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# DIFFERENCES IN THE CONTENT OF METALLOTHINEIN IN RODENT BRAIN UNDER POSTNATAL DEVELOPMENT AND CADMIUM INTOXICATION

***Introduction.*** *Metal-binding metallothionein genes are found in a vast population of organisms. These proteins are non enzymatic and very rich in cysteine residues. The various metallothionein isoforms in different brain regions arguably change over time.*

***The aim of the study*** *– evaluating the distribution of metallothionein in different brain regions of gerbils and Wistar rats at different stages of postnatal development (PND), under standard and low dose Cd-induced conditions.*

***Materials and Methods.*** *18 Mongolian gerbils and 36 Wistar rats were divided into 6 groups (n=6), by age and condition of experiment: groups 1, 2, 3, 4 – 1, 30, 90 and 180-days old were exposed to standard conditions; group 5 and 6 – 180-days old+0.1 or 1.0 μgμg $Cd^{2+}$ per animal everyday for 36 days. The metallothionein content in the hippocampus, cerebellum, and thalamus were detected by the ELISA.*

***Results and Discussion.*** *Obtained data was shown the dynamic of metallothionein distribution in different brain regions of gerbils and Wistar rats depending on the stage of postnatal development and functional capacities. The content of metallothionein in the hippocampus continually decreased in both animal types but the cerebella metallothionein distribution pattern was different from that of the hippocampus, but identical in both rodents, rising from day one before decreasing on day 30. The low levels of metallothionein under the influence of Cd were proportional to the doses administered.*

***Conclusions.*** *The level of metallothionein in the brain varies depending on the stage of development of the functional capacities of the brain parts. The significant down-regulation of metallothionein in the investigated brain regions under Cd influence suggests that a decrease in metallothionein levels depends on the dose of Cd and on the time necessary for its accumulation.*

KEY WORDS: **metallothionein; brain; postnatal development; gerbil; rat; Cd.**

INTRODUCTION. Metallothionein (MT) is a metal-binding, non-enzymatic protein found in many organisms, including vertebrates, invertebrates, higher plants, protozoa, yeasts, and some prokaryotes [1]. Since the first isolation of MTby Margoshes and Vallee in (1957) [2], many experimental reports have subsequently illustrated the probable locations and functional properties of its isoforms in various parts of different organisms, such as in the midgut gland of terrestrial snail *Helix pomatia* [3], in Freshwater Mussel *Anodontawoodiana* [4], and in many more [1].

In eukaryotes, MT genes are present in multiple copies, while not every prokaryote is capable of manifesting MT genes. Mammalian MT consists of four isoforms; MT-I, MT-II, MT-III, and MT-IV. Humans have at least 16 MT genes clustered on chromosome 16. 7 functional MT-I genes (MT-IA, -B, -E, -F, -G, -H, and -X) and a single gene encoding each of the other MT isoforms, namely MT-II (the MT-IIA gene), MT-III, and MT-IV constitute the MT genes that are tightly clustered in the q13 region of chromosome 16 [5]. Most recent findings have suggested that the first two members of the mammalian MT isoforms are equally of significant importance in the brain [6]. The extensively studied MT gene structure in mice is much simpler. Only one functional gene exists for each isoform MT-I to MT-IV, and they are all located on chromosome 8.

MT is remarkably rich in cysteine residues. These residues manage the exchange and maintenance of zinc (Zn) and copper (Cu) under physiological conditions, a function known as metal homeostasis. Besides metal homeostasis, other functions of MT in the central nervous system (CNS) include transport of physiologically essential metals (Cu, Zn), metal detoxification (Cd, mercury (Hg)), the protection against oxidative stress, the maintenance of intracellular redox balances, the regulation of cell proliferation and apoptosis, the protection against neuronal injury and degeneration, and the regulation of neuronal outgrowth [5].

Cadmium (Cd) is one of toxic transition metal of continuing occupational and environmental concern, and its exposure leads to a variety of adverse effects. The extremely long biological half-life of Cd essentially makes it a cumulative toxin, so long past exposures could still result in direct toxic effects of the residual metal. Unfortunately, there are no proven effective treatments for chronic Cd intoxication. The metabolism of toxic metals often is dictated by the essential elements they may mimic. Cd appears to mimic Zn and to a lesser extent calcium (Ca). The toxic effects of Cd often stem from interference with various Zn mediated metabolic processes, while Zn treatments frequently reduce or abolish the adverse effects of Cd. The various regulatory agencies have concluded that there is adequate evidence that Cd is a human carcinogen. This designation was largely prompted by repeated findings of a link between occupational Cd exposure and lung cancer, as well as very strong data in rodents showing the pulmonary system as a target site after Cd inhalation [7].

The objectives of our study were 1) the evaluation of quantitative distribution of MT in the differ brain regions (hippocampus and cerebellum) of gerbils and Wistar rats; 2) and, the effect of low doses (0.1 and 1.0 μg) of Cd on the levels of MT in the said brain regions of rodent.

MATERIALS AND METHODS. 18 gerbils (Mongolian gerbils) and 36Wistar rats were divided into 3 and 6 groups (n=6), by age and condition of experiment: group 1 – newborns (1-day old);groups 2, 3, 4 – 30, 90 and 180-days old were exposed to standard conditions; group 5 – 180-days old+0.1 μg $Cd^{2+}$ per animal everyday for 36 days and group 6 – 180-days old+1.0 μg $Cd^{2+}$ per animal everyday for 36 days were used for the comparative experiment. The rats were placed under standard conditions with natural changing of lights and with compliance to general emergency diet. All the animals had free access to food and water. The experiment was carried out in conformation with the regulations on the use of animals in biochemical research [8]. The administration of Cd was carried out intragastrically through a sterile stainless steel probe integrated with adjustable dispenser. The two doses were prepared from high-purified $CdCl_2x2.5H_2O$ (Sigma, USA) with pure deionized water "Malyatko" (Econiya, Ukraine) and administered daily.

At the end of the experiment, the rats were decapitated under mild anesthesia (thiopental, 60 μg/kg). The hippocampus and cerebellum were isolated, and used to produce soluble protein fractions. The fractions were obtained by differential centrifugation [9]. The output buffer contained 25 mM Tris (pH 7.4), 1.0 mM ethylenediaminetetraacetate (EDTA), 2.0 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride (PMSF), 0.01 % sodium nitride ($NaN_3$), (indicated reagents were purchased from Sigma, USA).

The MT content in the hippocampus and cerebellum were determined by the method of solid-phase enzyme-linked immunosorbent assay (ELISA),using polyclonal monospecific antibodies against the MT (Santa Cruz Biotechnology Inc., USA), purified MT-standard, and secondary antibodies against rabbit IgG conjugated with horseradish peroxidase (Sigma, USA). The results were obtained using ELISA reader Anthos 2010 (Finland) at 492 nm. The amount of MT was expressed in micrograms per 100 mg of tissue.

Statistical processing of the data was performed using the Student's t-test and the difference was considered significant at $p<0.05$.

RESULTS AND DISCUSSION. The evaluation for the quantitative distribution of MT in the hippocampus and cerebellum of gerbils and rats, at different ages post birth, presented matching results with respect to the patterns shown by both mammals, but different results when the different brain regions were compared to each other. The level of MT in the hippocampus of the newborn was higher than the level of MT in any other region at any other stage. The hippocampal MT then decreased with time in gerbils, dropping from 10.8±0.4 μg/100 mg of tissue in the 1-day old gerbils to 9.9±1.6 μg/100 mg of tissue in 30-days old gerbils, and significantly to 8.6±1.4 μg/100 mg of tissue in 90-days old gerbils (Fig. 1). Similar effects were observed in rats during the same time frame, with MT content registered at 15.3±0.6 μg/100 mg of tissue in 1-day old, leading to largely more significant down-regulations; 7.3±1.6 μg/100 mg of tissue in 30-days old, 6.5±0.5 μg/100 mg of tissue in 90-days old, and 4.7±0.3 μg/100 mg of tissue in 180-days old.

Contrary to the distribution of MT in the hippocampus, that in the cerebellum was not continuously decreasing. MT levels in this region of the gerbil and rat brains in 1-day old were at the lowest; 4.8±1.04 μg/100 mg of tissue and 1.6±0.1 μg/100 mg of tissue respectively, and seemed to gradually increase with time. The levels in the 30-days old rodents were higher; 7.6±2.6 μg/100 mg of tissue in gerbils and significantly higher; 5.5±1.9 μg/100 mg of tissue in rats. However, the levels recorded in 90-days old were down-regulated to 6.3±0.6 μg/100 mg of tissue in gerbils and significantly down-regulated to 2.8±0.6 μg/100 mg of tissue in rats, when compared to 30 days old but still higher than their respective 1-day olds (Fig. 1). Furthermore, the MT content in 180-days rats increased on that of 90-days old.

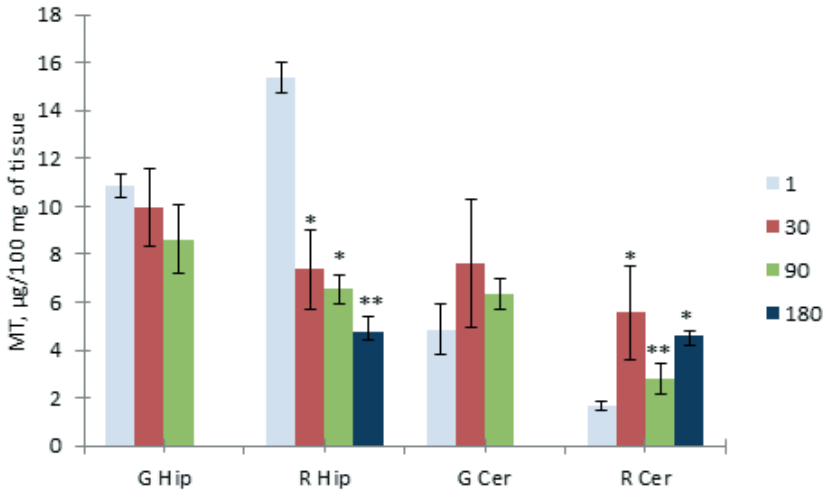


Fig. 1. The level of MT in rodents' brain under postnatal development.

Note. G Hip – gerbil hippocampus, R Hip – rat hippocampus, G Cer – gerbil cerebellum, R Cer – rat cerebellum; 1, 30, 90, 180 – postnatal days; * – significant difference with respect to 1 pd, ** – significant difference with respect to all other groups; p≤0.05; n=6.

In the hippocampus, as observed on Fig. 2, the more the rats were exposed to cadmium, the more significant the down-regulation in MT was observed. The values recorded disclosed sharp reductions in MT content; 3.4±0.08 µg/100 mg of tissue under 0.1 µg Cd effect and 2.7±0.1 µg/100 mg of tissue under 1.0 µg Cd effect, when compared to the controlled experiment, 4.7±0.3 µg/100 mg of tissue.

The cerebella MT quantities equally dropped in the Cd-induced animals but significantly only under 1.0 µg Cd concentration – 3.3±0.4 µg/100 mg of tissue, when compared to control.

The results shown in figure 1 are speculative projections of the regional distribution of MT in the brain of rodents at different stages of their postnatal existence. In this case, the quantitative expression of MT is projected to be higher in new born rodents than they are in adults. In earlier studies, MT isoforms in rodents have been reported to exist in different quantities [10, 11] and have different functions in their host organisms. According to Choudhuri et al., the quantitative order of expression of MT is MT-I>MT-III>MT-II, with MT-III and MT-II about 70 % and 50 % that of MT-I, as expressed in the mouse brain [10]. In Ono and Cherian's investigation of the regional brain distribution of MT in Sprague-Dawley [SD] and Lewis rats before, no significant difference was registered in the level of MT in the brains of both types of rats. However, MT was more highly expressed in the white matter than in other examined regions in the SD rats. In contrast, MT concentrations were highest in the cortex in the Lewis rats. The MT levels in the cortex, corpus striatum, hippocampus, and thalamus plus hypothalamus were significantly lower in SD rats than in Lewis rats [11]. In our work, the pattern of expression of MT was similar in both Wistar rats and gerbils but the significant timely changes in the levels of MT of the young rodents were more pronounced in the Wistar rats than they were in the gerbils. The favored area of distribution of MT in both cases, however, being hippocampus>cerebellum.

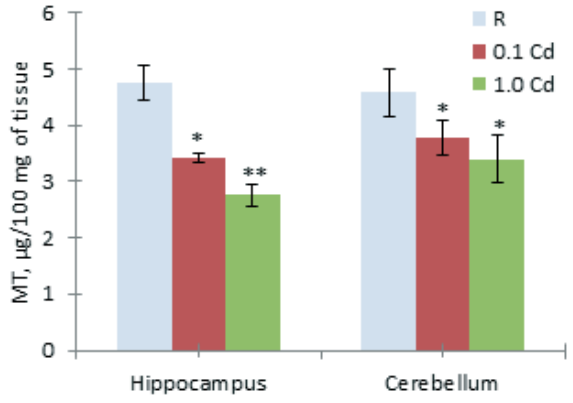


Fig. 2. The level of MT in rats' brain under cadmium effect.

Note. R – 180-days old rats; 0.1 Cd – 180 days old rats exposed to 0.1 µg cadmium per animal everyday for 36 days; 1.0 Cd – exposed to 1.0 µg cadmium per animal everyday for 36 days; * – significant difference with respect to control rats, ** – significant difference with respect to 0.1 µg Cd; p≤0.05; n=6.

The common expression pattern in both the young rodents let us to believe that this pattern could be somewhat identical in most mammals, if not exactly the same. A telling difference between the two species lies in the degree of changes in the MT content over time. With the exact reasons for these changes not yet established, functional constraints of the different brain regions and the stages of development are the most we can advance at the moment, pending a deeper research. At such an early stage after birth, it is understandable that functioning depends on the level of development of the different brain compartments.

As regards to other functional constraints that might account for the changes in the MT content, it has been shown that only about half the neurons

generated during development, after birth, survive to function in the adult. Entire populations of neurons are removed through apoptosis or programmed cell death – which involves the normal loss of more than half of brain neurons and synaptic exuberance and pruning – in which there is massive excess production of connections, initiated in the cells [12, 13]. Apoptosis is a process that induces high levels of MT [15], and given that MT is able to regulate this process, this may explain why postnatal MT is highly expressed in the brain. The substantial decrease in the levels of MT, especially in the hippocampus, is characteristic of MT behavior during regulatory processes.

Under cadmium induced stress, the down-regulation of MT may perhaps be accounted for by their presumed functional involvement in metal regulation [6].Given that Cd has a long half-life when bound to MT [7], it is clear to understand why levels of MT significantly reduce upon intoxication by Cd. MT plays a homeostatic role in the control and detoxification of Cd [14].

It is known that Cd uptake involves competition with calcium (Ca), Fe and Zn and makes use of their transport systems. Once taken up enterally, Cd reaches the liver where it binds to MT, glutathione (GSH) and other proteins or peptides. MT induced upon cadmium exposure can act in two ways. On one hand MT binds to Cd, thereby detoxifying and removing it from the cellular environment. On the other hand, due to its thiol groups, MT can scavenge ROS that are produced as a result of Cd-induced oxidative stress. However, the latter results in Cd dissociation from MT due to the corresponding decreased metal binding stability [15]. Cd exposure results in pathological conditions in the liver, testis, brain, and nervous system, kidney, spleen, and bone marrow, locations that have been shown to express MT upon induction. Its exposure can also lead to apoptosis in testes of rat, mouse liver, and human T-cells [16]. Having registered continuously decreasing MT concentrations in the brain under two different low doses of Cd, we observed that MT plays a vital role in heavy metal regulation. Most of the harmful effects of heavy metals on human health, and in rodents, are mediated through oxidative stress [17] – a process very well regulated by MT [18]. It is worth noting that the expression of MT under other different toxic conditions is classically up-regulated, like in blood [6], but in the case of Cd, it is down-regulated.

CONCLUSIONS. The brain parts of newborn rodents definitely differ in their content of MT to those of older organisms. In the postnatal development, it is clear that MT distribution is characteristic of the periods of maturation of the functional capacities of different brain parts: in the hippocampus – at the early stages after birth, in the cerebellum – during the first month of life. After this point, the content of MT in rodents, and presumably other mammals, generally decrease with age and the subsequent exposuresto conditions other than standard.

The intoxication of Wistar rats with small doses of Cd, over time, causes the gradual exhaustion of the levels of MT in the brain, depending on the dose administered and the time required for the accumulation of this metal.

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**О. Н. Шиїнтум, Г. О. Ушакова**
*ДНІПРОВСЬКИЙ НАЦІОНАЛЬНИЙ УНІВЕРСИТЕТ ІМЕНІ ОЛЕСЯ ГОНЧАРА*

## ВІДМІННОСТІ ВМІСТУ МЕТАЛОТІОНЕЇНІВ У МОЗКУ ГРИЗУНІВ ЗА УМОВ ПОСТНАТАЛЬНОГО РОЗВИТКУ ТА КАДМІЄВОЇ ІНТОКСИКАЦІЇ

**Резюме**

***Вступ.*** *Гени металозв'язувальних металотіонеїнів містяться в більшій популяції організмів. Ці білки не є ферментами і багаті на залишки цистеїну. Різні ізоформи металотіонеїнів у різних ділянках головного мозку, можливо, змінюються з плином часу.*

***Мета дослідження*** *– прослідкувати розподілення металотіонеїнів у різних відділах мозку піщанок і щурів на різних етапах постнатального розвитку за нормальних умов та при впливі малих доз кадмію.*

***Методи дослідження.*** *Тварин (18 монгольських піщанок і 36 щурів лінії Вістар) за віком та умовами експерименту було поділено на шість груп (n=6): 1–4 групи– 1, 30, 90 і 180 дні постнатального розвитку за нормальних умов; 5-та і 6-та – 180 п.д.+0,1 або 1,0 мкг $Cd^{2+}$ на тварину кожного дня протягом 36 днів. Кількість металотіонеїнів у гіпокампі, мозочку і таламусі визначали за допомогою імуноферментного аналізу.*

***Результати й обговорення.*** *Отримані дані показали динаміку розподілення металотіонеїнів у різних відділах мозку піщанок і щурів лінії Вістар залежно від терміну постнатального розвитку та функціональних можливостей. Рівень металотіонеїнів у гіпокампі поступово знижувався як у піщанок, так і в щурів, тоді як у мозочку він збільшувався з 1-го до 30-го дня розвитку, а потім поступово зменшувався в усіх піддослідних тварин. Зниження кількості металотіонеїнів у мозку за умов впливу кадмію було пропорційним застосованим дозам.*

***Висновки.*** *Кількість металотіонеїнів у різних ділянках мозку піщанок і щурів залежить від стадії постнатального розвитку та функціонального навантаження. Значиме зниження регуляції металотіонеїнів у досліджуваних відділах головного мозку під впливом кадмію передбачає, що зменшення їх рівня залежить від дози кадмію та часу, необхідного для його накопичення.*

КЛЮЧОВІ СЛОВА: **металотіонеїни; мозок; постнатальний розвиток; піщанки; щури; кадмій.**

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## ОТЛИЧИЯ СОДЕРЖАНИЯ МЕТАЛЛОТИОНЕИНОВ В МОЗГУ ГРЫЗУНОВ ПРИ ПОСТНАТАЛЬНОМ РАЗВИТИИ И КАДМИЕВОЙ ИНТОКСИКАЦИИ

**Резюме**

***Вступление.*** *Гены металлосвязывающих металлотионеинов находятся в большей популяции организмов. Эти белки не являются ферментами и богаты на остатки цистеина. Различные изоформы металлотионеинов в разных областях головного мозга, возможно, изменяются с течением времени.*

***Цель исследования*** *– проследить распределение металлотионеинов в разных отделах мозга песчанок и крыс на разных этапах постнатального развития в нормальных условиях и при влиянии малых доз кадмия.*

***Методы исследования.*** *Животных (18 монгольских песчанок и 36 крыс линии Вистар) по возрасту и условиям эксперимента разделили на шесть групп (n=6): 1–4 группы – 1, 30, 90 и 180 дни постнатального развития в нормальных условиях; 5-я и 6-я – 180 п.д.+0,1 или 1,0 мкг $Cd^{2+}$ на животное ежедневно в течение 36 дней. Количество металлотионеинов в гиппокампе, мозжечке и таламусе определяли с помощью иммуноферментного анализа.*

***Результаты и обсуждение.*** *Полученные данные показали динамику распределения металлотионеинов в разных отделах мозга песчанок и крыс линии Вистар в зависимости от срока постнатального развития и функциональных возможностей. Уровень металлотионеинов в гиппокампе постепенно снижался как у песчанок, так и у крыс, тогда как в мозжечке он увеличивался с 1-го до 30 дня развития, а затем постепенно уменьшался у всех подопытных животных. Снижение количества металлотионеинов в мозгу при воздействии кадмия было пропорционально примененным дозам.*

***Выводы.*** *Количество металлотионеинов в разных областях мозга песчанок и крыс зависит от стадии постнатального развития и функциональной нагрузки. Значимое снижение регуляции металлотионеинов в исследуемых отделах головного мозга под влиянием кадмия предусматривает, что уменьшение их уровня зависит от дозы кадмия и времени, необходимого для его накопления.*

КЛЮЧЕВЫЕ СЛОВА: **металлотионеины; мозг; постнатальное развитие; песчанки; крысы; кадмий.**

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