

RNA AND PROTEIN SYNTHESIS IN DIFFERENT ORGANS OF RAT OFFSPRING AFTER CHRONIC CADMIUM EXPOSURE DURING PREGNANCY

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Abstract. Despite binding by placental metallothionein, cadmium (Cd) relatively easily enters fetal circulation and may be harmful to tissues and organs of offspring. Although Cd toxicology is relatively well described in the literature there are only few studies on Cd toxicity exerted during fetal life. We examined the influence of cadmium exposure during pregnancy on RNA and protein synthesis in different organs of the rat offspring.

Their dams were fed diet containing cadmium chloride-treated drinking water during the whole pregnancy period at 50 ppm dose level. The offspring, 6-weeks-old male Wistar rats, weighing 105 ± 10 g were subjected to examination. Synthesis of RNA and proteins was quantitated by scintillation technique, which measured incorporation of tritiated uridine and alanine, respectively. A set of 17 organs and tissues was examined.

RNA synthesis increased significantly in buccal mucosa, tongue, parotid gland, cardiac muscle, brain and bone marrow. A strong induction of RNA synthesis in all four studied brain regions attracts special attention. The activation of RNA metabolism may be partly explained by the increased expression of genes involved in detoxication and adaptation (e.g., metallothionein, stress response proteins, etc.). A profile of protein synthesis was much more heterogeneous with elevated H³-alanine uptake in 12 organs of experimental animals, however without any statistical significance. Since the study of protein synthesis did not demonstrate any significant changes in Cd-treated animals, the profile of RNA synthesis cannot be simply extrapolated on protein synthesis, probably because of complex post-transcriptional and post-translational genetic modifications.

Key words:

Cadmium, RNA, Protein synthesis, Metallothionein, Scintillation technique

INTRODUCTION

Human and rodent placenta contains inducible metallothionein (MT), an intracellular low molecular mass protein with high affinity to cadmium (Cd). MT binds Cd and protects the fetus from Cd toxicity [1–4]. Nevertheless, Cd administered to dams of experimental animals during their pregnancy to some extent passes the placenta, accumulates in fetal tissues, and induces a number of patho-

logical and metabolic alterations [5–7]. The influence of Cd on RNA and protein synthesis was reported earlier in relation to individual organs, mostly liver and kidneys [8–10]. To our best knowledge, there are no previous reports describing alterations of RNA and protein synthesis in a large set of rat organs, including studies on transplacental intoxication. We decided to examine the influence of maternal exposure to Cd on the synthesis of RNA and

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protein in rat offspring in order to determine the tissue distribution and extent of transplacental Cd toxicity.

MATERIALS AND METHODS

Two groups (each containing 5 animals) of 6-weeks-old male Wistar rats, weighing 105 ± 10 g, were used in the study. Their dams were fed diet containing cadmium chloride-treated drinking water during the whole pregnancy period at a 50 ppm dose level, as described previously [11,12]. As controls, two groups (each containing five animals) of healthy 6-weeks-old Wistar males, weighing 120 ± 12 g, were used. It was assumed that incorporation of 6-H^3 uridine reflects the rate of RNA synthesis, and incorporation of 6-H^3 alanine is a measure of protein synthesis in cells. This assumption was verified in earlier reports on Cd toxicity [13–16]. In order to quantitate the RNA and protein synthesis, 6-H^3 uridine or 6-H^3 alanine (Amersham) was given intraperitoneally to the appropriate group of the rat offspring at the dose of $1 \mu\text{C}$ per 1g of body weight, applied in 0.2 ml volume of aqueous solution. The 6-H^3 uridine and L-[2,3 - H^3]-alanine specific activity was 1.1 TBq/mmol, and 1.5 TBq/mmol, respectively. All the animals were killed by decapitation 4 h after administration of labeled precursor. Specimens were collected during autopsy from the following organs: liver, pancreas, small intestine, stomach, brain, tongue, parotid gland, buccal mucosa, spleen, thymus, bone marrow, kidney, suprarenal gland, lung, skin, cardiac and skeletal muscles. In the brain, four regions were collected separately: frontal cortex, striatum, thalamus with hypothalamus and cerebellum with brainstem. The details of scintillation quantitation of tritiated uridine and alanine uptake in studied tissues were described in our previous study [12]. The recorded impulses were expressed as a number of radioactive disintegrations per minute (DPM). Three records in each specimen were averaged and the mean number of DPM/100 mg of tissue and standard deviation were calculated in each group of rats.

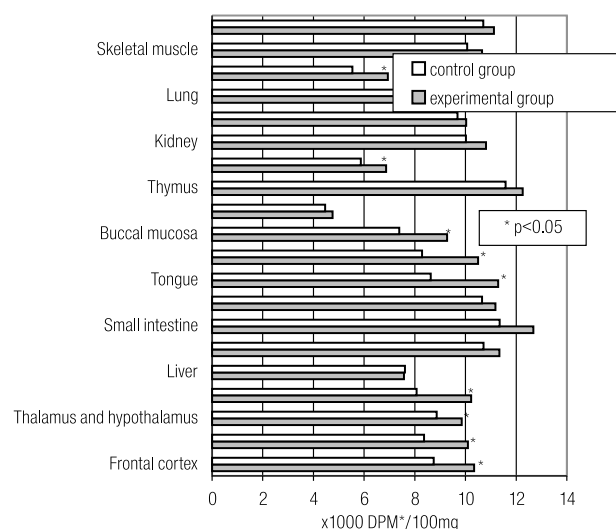
Statistical analysis

The Mann-Whitney U test was used to compare H^3 -uridine and H^3 -alanine uptake means in studied groups of rats. The differences were considered significant at the p

value < 0.05 . The correlation between changes in uridine and alanine incorporation in the control and experimental groups were assessed with correlation coefficient. The statistical analysis was performed using the Epi Info 5.01b software. Excel 97 for Windows (Microsoft) software was used for graphic data presentation.

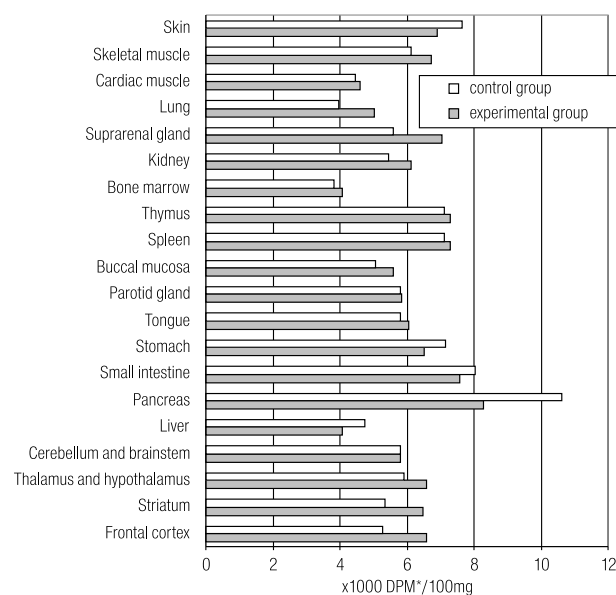
RESULTS

The results are presented in Figs. 1 and 2.



* DPM – disintegrations per min.

Fig. 1. Incorporation of H^3 -uridine in rat offspring organs and tissues after cadmium exposure in prenatal life.



* DPM – disintegrations per min.

Fig. 2. Incorporation of H^3 -alanine in rat offspring organs and tissues after cadmium exposure in prenatal life.

Control group

Incorporation of labeled uridine was most prominent in thymus, small intestine, pancreas, skin and stomach. The profile of labeled alanine incorporation, most pronounced in pancreas, small intestine, skin, stomach and thymus, corresponded with uridine incorporation.

Experimental group

In the 6 of 17 organs, the incorporation of labeled uridine increased significantly after cadmium exposure, most strikingly in the tongue, buccal mucosa, parotid gland and frontal cortex of brain. Generally, the RNA synthesis in all organs but liver was higher in the experimental group. Regarding incorporation of labeled alanine, no significant changes were noted after cadmium exposure. The insignificant increase in incorporation was found in the brain, suprarenal gland and lung, and insignificantly decreased incorporation in the liver, pancreas and skin.

The changes in RNA and protein synthesis after cadmium exposure correlated very weakly (correlation coefficient $r = 0.12$).

DISCUSSION

The influence of Cd exposure on RNA and protein synthesis have been studied since the 1970s. However, individual organs were usually examined, mostly liver, kidney or lung. Furthermore, experimental animals were most frequently directly subjected to Cd exposure, so the issue of transplacental effect of Cd was not addressed. Cd enters fetal circulation through the placenta. Due to MT produced by trophoblast, Cd concentration in human umbilical cord blood vessels is reduced up to 40–70% of values in maternal blood [6,17,18]. In rats, the transplacental Cd transfer can even be more restricted [19].

Results of experimental studies reported in the literature are contradictory. The authors describe enhanced RNA and protein synthesis in selected organs in Cd-treated animals and cell cultures [10,14,20]. These trends were explained by the increased mRNA activity and stimulation of RNA polymerase [10,16]. An increase in RNA and protein synthesis after chronic Cd exposure could be

attributed to regeneration in different organs, like in the liver after methylmercury chloride intoxication as already suggested [10].

On the other hand, Cd is known to inhibit the synthesis of RNA and proteins in different organs *in vivo* [9,21,22] and *in vitro* [13,16,23]. Cd inhibited RNA polymerase activity and protein synthesis in the rat liver *in vivo*. These findings were probably irrelevant since a peak of protein synthesis preceded maximum RNA synthesis in the experiment [8]. A transient decrease with secondary increase in pancreatic RNA level was demonstrated in rats exposed to Cd [24]. A secondary regeneration (cell renewal) after primary lesion was suggested. Inhibition of RNA synthesis in *Physarum polycephalum* was ascribed to the damage to nucleoli structure and function caused by Cd [25]. Thus, the nucleolus (aside from nucleus itself) was supposed to be the main cellular target for Cd toxicity. A slowdown in the transport and processing of nucleolar RNA, seen in HeLa cells [26], probably reflects these alterations.

In our experiment, Cd effect was generally much stronger on the RNA than protein synthesis. A significant increase in RNA synthesis, observed in most of studied organs, suggests a generalized metabolic effect of Cd. The uridine incorporation in control animals generally corresponded with incorporation of labeled thymidine in the same organs studied previously [12].

The increase in protein and especially RNA synthesis (statistically significant) in all studied regions of the brain deserves a separate mention. Mammalian brain is characterized by intensive cellular proliferation and differentiation during pre- and postnatal development, defined e.g., by short half-life of histones during first weeks after birth [27]. Our results probably reflect this enhanced metabolism in developing rat brain, exaggerated due to repair processes after Cd intoxication. Otherwise, the selective Cd accumulation in brains of rat offspring demonstrated previously [11] is in agreement with this hypothesis.

The effect of cadmium on RNA metabolism, exerted mainly by stimulation of tRNA and mRNA, especially related to MT metabolism has been reported [28]. Induction of MTmRNA and MT itself was noted in animal liver, testes and kidneys [28–31]. However, as this protein accounts

for merely a small part of the protein pool, its upregulation should not affect the total value of protein synthesis. There is plenty of proteins, their genes and mRNAs, apart from MT, which are induced by Cd exposure. They include glutamylcysteine synthetase [32], stress proteins, e.g., hsp70 [33–35], acute phase proteins [36], “primary response” (TIS) genes [37], metal-responsive transcription factors [35], and oncogenes, e.g., c-fos, c-myc [34]. The upregulation of these and other proteins and mRNAs probably contributes to an elevated uptake of precursors observed in our study. The increase in RNA synthesis after cadmium exposure did not correlate with protein synthesis in the corresponding tissues. Obviously, the protein synthesis cannot be simply regarded as a function of RNA metabolism. Different efficiency of mRNA transfer to cytoplasm and a set of post-transcriptional (e.g., mRNA splicing) as well as post-translational modifications are presumably responsible for these discrepancies. Our data may provide a baseline for future studies on transplacental Cd toxicity in regard to quantitative changes and their topographic distribution.

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