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Original Research

Effect of Pre- and Postnatal Exposure to Zinc on [^{3}H]glucose Uptake in the Brain and Peripheral Tissues of Adult Rats

J. Konecki[1], G. Bielaczyc[2], P. Nowak[2], R. Szkilnik[2], G. Szczerbak[2], M. Swoboda[2], A. Kwieciński[2], R.M. Kostrzewa[3], R. Brus[2]*

[1]Department of Histology and Embriology, Medical University of Silesia, 41-808 Zabrze, Poland
[2]Department of Pharmacology, Medical University of Silesia, 41-808 Zabrze, Poland
[3]Department of Pharmacology, Quillen College of Medicine, East Tennessee State University, Johnson City, TN 37614, USA.

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Abstract

To determine the susceptibility of developing brain and other tissues to accumulate zinc, rats were exposed to zinc at different periods of ontogeny. For the prenatal group, pregnant Wistar rats received 50 ppm of zinc ($ZnSO_4 \cdot 7H_2O$) in drinking for the entire duration of pregnancy. On the day of delivery zinc was removed from the drinking water. Another group, dams, received 50 ppm of zinc in drinking water only during the suckling period (from delivery until the 21st day of postnatal life). Their offspring were weaned on the 21st day, at which time zinc was removed from the drinking water. The control group drank tap water only. At 3 weeks after birth, the level of zinc was estimated in the brain, liver, mandibular bone and kidney of offspring from all groups. At 8 weeks after birth 6-[^{3}H]D-glucose (500 μCi/kg) was administered IP to male offspring, 15 minutes before sacrifice. By liquid scintillation spectroscopy, ^{3}H-activity (expressed as disintegrations per minute [DPM]) was determined in discrete parts of the brain and some peripheral tissues, and expressed as DPM/100 mg of tissue, wet weight. It was found that the highest amount of zinc was accumulated in the brain and liver of rat offspring that were exposed to zinc postnatally. [^{3}H]-activity was at lower levels, in comparison, in nearly all other parts of the brain of rats exposed to zinc postnatally. In offspring receiving zinc prenatally, zinc levels were at similar or lower amounts in the brain and peripheral tissues, vs. the group with postnatal exposure. From this study in rats we conclude that zinc accumulates to the highest extent in brain, following a later ontogenetic (postnatal) exposure period, and by this, there is also greater disturbance of metabolic processes associated with glucose utilization.

Keywords: zinc, 6-[^{3}H]D-glucose, brain, development, rats

Introduction

Zinc (Zn) is a nutritionally essential metal, and Zn-deficiency results in severe health consequences. Conversely, excessive exposure to Zn is relatively uncommon and a heavy body-burden is found only after heavy exposure [1].

Zn is ubiquitous in the environment, present in most foodstuffs, water and air. Zn exposure may be increased if drinking water is in contact with galvanized copper or plastic pipes. Zn applied to soil is taken up by growing

*Corresponding author; e-mail: pharbrus@slam.katowice.pl

vegetables. Zn atmospheric levels are increased over industrial areas. The average daily intake is approximately 12 to 15 mg, mostly from food [1].

About 20 to 30 percent of ingested Zn is absorbed. The mechanism is thought to be homeostatically controlled and is probably a carrier-mediated process [2]. It is influenced by prostaglandins E_2 and F_2, and Zn is chelated by picolinic acid – a tryptophan derivative. Deficiency of pyridoxine or tryptophan depresses Zn absorption. Within the mucosal cell, Zn induces metallothionein synthesis and, when saturated, may depress zinc absorption. In the blood, about two-thirds of the Zn is bound to albumin, and most of the remainder is complexed with β_2-macroglobin [3].

Zn concentration in tissues varies widely. Liver receives up to about 40 percent of a tracer dose. Concentrations of Zn in this organ are influenced by humoral factors including adrenocorticotropic hormone, parathyroid hormone, and endotoxin. In the liver, as well as in other tissues, Zn is bound to metallothionein. The greatest concentration of Zn in the body is in the brain and in the prostate, probably relative to the rich content of the Zn-containing enzyme acid phosphatase [1].

Hundreds metalloenzymes require Zn as a cofactor, and Zn-deficiency results in a wide spectrum of clinical effects depending on age, stage of development, and deficiencies of related metals.

Zn deficiency in humans was first characterized by Prasad and coworkers [4] in adolescent Egyptian boys with growth failure and delayed sexual maturation, and is accompanied by protein-caloric malnutrition, pellagra and iron or folate deficiency. Zn deficiency in the newborn may be manifested by dermatitis, loss of hair, impaired hearing, susceptibility to infections, and neuropsychologic abnormalities.

Zn toxicity from excessive ingestion is uncommon, but gastrointestinal distress and diarrhea have been reported following ingestion of beverages standing in galvanized cans or from the use of galvanized utensils. However, evidence of hematologic, hepatic, or renal toxicity has not been observed in individuals ingesting as much as 12 g of elemental Zn over a two-day period [1]. In animals, teratogenicity and carcinogenicity (mostly of sex organs) was observed [5-7].

Zn easily penetrates the placenta and blood-brain barriers, and is transferred to a suckled child in mother's milk [8-10]. Among the different organs, the brain of developing animals is most sensitive to the neurotoxic effects of Zn and other heavy metals.

There are only sparse data concerning the effect of Zn on the central nervous system, particularly relative to the effect of Zn exposure during early stages of ontogenetic development. Accordingly, the first aim of this study was to examine the effect of prenatal (during intra-uteral development) and postnatal (during the first 3 weeks of the suckling period) zinc exposure on central nervous system function in adulthood. A second objective was to determine if excess zinc exposure in ontogeny affected the basic process of tissue uptake of glucose in adulthood, and this was examined by assessing the uptake of tritium-labeled glucose.

Material and Methods

Adult female Wistar rats ab 250 BW were used for this study. Animals were single housed in a room at 22 ± 1°C, with an alternating light/darkness cycle of 12 hours (light on 07.00). Rats had free access to standard food pellets (Labofeed, A. Morawski's Animal Food Works, Kcynia, Poland). All studies were approved by the Bioethic Committee of the Medical University of Silesia for Animals (permission # 24/02, issued on 17.09.2002).

Pregnant rats were divided into 3 groups. The control group received tap water during pregnancy. Another group received tap water with Zn ($ZnSO_4 \cdot 7H_2O$; 50 ppm) throughout pregnancy. On the day of delivery tap water replaced the water with Zn. The third group received tap water during pregnancy, but from the day of delivery and for the next 21 days this group received water with added Zn (50 ppm). Fluid consumption was monitored.

At 21 days after birth offspring from each of the 3 groups were weaned and housed by sex. Tissue estimations of Zn were performed at 22 days after birth, while [^{3}H]glucose uptake was assessed at 8 weeks after birth.

Assessment of Tissue Uptake of [^{3}H]glucose

At 8 weeks after birth male rats from each group were placed in individual cages for one hour of adaptation. Then all animals from each group were injected intraperitoneally (IP) with 6-[^{3}H]D-glucose (Amersham Radiochemicals, Pittsburg, PA, USA; 41 Ci/mmol), at a 500 μCi/kg BW dose. After 15 min, rats were sacrified and brains were immediately excised and placed on ice, while the striatum, hippocampus, frontal cortex, hypothalamus with thalamus, cerebellum and pons were removed, weighed, and placed in 20-ml scintillation vials. In addition, the liver, heart muscle, kidneys and thoracic aorta were removed. Soluene-350 (Pacard Inc., Downers Grove, III, USA; 1 ml) was added to each vial, and the tightly-closed vials were incubated at 37°C for 48 hours, by which time the tissues were completely solubilized. Then ten ml of scintillation cocktail (Dimilume-350, Pacard Inc.) was added, and the vials were briefly vortexed and placed in a scintillation counter (Liquid Scintillation Counter: DSA 14091, Wallac, Finland). Radioactivity was assessed twice for 2 minutes each time, and the mean ± SEM of DPM (Disintegrations per minute) per 100 mg wet tissue was calculated for each group [11].

Each group consisted of 4-5 rats (tissues).

Zinc Estimation

Offspring, at 22 days after birth, were decapitated for estimation of Zn content in brain, liver, kidney and mandibular bone (ca. 100 mg each). Tissues were weighed and dissolved in 1 ml of ultra-pure nitric acid. Then the level of Zn was estimated by means of atomic-absorption mass

Table 1. Effect of zinc prenatal and postnatal exposure on radioactivity (DPM/100 mg of wet tissue) in the brain and some peripheral tissues of adult male rats (x ± SEM; n = 4-5).

No.	Examined Tissue	Group - Period of exposure		
		Control	Zinc prenatally	Zinc postnatally
1.	Striatum	53747 ± 9184	37839 ± 6149	23995 ± 7829*
2.	Frontal cortex	59485 ± 1088	48631 ± 3623	35208 ± 4360*
3.	Hippocampus	49994 ± 5752	41943 ± 5851	27816 ± 3878*
4.	Thalamus with hypothalamus	52341 ± 10188	41258 ± 4295	29078 ± 7798*
5.	Cerebellum	53645 ± 9502	43111 ± 5170	30022 ± 4978*
6.	Pons with medulla prolongata	50739 ± 8189	40438 ± 5452	29513 ± 6162*
7.	Heart muscle	48470 ± 7013	57697 ± 6378	42654 ± 1797*
8.	Thoracic aorta	74385 ± 2015	52987 ± 9697	66527 ± 4085
9.	Liver	107868 ± 98774	90525 ± 7285	65690 ± 2536
10.	Kidney	110064 ± 11413	100061 ± 8925	71213 ± 2475

Explanation: * $p < 0.05$ as compare to the control group.

Table 2. Zinc level (µg/g of wet tissue) in the brain, liver, mandibula bone and kidney of 3 weeks old offspring exposured to Zn pre- and postnatally (x ± SEM; n = 4).

No.	Examined Tissue	Group - Period of exposure		
		Control	Zinc prenatally	Zinc postnatally
1.	Brain	133 ± 39	251 ± 63	265 ± 19*
2.	Liver	1141 ± 182	1459 ± 222	1720 ± 51*
3.	Mandibula bone	761 ± 44	589 ± 69	658 ± 33
4.	Kidney	471 ± 36	412 ± 20	550 ± 63

Explanation: * $p < 0.05$ as compare to the control group.

spectrometry using an SP-2900 Pye Unicam spectrometer [12]. Results were expressed in µg/g of wet tissue. Each group consisted of 4 animals (tissues).

Statistical Analyses

Analysis of variance (ANOVA) and the post-ANOVA test of Neuman-Keuls was used to compare the differences between groups for significance. A "p" value of 0.05 or less was considered a significant difference between groups.

Results

Pregnant rats drank an average of 14.5 ml/100g BW of tap water (control group), and 13.7 ml/100g of tap water with added Zn (50 ppm) (study group). Rats that nursed their litters drank 15.2 ml/100 g BW of water with added zinc.

In rats injected at 8 weeks after birth with 6-[^{3}H]D-glucose (500 µCi/kg) there was no significant change in tritium uptake in any tissue of the group exposed to Zn in utero (Table 1). In rats exposed to Zn during the first 21 days of postnatal life (suckling period) there was a reduction in tritium accumulation in most brain regions, but not in peripheral tissues (Table 1).

At 21 days there was a significant increase of Zn in the brain of pre- and postnatal exposed rats vs. the control group (Table 2). Also, in the group exposed to Zn during the 21-day suckling period, there was a moderate increase of Zn in the liver. No change in Zn level was observed in the bone or kidney of groups exposed to Zn either in utero or during the suckling period (Table 2).

Discussion

Cerebral function requires a continous supply of glucose, although glucose transport into brain is not dependent on insulin. In the brain glucose is not stored,

but immediately metabolized to obtain energy (ATP). Brain glucose uptake is relatively high and this process is dependent on the activity of the central nervous system neurons; it also is influenced by hormons, drugs and other agents [13]. Dopaminergic neurons are particularly influenced by glucose uptake. A single injection of glucose suppresses firing of nigrostriatal dopamine-containing neurons and lowers dopamine metabolite levels [14, 15], while chronic hyperglycemia in diabetic rats reduces dopamine metabolite levels and their rate of synthesis [16, 17]. Moreover, insulin-induced coma is an effective treatment for schizophrenia, and disturbances in glucose metabolism were reported in other disorders and are believed to involve hyperactivity of brain dopaminergic transmission [18, 19]. In our previous study we confirmed that the central dopamine receptor agonists quinpirole, 7-OH-DPAT and SKF 38393 (D_2, D_3 and D_1 respectively) influenced glucose utilization in rat brain [20, 21].

In another of our studies we examined the influence of some environmental metals like lead, mercury, cadmium, manganese and aluminum on exogenous glucose uptake in the rats [22-26], and found heavy metal exposure during pregnancy affected [^{3}H]glucose uptake in the brain of offspring in adulthood.

In the present study we found that Zn decreased [^{3}H]glucose uptake in adult rats exposed to Zn, primarily during the postnatal period. There is only scarce data concerning the effect of Zn on glucose metabolism in mammals. Adipocytes exposed to Zn exhibited increased glucose uptake in vivo [27]. Others find that Zn mimics insulin action and facilitates glucose transport via the plasma membrane [28]. Zn attenuates the inhibitory effect of cadmium and copper on glucose transport across the plasma membrane [29].

In the present experiment the observed decrease of [^{3}H]glucose uptake in the brain of rats exposed to Zn during early stages of ontogenetic development can be attributed to the neurotoxic effect of Zn. It is unclear if that effect is associated with the influence of Zn on the central dopamine system. As mentioned, Zn is a cofactor for hundreds of enzymes regulating biological processes, and by this Zn can modify the biological function of many tissues and organs – and this is likely to be further influenced by exposure to Zn during ontogeny.

It is worth noting that in the present study, the reduced [^{3}H]glucose uptake was more altered in the offspring of rats exposed to Zn postnatally, and this is in accord with other findings [30, 31]. Zn is accumulated in the dam's milk and thus transmitted to nursing pups [8, 9]. When administered to pregnant rats Zn must cross the placental barrier, which synthesizes and retains high levels of metalothionein [32]. This protein binds and inactivates heavy metals, thereby reducing transfer to the fetus. Another barrier to transfer is the liver. In this organ Zn induces metalothionein production in a dose-dependent manner [33]. In our experiment the highest level of Zn was was observed in the liver of rats exposured to Zn postnatally.

Although dopamine receptors are present in the peripheral tissues examined [34], there was no difference in tritium accumulation in any of the groups treated at 8 weeks with [^{3}H]glucose. It therefore appears that Zn did not exert a toxic effect relating to glucose accumulation in the peripheral tissues.

Our results demonstrate that exposure of rats to zinc during the suckling period can be harmful for the developing brain. From this, we conclude that uncontrolled supplementation with Zn during pregnancy or in the suckling period can be disadvantageous for mammalian brain development.

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