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EFFECTS OF CADMIUM ON THE ACTIVITY OF MATRIX METALLOPROTEINASES AND METALLOTHIONEIN LEVEL IN THE RAT BRAIN

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We studied the effects of long-lasting treating with cadmium in two different doses (0.1 and 1.0 μg/kg) on the activity of matrix metalloproteinases (MMP2 and MMP9) and metallothionein level in the rat brain. Cadmium in a dose of 1 μg/kg caused a decrease in MMP2 and increased the proMMP9 activity in the brain. The level of MT in the hippocampus and cerebellum dropped under usage of Cd different doses. Thus, even small Cd doses exert specific effects on the MMPs activity and MT level in the brain.

Keywords: metalloproteinases, MMP2, MMP9, metallothionein, rat brain, cadmium.

INTRODUCTION

Among the exogenous risk factors for the development of neurodegenerative diseases, special attention should be paid to cadmium (Cd), a metal widely used in the industrial production of alloys and pigments and in the electrical industry. Cadmium in the air may appear as by-products of smelting lead and zinc, due to burning of plastics, and due to disruption related to utilization of cadmium-nickel batteries. The intake of Cd in the body is also possible with cigarette smoke and with food; blood absorbs up to 40-50% Cd entered by inhalation and up to 3-7% when eating [1]. The effect of Cd is long-lasting as it is slowly excreted from the body. The Cd half-life in the liver is 19 years, and in the kidneys it is 38 years. This heavy metal can be accumulated in bones, kidneys, pancreatic and prostatic glands, testicles, and placenta [2]. A growth in its content in tissues can lead to the development of renal failure and emphysema; it increases the risk for coronary insufficiency, cancer, and neurodegenerative diseases.

Cadmium can displace zinc and copper ions from metal-containing enzymes and proteins. Such substitution leads to inhibition of the activity of the latter and the development of pathological conditions. However, the effect of Cd on the activity of Zn^{2+}-containing matrix metalloproteinases (MMPs) and metallotioneins (MTs) has not practically been investigated. Many pathological processes are associated with the disturbance of MMP activity; these are oncogenesis, atherosclerosis, and tissue fibrosis. These enzymes are involved not only in degradation of the intercellular matrix, but also in the processes of cell growth and migration, regulation of signaling pathways, and formation of the endothelium and atherosclerotic plaques. Gelatinases A (MMP2) and B (MMP9) have a broad substrate specificity and play an important role in the development of cardiovascular and neurodegenerative diseases.

Metal-binding metallothioneins are found in a vast population of organisms. These proteins are very rich in cysteine residues and do not manifest the enzymatic activity [3].

The aim of our work was to estimate the effects of low doses of Cd on the activity of MMP2 and MMP9 and on the metallothionein level in the rat brain.

METHODS

Wistar rats (6 month old, weighing 190-200 g) were randomly divided into three groups *(n* = 6 in each). These were group 1 (control animals kept under standard conditions on a standard diet), group 2 (rats given a cadmium-containing diet, 0.1 μg/kg body mass), and group 3 (animals given a diet with a greater content of cadmium, 1.0 μg/ kg body mass).

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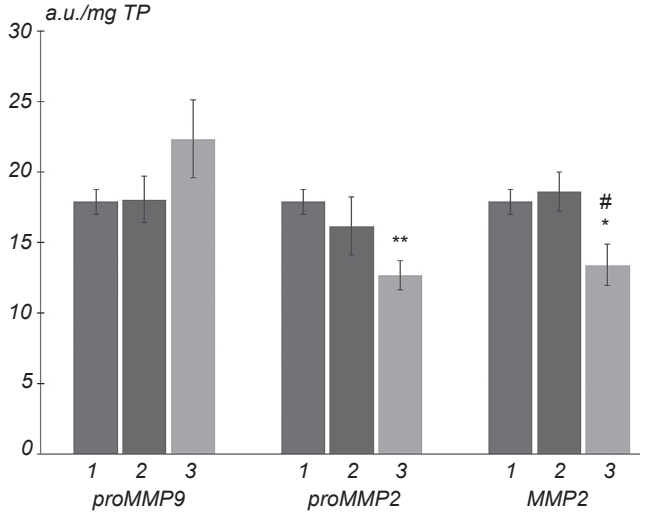
18 animals from the respective groups were used in the experiments. High-purified $CdCl_2 \cdot 2.5H_2O$ (Sigma, USA) and drinking water for babies containing no cadmium ions as a solvent were used for preparation of the cadmium solution. The latter was introduced perorally to the rats once a day before feeding. Water and food were freely available. The experiment lasted 37 days. At the end of the experiment, the animals were decapitated under thiopental anesthesia. The fraction containing water-soluble proteins was obtained by ultracentrifugation. The initial buffer contained 0.25 mM Tris (pH 7.4), 1.0 mM EDTA, 2.0 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride (PMSF), and 3 mM sodium azide (NaN_3) (Sigma, USA).

Activities of gelatinases A and B (MMP2 and MMP9, respectively) were estimated using direct enzyme zymography after vertical electrophoresis of the samples in 7.5% PAG containing 0.1% SDS and 1% gelatine (Sigma, USA). The zymograms were digitized, and gelatinase activity was calculated using the Videodensitometer Sorbfil 2.0 program. This activity was measured in arbitrary units (a.u.) relative to the respective activities in a standard sample taken as 1 a.u. Protein concentrations in brain tissues were measured by the Bradford method. The specific activity of the studied enzymes was calculated per 1 mg of protein.



F i g. 1. Relative average activities of gelatinases (a.u./mg total protein) in the fraction of soluble brain proteins. 1) Control group of the animals kept under standard conditions, 2 and 3) animals obtaining a cadmium-containing diet, 0.1 and 1.0 μg/kg body mass, respectively; $n = 6$ in all groups, $^{*}P < 0.05$, $^{**}P < 0.01$ in comparison with the control group; $^{\#}P < 0.05$ (in comparison the 2nd and 3rd groups.

Р и с. 1. Середня відносна активність желатиназ (ум. од./мг протеїну) у фракції розчинних церебральних протеїнів.

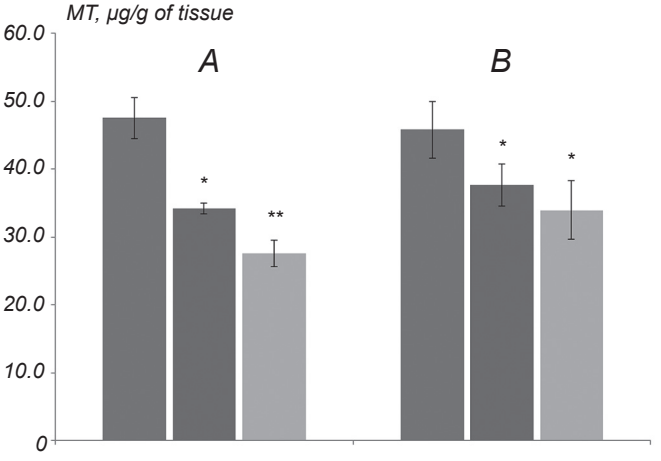
The metallothionein contents in the hippocampus and cerebellum were measured by ELISA using a monospecific antibody to MT (Santa Cruze Biotechnology, USA).

Statistical processing of numerical data was performed using «Microsoft® Excel 2000» (Microsoft®) and «STATISTICA® for Windows 6.0» (StatSoft Inc.). Intergroup differences were estimated by the Student *t*-test and Mann–Whitney criterion for small samplings. Values with $P \leq 0.05$ were considered as significant.

RESULTS AND DISCUSSION

The relative activity of gelatinases in the fraction of soluble brain proteins from rats treated for a long time with cadmium at a dose of 0.1 μg/kg remained practically unchanged. At the same time, in rats receiving higher doses of cadmium (1 μg/kg), the activity of proMMP9 increased to 22.4 ± 2.8 a.u./mg total protein (TP) in comparison with 17.9 ± 0.8 a.u./mg TP, with a decrease in the latent and mature forms of MMP2 to 12.7 ± 1.0 and 13.4 ± 1.5 a.u./mg TP, respectively. The respective values in the control group were 18.1 ± 0.9 and 17.8 ± ± 0.7 a.u./mg TP (Fig. 1).

The MT level in the hippocampus was found to significantly drop (to 58% in the 0.1 μg Cd group and to 42% in the 1.0 μg Cd group, as compared to the control). The same trend was found in the cerebellum, but the decrements were smaller than in the hippocampus (Fig. 2).



F i g. 2. The level of metallothionein, μg/g of tissue in the rat brain (A – hippocampus, B – cerebellum) under conditions of Cd intoxication. Designations are similar to those in Fig 1.

Р и с. 2. Рівень металотіонеїну (мкг/г тканини) у мозку щурів (*А* – в гіпокампі, *В* – у мозочку) в умовах інтоксикації кадмієм.

The intake of cadmium into the body occurs through the respiratory and digestive tracts with the help of specific metal transporters (for zinc, iron, magnesium, and calcium), such as Zrt, Irt-like protein (ZIP-8), and a divalent metal transfer protein (DMT-1) [2]. With the blood flow, cadmium is transported as a complex with metallothioneins, which have a great affinity for heavy metals and perform a protective role [4]. Accumulation of this metal in brain tissues can affect the state of matrix proteins and alter intercellular interactions, resulting in pathological shifts.

Cadmium is accumulated primarily in the liver and kidneys where it is bound to MT; it is believed that cadmium bound to MT is essentially detoxicated (at least temporarily) through this high-affinity sequestration [5]. A number of functions have been suggestively allocated to MT; among those, there are homeostasis and transport of physiologically essential metals (Cu, Zn), metal detoxication (Cd, Hg), protection against oxidative stress, regulation of cell proliferation and apoptosis, protection against neuronal injury and degeneration, and regulation of neuronal outgrowth [6]. Recently, researches showed that MT 1E could enhance tumor proliferation and invasion of malignant glioma, and these effects are mediated by regulation of activation and expression of MMPs [7].

It is known that toxic metals/xenobiotics (mercury, lead, arsenic, cadmium, etc.) are able to form covalent bonds with sulfhydryl groups in the molecules of glutathione, cysteine, and homocysteine and to reduce antioxidant capabilities of the latter. This leads to increase in the amount of active forms of oxygen and to activation of lipid peroxidation. The respective influences lead to damage of the cell membranes and microtubules, oxidation of amino acid residues in protein molecules, and changes in the conformation and biological activity of the latter.

Cadmium mimics the effect of zinc and, thus, can be involved in the processes where normally zinc participates. As is known, transition of MMPs from the zymogenic form to the active one takes place with the participation of zinc.

It was found that Cd affects the MMP9 activity in endothelial cells; it increases the production of reactive oxygen species and phosphorylation of epidermal growth factor (EGFR). This leads to activation of Erk1/2 and JNK1/2 kinases, phosphorylation of the AP-1 transcription factor, activation of Akt protein kinase, and activation of NF-κB transcription factor. These shifts ultimately induce an increase in the MMP9 levels [8].

When studying the effect of Cd (in animals exposed of 10 μM Cd for 8 weeks) on prostate epithelial cells, it was found that this metal causes degeneration of above cells and their conversion into tumor cells accompanied by a significant increase of gelatinase activity. The MMP2 activity increased to 250%, while that of MMP9 increased fourfold [9].

Lacorte et al. [10] showed that small doses of Cd (15 ppm) for 20 weeks induced a decrease in the MMP2 and MMP9 activity in the prostate. Exposure of 5 or 20 mM $CdCl_2$ caused inhibition of these activities by 80% and 100%, respectively.

Our data allow us to conclude that prolonged (for 37 days) Cd introduction in a small dose (0.1 μg/kg) leads to a significant decrease in the level of MT in the brain but does not change the MMP2 and MMP9 activity. It can be provided by removal of a Cd-binding form of MT from the brain (detoxication). However, prolonged Cd exposure with 1.0 μg/kg results in decreases in both MT and MMP2 levels but to an increase in the activity of MMP9.

The possible mechanism of the effects of different doses of cadmium on the expression and activity of gelatinases can be connected with the increasing reactive oxygen species production and the replacement of zinc in the active site of these enzymes.

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ВПЛИВ КАДМІЮ НА АКТИВНІСТЬ МАТРИКСНИХ МЕТАЛОПРОТЕЇНАЗ ТА РІВЕНЬ МЕТАЛОТІОНЕЇНУ В ГОЛОВНОМУ МОЗКУ ЩУРІВ

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Р е з ю м е

Досліджували впливи тривалого введення кадмію в різних дозах (0.1 і 1 мкг/кг на добу) на активність матриксних металопротеїназ MMP2 та MMP9, а також рівень металотіонеїнів (МТ) у головному мозку щурів. Пероральне введення кадмію в дозі 1 мкг/кг викликало помітне зниження активності MMP2, але підвищувало активність про-MMP2. При тривалій дії кадмію в зазначених дозах рівень МТ у гіпокампі й мозочку значно знижувався. Таким чином, кадмій навіть у малих дозах спричиняє дозозалежну і специфічну для різних відділів мозку дію.

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