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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 higher dose (1 μg/kg) caused a decrease in MMP2 activity but increased that of proMMP9 in the brain. The level of MT in the hippocampus and cerebellum dropped with both Cd doses. Thus, even small Cd doses exert specific effects on the MMP activity and MT level in the brain.

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

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 entering 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 substitute zinc and copper atoms in 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 metallothioneins (MTs) has practically not 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, formation of the endothelium, and that of atherosclerotic plaques. Gelatinases A (MMP2) and B (MMP9) are characterized by 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 enzymatic activity [3].

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

METHODS

Eighteen 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),

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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). Highly-purified $CdCl_2 \cdot 2.5\ H_2O$ (Sigma, USA) and drinking water for babies containing no cadmium ions (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 Videodensitometer Sorbfil 2.0 software. This activity was measured in arbitrary units (a.u.) relative to the respective activities in a standard sample taken as 1.0 a.u. Protein concentrations in brain tissues were measured using the Bradford method. The specific activity of the studied enzymes was calculated per 1 mg of protein.

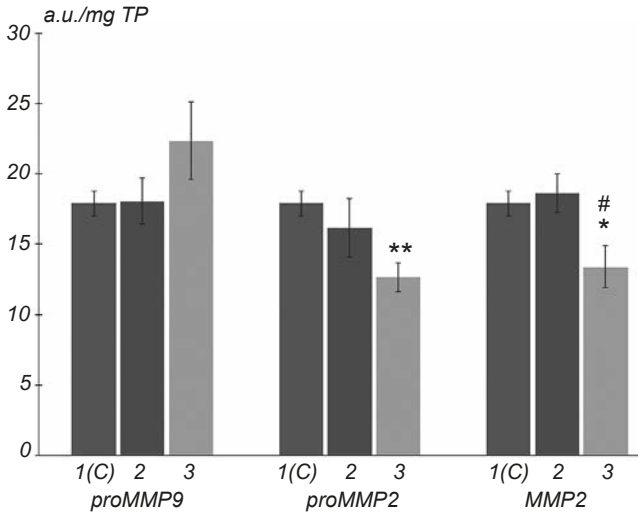
The metallothionein contents in the hippocampus and cerebellum were measured by ELISA using a monospecific antibody to MT (Santa Cruz Biotechnology, USA).

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

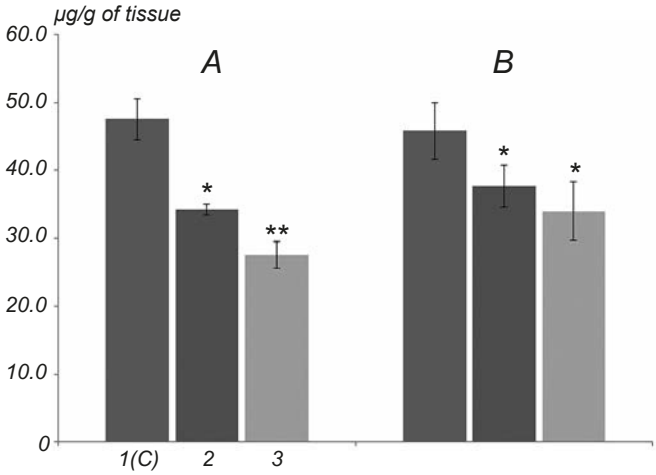
RESULTS AND DISCUSSION

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 (1.0 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. There were parallel decreases in the latent and mature forms of MMP2 to 12.7 ± 1.0 and 13.4 ± 1.5 a.u./mg TP, respectively (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 drop significantly (to 72% in the 0.1 μg Cd group and to 58% 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. 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.



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.