicating the destruction of myelin sheaths. The concentration of the neuroprotective protein PEDF decreased slightly.

The results of measuring the concentration of neuropeptides in the blood serum of rats 14 days after the last administration of mercury acetate are presented in Table 2.

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Table 2. Concentration of neuron-specific markers 14 days after the last administration of mercury acetate.

Parameters investigated	Animal groups	
	Control group	Experimental group
S-100, ng/ml	7,26±0,76	3,12±0,81*
NSE, mcg/ml	11,51±0,71	16,64±0,76*
GFAP, pg/ml	21,61±2,27	26,36±2,27
MBP, ng/ml	13,99±1,57	22,30±2,07*
PEDF, ng/ml	111,00±7,47	130,33±9,03

* – significantly compared to the control group (at $p \leq 0.05$; Mann–Whitney U test).

It was found that 14 days after the last administration of mercury acetate, the concentrations of neurospecific proteins in the blood serum of poisoned animals were still altered compared to the control group, and some indicators changed more significantly. Thus, in the group of poisoned animals, the concentration of S-100 protein was reliably lower by 1.3 times, while the content of myelin basic protein increased by 59.4% compared to the control group. Also, on day 44 of the study, the content of neuron-specific enolase turned out to be 44.6% higher in the experimental group of rats compared to the control group.

3. Discussion

In clinical practice, the determination of the neurospecific proteins concentration in blood serum is used to diagnose various pathological conditions of the central nervous system, such as traumatic brain injury, neurodegenerative diseases, mental disorders, etc.

The group of neurospecific proteins includes the calcium-binding protein S-100, which regulates the level of intracellular calcium. This protein is one of the functional activity markers of neuroglia cells. Nanomolar concentrations of S-100 protein have an autocrine effect on astrocytes, stimulating their proliferation in vitro [16], while its dimer modulates long-term synaptic plasticity [17] and has a trophic effect on both developing and regenerating neurons [18]. In micromolar concentrations, homo- or heterodimers of extracellular S-100 can have neurotoxic effects on neurons and glia, inducing both apoptosis and cell necrosis [19]. The latter effect is based on the ability to induce pro-inflammatory cytokines, oxidative stress enzymes, in particular iNOS, and amplify other signals directed at neurons and glial cells [20]. Contradictory data on the concentration of S-100 protein in blood in cases of heavy metal poisoning can be found in the literature. An increase in the concentration of S-100 in the blood of laboratory animals is observed when exposed to lead, metallic and organic forms of mercury, and arsenic [21]. Chronic intoxication caused by inhalation of metallic mercury vapors leads to a decrease in the expression of S-100 protein in the nervous tissue of white rats [22]. The immunohistochemical method revealed a decrease in the S-100 content even long after mercury vapor intoxication [23].

In our study, enzyme-linked immunosorbent assay (ELISA) demonstrated a significant decrease in the concentration of calcium-binding protein S-100 in blood serum of the experimental animal group 30 days after chronic mercury acetate intoxication and 14 days following the last administration of the toxicant. These results may be due to mercury's impact on calcium metabolism in cells by reducing the rate of S-100 protein synthesis, which indicates a reduction in neurotrophic activity of nervous tissue.

Mercury actively binds to the sulfhydryl groups of various proteins that decreases the activity of many enzymes, including the cell's antioxidant defense system. Moreover, binding of mercury to respiratory chain enzyme complexes leads to decoupling of respiration and phosphorylation processes and, consequently, to hyperproduction of reactive oxygen species (ROS). The excess ROS leads to an intensification of lipid peroxidation in biological membranes of the cells with the formation of highly cytotoxic reactive aldehydes such as malondialdehyde. The accumulation of ROS and the enhanced lipid peroxidation lead to the destruction of myelin sheaths of nerve cells. Therefore, the increase in the concentration of myelin basic protein in blood serum of the

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experimental animal group after 30 days of mercury acetate administration indicates neurodestructive processes. After 14 days, the neurodestructive effect of mercury acetate still persists, which is manifested in an increased concentration of myelin basic protein in the group of poisoned animals compared to the control values.

The literature data on the concentration changes of other neurospecific proteins after poisoning with heavy metals are also ambiguous. Exposure to mercury, lead, and cadmium causes pathological proliferation of glial tissue, which manifests itself in an increase in the content of GFAP in animal blood [24–26]. Exposure of laboratory rats to a mixture of heavy metals (arsenic, lead, and cadmium) leads to a decrease in GFAP expression [27]. Determination of the neuron-specific enolase content is used as a marker of changes in the permeability of the blood–brain barrier and oxidative damage to brain cells [28,29]. There is an increased expression of neuron-specific enolase (NSE) after prolonged exposure to mercury [22,23,30]. There is no data on changes in the NSE content in the blood after exposure to other heavy metals. Concentration changes in the pigment epithelial-derived factor (PEDF) were detected after exposure to barbiturates [31].

In our study, after 30 days of daily exposure to mercury acetate, the levels of GFAP, NSE and PEDF in the blood serum of experimental animals did not show any statistically significant differences compared to the control group. However, on the 14th day after the last administration of mercury acetate, a significant increase in the concentration of NSE in the blood serum of the experimental group of animals was observed. This fact indicates an increase in the rate of glycolysis in CNS tissues due to energy deficiency in the long-term period after chronic poisoning. In addition, this marker may indicate a change in the permeability of neuronal cell membranes. The data obtained are consistent with the results of a study on the apoptotic proteins expression in brain tissues indicating the activation of apoptosis processes [32] after exposure to neurotoxicants.

The results obtained show the important role of neurospecific peptides in the pathogenesis of cognitive impairment during mercury intoxication. After conducting additional research, the studied indicators can be used as convenient markers of CNS disorders in chronic heavy metal poisoning.

4. Materials and Methods

4.1. Animals

The study used sexually mature male Wistar rats weighing 160–200 g, which were bred at the Rappolovo nursery (Russia). The experimental animals were kept at the Testing Center for Preclinical Research of the Golikov Research Center of Toxicology in accordance with international requirements [33]. The laboratory animals were kept under standard quarantine conditions for 14 days. The relative humidity in the vivarium premises was maintained at 50–65%, and the air temperature was 20–25 °C. The following light regime was observed: 12 hours — day; 12 hours — night. The age of the animals at the time of the experiment was one month. Throughout the experiment, the rats had free access to food and drinking water.

4.2. Structure of the Experiment

The rats were divided into two groups: control and experimental. The animals in the control group received 1 ml of distilled water intragastrically daily throughout the study. The animals in the experimental group were administered an intragastric dose of 0.08% aqueous solution of mercury acetate at a dose of 4 mg/kg (0.1 LD50) for 30 days. The low-toxic dose for administration to the animals of the experimental group was determined according to the toxicity data sheet of mercury acetate (the semi-lethal dose with the intragastric route of administration for laboratory rats is 40.9 mg/kg). Thus, over the whole period of toxicant administration, each experimental animal received a total of 120 mg of mercury acetate per kg of animal body weight, which corresponds to 18 mg of mercury per kg.

On the 30th and 44th days, the animals were euthanized in a CO_2 chamber followed by decapitation. The procedure was carried out in accordance with international requirements [33,34] and standard operating procedures developed at the Testing Center for Preclinical Research of the

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Golikov Research Center of Toxicology under the supervision of a veterinarian from the testing center.

4.3. Blood and Tissue Sampling

Blood samples for biochemical studies were taken into Vacuette plastic blood collection tubes (Austria) containing EDTA potassium salt. The samples were incubated for 30 minutes at a temperature of +4 °C, and then centrifuged at 3000 rpm for 10 minutes. Serum without signs of hemolysis was used for biochemical studies.

4.4. Enzyme-Linked Immunosorbent Assay (ELISA)

The serum neuropeptides were determined by enzyme-linked immunosorbent assay (ELISA) using ELISA Kit sets from Cloud-Clone Corp. (USA). The following sets were used: «S-100 EIA», «Glial fibrillary acidic protein (GFAP)», «Neuron-specific enolase (NSE)», «Pigment epithelium-derived factor (PEDF)», and «Myelin basic protein (MBP)».

4.5. Statistical Data Processing

Statistical processing of the results was carried out using Statistica 6.0 software. Average values and mean errors (M±m) were calculated for a sample of n=10, and the level of statistical significance of differences in average data was assessed using the Mann-Whitney U-test at a significance level of 0.05.

5. Conclusions

The results of determining the neurospecific markers concentration in the blood serum showed that chronic mercury acetate poisoning was accompanied by an imbalance between neurodestruction and neuroprotection processes. A decrease in S-100 protein concentration reflects a disruption in calcium metabolism in neurons, which can lead to impaired learning and memory. An increase in MBP concentration indicates the activation of demyelination processes in nervous system tissues, which can result not only in impaired cognitive functions but also in motor function impairment. The increased content of neuron-specific enolase is a consequence of energy deficiency in nerve tissue. Thus, mercury accumulates in the rat brain and has toxic effects on the central nervous system even at low exposure levels over a long period.

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