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Original Research

Effects of Pre- and Postnatal Zinc Exposure on Adult Rat Brain Dopamine Activity and Behavior

P. Nowak, G. Bielaczyc, R. Szkilnik, Ł. Labus, J. Dąbrowska, A. Bortel, G. Szczerbak, M. Swoboda, A. Kwieciński, R. Brus*

Department of Pharmacology, Medical University of Silesia, ul. Jordana 38, 41-808 Zabrze, Poland

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Abstract

Pregnant Wistar rats received 50 ppm of zinc ($ZnSO_4 \cdot 7H_2O$) in their drinking water 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 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. In 8-12-week-old offspring of all three groups the DA, DOPAC, HVA, 3-MT, 5-HT, 5-HIAA, NA, and MOPEG synthesis rate in the brain was estimated by HPLC/ED technique. Independent behavioral exam were performed such as locomotor and exploratory activity, irritability, yawning and oral activity, stereotype behavior, catalepsy and others. For the above, central DA receptor agonists (quinpirole, SKF 38393, apomorphine, 7-OH-DPAT) or antagonists (haloperidol, SCH 23390) were used. It was found that exposure to zinc during early stages of ontogenic development produce changes in the central dopaminergic system activity of adult offspring. From the above we concluded that uncontrolled supplementation with zinc during pregnancy or lactation can induce disturbance of the central dopaminergic system in adult mammal.

Keywords: zinc, rats, brain, biogenic amines, behavior, dopaminergic

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

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

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 its absorption. In the blood, about two-thirds of 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, parathy-

roid 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 of 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 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 [4]. On the other hand Zn deficiency induced Prasad's syndrome in humans [5].

There are some data on the toxic effect of Zn exposure on the central dopaminergic system in mammal. Zn applied to the brain's substantia nigra of rats decreased dopamine (DA) content and tyrozine hydrooxylase activity in the examined structure [6]. Some authors suggested a role for Zn in the etiology of Parkinson's disease because of its ability to generate free radical oxygen species [7-9]. In the post-mortem study of a person who died of Parkinson's disease the Zn level in the brain was 31-35% higher as compared to the control [10]. Zn also can induce apoptosis and neurodegeneration [11]. Agents chelating Zn prevent its neurotoxic activity *in vitro* [7]. In another study it was shown that Zn regulates neuronal uptake of the DA [12, 13], influencing the function of the DA membrane transporter (DAT) [14-16]. Zn also modulates activity of MAO [17] and decreases specific antagonist binding to the central DA receptors [12, 18].

Zn easily penetrates the placenta and blood-brain barriers, and is transferred to a suckled child *via* mother's milk [19-21]. Among the different organs, the brain of developing animals is most sensitive to the neurotoxic effects of Zn and other heavy metals.

There is only scarce data concerning the effect of Zn on the central nervous system, particularly related to the effect of Zn exposure during early stages of ontogenetic development. It is important to know more, because of the tendency to supplement and overdose vitamins and trace elements during pregnancy and lactation. 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) Zn exposure on the central dopaminergic nervous system function in adulthood. For this study biochemical and behavioral methods were used, induced by specific DA receptors agonists and antagonists.

Material and Methods

Adult female Wistar rats about 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. The second group received tap water with Zn ($ZnSO_4 \cdot 7H_2O$; 50 ppm) throughout pregnancy. On the day of delivery water with Zn was replaced by tap water. The third group received tap water during pregnancy, and from the day of delivery 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.

A following behavioral and biochemical study was performed on male offspring 8-12 weeks old.

Biochemical Estimations

Biogenic Amines Assay

Two-month old male offspring from all three study groups were sacrificed by decapitation, and the brains were immediately excised and placed on ice. The striatum, hippocampus and frontal cortex were separated, placed on dry ice, weighed and stored at -70°C, pending assay. Brain specimens were homogenized in ice-cold 0.1 M trichloracetic acid, containing 0.05 mM ascorbic acid. After centrifugation (5000 x g for 5 min), the supernatants were filtered through 0.2 μm cellulose membranes (Titan MSF Microspoin Filter Scientific Resources Inc., Eatontown, GB) and the supernatant was injected into the HPLC/ED column. In the above structure dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxytyramine (3-MT), serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), noradrenaline (NA), 4-hydroxy-3-methoxyphenylethylglycol (MOPEG) was estimated according to Magnusson et al. [22] and expressed in ng/g of wet tissue.

DA Synthesis Rate

The study was performed in separate adult offspring rats from the three examined groups. Animals were injected with the aromatic amino acids decarboxylase inhibitor NSD-1015 100.0 mg/kg IP [23], and decapitated 30 minutes later for excision of the corpus striatum, hippocampus and frontal cortex. Brain specimens were stored at -70°C pending assay. Then in the above tissues L-DOPA was estimated according to Magnusson et al. [22]. The level of the above aminoacid in the examined brain's part was expressed indirectly as a DA synthesis rate [23].

Each group consisted of 5-6 rats (tissues).

Behavioral Study

Locomotor Activity [24]

Rats from each group were individually placed in transparent glass cages 48 x 26 x 36 cm, and were allowed to acclimate for 30 minutes. Then 1.0 ml/kg saline was injected IP to each rat, and 10 minutes later locomotor activity (time spent walking, sniffing, grooming and rearing) was recorded in seconds during 10 minutes. Simultaneously, grooming time (sec) was recorded as well as numbers of rearings. After completing the above observation irritability was evaluated according to Nakamura and Thoenen [25].

Then quinpirole (a central DA receptor D_2/D_2 agonist) 3.0 mg/kg IP or SKF 38393 1.0 mg/kg IP was injected, and 30 and 60 minutes later all observations as above were repeated.

Exploratory Activity [26]

After rats had acclimated to the laboratory environment for 30 minutes, each rat from the three examined groups was injected IP with saline 1.0 ml/kg. Ten minutes later each rat was placed in the center of a wooden platform, 100 cm square, surrounded by a 40-cm fence to prevent escaping. The flat platform had 4 rows of 4 holes each, 7 cm in diameter and 20 cm apart. The number of times (during a three minute period) that each rat stuck its head beneath the interaural line, into any hole, was counted and recorded. Then quinpirole 3.0 mg/kg IP or SKF 38393 1.0 mg/kg IP was injected, and 30 and 60 minutes later observation as above was repeated.

Yawning Activity

Yawning activity was evaluated by 7-OH-DPAT (a central DA D_3 receptor agonist), according to Kostrzewa and Brus [27]. After one-hour adaptation to the laboratory environment, each rat was injected IP with saline 1.0 ml/kg, and the number of yawns was counted for 60 minutes. Then, each rat was injected IP with a low dose (0.0325 mg/kg) of 7-OH-DPAT (a selective D_3 receptor agonist) [28], and the number of yawns was counted for an additional 60 minutes. The same male rats were challenged on subsequent days with escalating doses of 7-OH-DPAT (0.065 and 0.130), one dose per day, and were observed as above.

Oral Movements

Oral movements (vacuous chewing) were evoked by SKF-38393, a selective central D_1 agonist [24]. After acclimating to the laboratory environment, rats were injected with SKF-38393 in escalating daily doses of 0; 0.1; 0.3 or 1.0 mg/kg IP.

Stereotyped Behavior

Rats were individually placed in transparent glass cages 48 x 26 x 36 cm, on fresh wood-chip bedding, and were allowed to acclimate for 30 minutes. Then, all rats were injected SC with 1.0 mg/kg apomorphine, a non-selective DA receptor agonist. Every 15 minutes after the injection, up to 90 minutes, stereotyped behavior of each rat was measured by the scoring method according to Creese and Iversen [29], on a scale of 0-6.

Catalepsy

Catalepsy was evaluated as described by Kostrzewa and Kastin [30], using 0.5 mg/kg IP of haloperidol (a selective central D_2 receptor antagonist), or SCH 23390 (a selective central D_1 receptor antagonist), or saline. Each rat was placed on a 25x50 cm wire mesh screen, forming 1x1 cm squares, and was inclined by 60° to the horizontal plane. The time (in seconds) taking each rat to move any paw along at least one screen division within 60 seconds was recorded. Measurements were performed 5 times: at 15, 30, 45, 60 and 90 minutes, and the results of each observation were summarized.

Reaction to Painful Stimulus

Reaction to painful stimulus was performed using the hot plate method [26]. Each rat was placed on a 28 x 28 cm copper plate, maintained at 55°C and surrounded within plexiglass walls. The time interval between the moment when a rat had all four paws on the plate, and when it started to shake or lick one of its paws, was recorded in seconds. Each recording started 10 minutes after IP saline administration, and was performed 3 times, with 10 minute intervals. Results are expressed as the mean of the three measurements. No central DA receptor agonists were used for this examination.

Locomotor Coordination [26]

After 30 minutes of adaptation to laboratory conditions, rats were injected IP with saline (1.0 ml/kg). Ten minutes later each rat was placed on a wooden bar, 3 cm in diameter. The bar rotated longitudinally five times per minute, and the length of time (in seconds) each rat managed to stay on the rotating bar was recorded. A rat that stayed on the bar for 300 seconds was taken off. This test was carried out on each rat twice, at 30-minute intervals, and the mean time was calculated for each rat. No central DA receptor agonists were used in this study.

Each group consisted of 8 rats.

Statistical Analyses

Data from all biochemical or behavioral studies were

analyzed by two-way ANOVA and post-ANOVA test of Neuman-Kuels. Differences in p value of < 0.05 were considered significant.

Results

Consumption of Fluids

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) per day. Rats that nursed their litters drank 15.2 ml/100 g BW of water with added Zn per day.

Biogenic Amines Assay (Table 1).

The DA level in the frontal cortex of the brain increased significantly in adult rats exposed to Zn prenatally and postnatally by ab., 37% and 54%, respectively as compared to the DA level in the control group. No differences in the level of all other examined biogenic amines between all three groups in the frontal cortex, striatum or hippocampus of adult rats were observed.

The L-DOPA level increased in the striatum only of adult rats postnatally exposed to Zn only by ab. 17% as compared to the control group. No significant changes in L-DOPA level was observed in the other examined structures of the adult rat's brain exposed to Zn pre- and postnatally.

Behavioral Study (Table 2)

Spontaneous locomotor activity (after saline) of the rats from the three examined groups was similar. Rats were active about 200 seconds during 600 seconds of observation. Quinpirole (a central DA D_2/D_3 receptor complex agonist) 3.0 mg/kg IP increased locomotor activity 30 and 60 minutes after its injection in all three examined groups. The strongest effect was observed in the adult rats exposed to Zn postnatally as compared to the control. SKF-38393 (a central DA D_1 receptor agonist) 1.0 mg/kg IP did not influence locomotor activity in any exposed group 30 and 60 minutes after its application in comparison to the respective controls.

Spontaneous grooming time (after saline injection) was similar in all three examined groups. Rats groomed about 120 seconds. Quinpirole 30 minutes and 60 minutes after its injection dramatically decreased grooming time in all examined groups as compared to saline control. No grooming was observed in adult animals exposed to Zn postnatally. 60 minutes later grooming behavior was very short in all three examined groups of rats pretreated with Zn, but shortest in postnatally exposed rats as compared to the respective controls. SKF 38393 1.0 mg/kg IP did not influence grooming activity (30 and 60 minutes after its injection) in any examined group of adult rats as compared to respective controls.

The spontaneous number of rearings (after saline injection) was similar in all three examined groups, ranging from 1.5 (control) to 2.5 (Zn prenatal). Quinpirole 3.0 mg/kg IP increased the number of rearings in the control and Zn prenatally exposed rats 30 and 60 minutes after

Table 1. Effect of zinc pre- and postnatal exposure on the level of biogenic amines and l-DOPA in the brain of adult rats (x ± SEM; n = 5-6).

BIOGENIC AMINES ng/g of wet tissue		DA	DOPAC	HVA	3-MT	5-HT	5-HIAA	NA	MOPEG	L-DOPA
Part of the brain	Group									
STRIATUM	CONTROL	10024.2 ± 416.8	759.9 ± 47.1	669.9 ± 45.5	159.8 ± 12.7	419.2 ± 25.6	320.3 ± 16.8	142.8 ± 11.4	=	1393.0 ± 83.8
	Zn PRENATAL	9741.5 ± 295.7	711.2 ± 40.2	697.9 ± 37.5	140.8 ± 14.1	404.3 ± 26.2	303.2 ± 10.6	110.9 ± 5.8	=	1389.6 ± 114.0
	Zn POSTNATAL	11160.9 ± 592.2	744.2 ± 42.2	691.4 ± 33.8	124.6 ± 11.7	429.9 ± 16.3	339.3 ± 21.8	127.2 ± 11.5	=	1638.8* ± 66.9
HIPPOCAMPUS	CONTROL	=	=	=	=	296.2 ± 20.5	160.9 ± 10.6	528.3 ± 28.7	116.8 ± 10.5	91.9 ± 4.0
	Zn PRENATAL	=	=	=	=	287.9 ± 26.7	195.8 ± 19.0	425.1 ± 28.9	112.3 ± 7.3	89.3 ± 6.0
	Zn POSTNATAL	=	=	=	=	267.1 ± 16.3	169.5 ± 10.2	490.2 ± 42.9	118.5 ± 6.5	95.1 ± 6.9
FRONTAL CORTEX	CONTROL	198.5 ± 23.8	43.7 ± 23.8	58.1 ± 3.8	=	400.4 ± 21.1	141.3 ± 7.6	372.3 ± 69.2	122.7 ± 8.9	156.4 ± 17.6
	Zn PRENATAL	272.1* ± 13.9	41.0 ± 3.6	47.8 ± 8.2	=	350.7 ± 27.2	138.2 ± 8.1	326.2 ± 10.6	146.0 ± 12.5	158.3 ± 7.1
	Zn POSTNATAL	365.2* ± 46.3	53.2 ± 5.3	66.8 ± 5.0	=	362.0 ± 24.1	151.4 ± 14.5	483.0 ± 65.5	148.2 ± 9.6	157.6 ± 9.7

Explanation: * $p < 0.05$ as compared to the control, = not detectable

Table 2. Effect of zinc pre- and postnatal exposure on locomotion irritability and exploratory activity of the adult rats after quinpirole and skf 38393 application (x ± sem; n = 8).

TYPE OF BEHAVIOR		SALINE	QUINPIROLE 3.0 mg/kg/IP		SALINE	SKF 38393 1.0 mg/kg IP	
			30 min.	60 min.		30 min.	60 min.
LOCOMOTOR ACTIVITY seconds	CONTROL	190.7 ± 48.7	472.1 ± 47.9*	499.0 ± 48.2*	245.0 ± 42.4	263.8 ± 60.9	168.2 ± 64.6
	Zn PRENATAL	162.3 ± 64.9	332.8 ± 61.5*	533.5 ± 24.0*	163.3 ± 36.6	168.7 ± 76.3	86.0 ± 42.5
	Zn POSTNATAL	222.1 ± 44.1	412.4 ± 70.6*	557.1 ± 19.0*	308.6 ± 56.0	293.0 ± 42.7	117.5 ± 32.1
GROOMING seconds	CONTROL	119.2 ± 42.5	11.6 ± 4.5*	27.0 ± 19.7*	111.2 ± 31.2	142.1 ± 48.3	82.6 ± 31.8
	Zn PRENATAL	118.1 ± 32.2	20.8 ± 12.8*	7.6 ± 5.1*+	84.8 ± 24.4	106.6 ± 53.1	33.7 ± 12.1
	Zn POSTNATAL	127.6 ± 43.0	0.0 ± 0.0*+	2.2 ± 1.0*+	125.0 ± 43.9	138.3 ± 38.1	46.0 ± 16.2
REARINGS number	CONTROL	1.5 ± 0.9	19.7 ± 8.1*	29.6 ± 12.4*	2.2 ± 1.7	2.0 ± 0.6	2.2 ± 1.1
	Zn PRENATAL	2.5 ± 1.4	14.0 ± 9.9	13.0 ± 10.6	1.2 ± 0.5	1.6 ± 0.9	1.6 ± 0.9
	Zn POSTNATAL	1.6 ± 0.8	13.2 ± 7.2*	7.1 ± 4.1*+	4.2 ± 1.3	2.2 ± 1.1	1.6 ± 0.6
IRRITABILITY Scores	CONTROL	1.0 ± 0.0	2.2 ± 0.5	1.7 ± 0.4	1.4 ± 0.2	1.1 ± 0.4	0.9 ± 0.3
	Zn PRENATAL	0.9 ± 0.2	1.0 ± 0.3	1.0 ± 0.3	1.2 ± 0.1	0.5 ± 0.2	0.2 ± 0.1
	Zn POSTNATAL	1.0 ± 0.0	0.9 ± 0.2	0.7 ± 0.3	1.0 ± 0.0	0.7 ± 0.1	0.3 ± 0.2
EXPLORATORY ACTIVITY number of peepings	CONTROL	9.8 ± 2.4	5.9 ± 2.0	5.4 ± 1.4	13.8 ± 2.7	8.0 ± 1.5	4.2 ± 1.0
	Zn PRENATAL	6.1 ± 2.6	4.9 ± 0.6	4.7 ± 0.7	7.1 ± 1.8	10.2 ± 2.1	7.1 ± 2.2
	Zn POSTNATAL	14.9 ± 2.4	3.7 ± 0.9*	4.2 ± 1.2*	15.1 ± 2.0	12.7 ± 1.5	10.2 ± 1.9

Explanation: * $p < 0.05$ as compared to saline, + $p < 0.05$ as compared to control

its injection. SKF 38393 1.0 mg/kg IP did not change the rearing activity in all three examined groups of adult rats 30 and 60 minutes after its injection in comparison to respective control (saline).

Spontaneous irritability (after saline injection) was similar in all three examined groups (about 1.0). Quinpirole and SKF 38393 did not change irritability in any examined group 30 and 60 minutes after their application as compared to respective control (saline).

Exploratory activity (after saline injection) was similar in all three examined groups (about 10 peeps per 3 minutes of observation). Quinpirole 3.0 mg/kg IP decreased the number of peepings mostly in postnatally exposed rats 30 and 60 minutes after its injection as compared to respective control (saline). SKF 38393 1.0 mg/kg IP did not modify exploratory activity in any examined group.

Yawning activity (Fig. 1). The average number of yawns after saline injection in all examined groups was about 2.5, and steadily increased after 7-OH-DPAT up to a dose of 0.130 mg/kg IP. The greatest increase was observed in the control group (tap water), and the lowest number of yawns was noticed in rats exposed to Zn prenatally. The number of yawns after 7-OH-DPAT in the rats pretreated with Zn postnatally was in between the above groups.

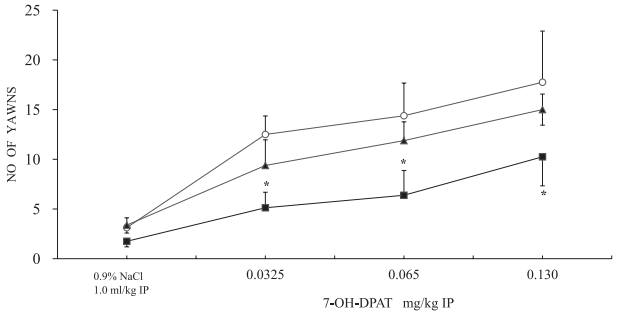

Fig. 1. Effect of pre- and postnatal zinc (Zn) exposure on yawning behaviour in adult rats after 7-OH-DPAT application (x ± SEM; n = 8)

Explanation: 1. – ○ – control, 2. – ■ – Zn prenatally 50 ppm in drinking water, 3. – ▲ – Zn postnatally 50 ppm in drinking water, * $p < 0.05$ as compared to the control

Oral activity (Fig. 2). There was a gradual increase in the number of oral movements in all three groups tested after SKF 38393 challenges. The lowest effect was observed in prenatally exposed group as compared to the control. The group exposed to Zn postnatally was in between the values of control and Zn prenatally exposed adult rats.

Stereotyped behavior (Fig. 3). Scores for stereotyped behavior peaked in all three groups at 15 to 30 minutes after apomorphine 1.0 mg/kg SC administration, then gradually declined. There were insignificant differences in the scores between the three examined groups up to 45 minutes of observation. In 60 and 75 minutes of observation significantly higher scores were observed in the prenatally exposed rats as compared to the other two groups.

No differences in catalepsy intensity between the three examined groups were observed after haloperidol or SCH 23390 0.5 mg/kg IP application (results not presented).

Reaction to pain stimulus was significantly higher in both groups of adult rats exposed to Zn (pre- and postnatally) as compared to the control group, and higher in the Zn postnatally exposed group (6.09 ± 0.18; 7.26 ± 0.41 and 5.08 ± 0.40 seconds respectively).

Locomotor coordination expressed in seconds was significantly shortest in the Zn prenatally pretreated rats and longer in postnatally exposed rats as compared to the control (13.35 ± 2.41; 122.8 ± 45.55 and 67.66 seconds, respectively).

Discussion

In the present study we exposed pregnant rats to Zn in the concentration of 50 ppm in drinking water. We have used similar concentrations of other heavy metals (eg. lead, cadmium, mercury) to expose pregnant rats and central dopaminergic system function study in adult offspring [31-33]. There was no such model in the literature and the concentration of Zn used cannot be related to any other data except ours. It must be stressed that daily requisition of Zn in humans is about 15 mg. Pregnant and lactating women have a tendency to take additional vitamin complexes where usually trace elements are added, among them Zn. As a consequence, overdosage of the metal can easily occur.

Zn is easily transferred through the blood-placenta and blood-milk barriers, and is therefore easily available in fetuses and in pups [19, 21, 34, 35]. For this reason we chose the pregnant rat as our study model for Zn intoxication of the offspring. The reason for choosing neurotransmitter-induced behavioral parameters as our criteria stems from the fact that the fetal and developing brains are much more susceptible to the toxic effects of Zn as compared to mature brain tissue, because of the slow development of the fetal blood-brain barrier. For this reason, uptake of Zn by the fetal rat brain during gestation is greater than its uptake by the brain after weaning. In our previous study we found that mostly postnatal Zn exposure increased metal accumulation in the brain of 3-week-old offspring [36]. It also has been demonstrated that Zn interferes with synaptic mechanisms of release of some neurotransmitters, and is responsible for the observed impairment of various neurotransmitter systems.

The central dopaminergic system has been demonstrated to have two main receptor classes, D1 and D2. To both classes of DA receptors belong different types of receptors: $D_1 - D_5$, which are involved in behavioral, neurobiological and psychotic disorders [37]. Many of their agonists and antagonists have been used for treating mental disturbances. In addition, nonselective and selective agonists such as apomorphine, SKF-38393, quinpirole, 7-OH-DPAT, and antagonists such as haloperidol and SCH-23390, have been widely used as pharmacological tools.

SKF-38393 is an agonist of the D_1 receptor complex, which includes D_1 and D_5 (D_1-like) receptor subtypes. The D_1 subtype is associated with SKF-38393-induced oral activity in rats, and this has been demonstrated to be a sensitive method for evaluating its binding [38]. Quinpirole and 7-OH-DPAT has been shown to be an agonist of the D_2 and D_3 (both D2-like) receptor subtypes, respectively. The D_2/D_3 receptor complex has also been implicated in quinpirole and 7-OH-DPAT-induced yawning or locomotor behavior. It has therefore been suggested that the D_3 receptor subtype may be the most important of the D_2 receptor isoforms, which is involved specifically in yawning [28, 39]. For this reason we have used the oral movement, locomotor and yawning activity induced by DA agonists, in order to assess the reactivity of DA receptors following pre- and postnatal exposure to Zn. Also apomorphine, a nonspecific central DA receptor agonist was used for stereotype behavior measurements.

Zn is an essential nutritional element, involved in the activity of many enzymes and in a variety of biochemi-

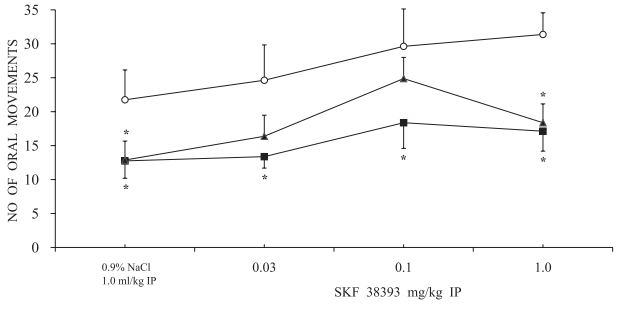

Fig. 2. Effect of pre- and postnatal zinc (Zn) exposure on oral activity in adult rats after SKF 38393 application (x ± SEM; n = 8). Explanations in Fig. 1.

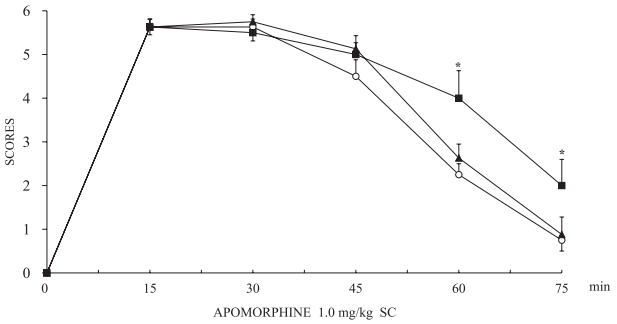

Fig. 3. Effect of pre- and postnatal zinc (Zn) exposure on stereotype behavior in adult rats after apomorphine application (x ± SEM; n = 8). Explanations in Fig. 1.

cal processes. It can interact with such metals as copper, iron, manganese, cadmium and lead. Administration of Zn together with cadmium decreases its hepatic and renal uptake and reduces the cadmium-induced inhibition of delta-aminolevulonic and dehydratase activity in serum. Increased dietary Zn content decreased cadmium level in blood and tissues, and its toxicity in mammals [40, 41]. Zn also protects cadmium and copper inhibition of glucose transport in the kidney in vitro [42]. Previously we found that single injection of Zn followed by consumption of cadmium-containing water during pregnancies of rats, prevent some disadvantageous effects on the central DA receptors reactivity to specific agonists and antagonists in their offspring [43].

Overdoses of Zn from excessive ingestion are uncommon, and result mostly from inhalation of Zn fumes during industrial processes. It may cause respiratory problems, fever, weakness, nausea and vomiting [1, 40].

As previously mentioned, Zn is an environmental element among many others suspected to induce neurotoxicity and Parkinson's disease development [7-9]. In our experiment we presented that pre- and postnatal exposure of rats to Zn-modified reactivity of the central DA receptors to their agonists and antagonists, and by this function the dopaminergic system, can be changed. Pretreatment with Zn also affected locomotor coordination and reactivity to pain stimulus. Observed behavioral changes were manifested mostly in prenatal Zn-exposed rats, in contrast to biochemical changes such as DA level and its turnover, and uptake of (^{3}H)glucose [36] was more expressed in postnatal exposed adult animals.

From the foregoing we conclude that prenatal exposure to Zn can change the function of the central dopaminergic system, and that uncontrolled supplementation of Zn during pregnancy or the suckling period can be disadvantageous for the developing mammalian brain (especially for the central dopaminergic neurotransmitter system).

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