Effect of Histochrome on the Severity of Delayed Effects of Prenatal Exposure to Lead Nitrate in the Rat Brain

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The effects of histochrome on the severity of delayed effects of prenatal exposure to lead nitrate were studied in the rat brain. Exposure of pregnant rats to lead nitrate during activation of free radical oxidation reduced activity of NADH- and NADPH-dehydrogenases in cortical neurons of their 40-day-old progeny, reduced the number of neurons in a visual field, increased the number of pathologically modified neurons, and stimulated rat motor activity in an elevated plus-maze. Two intraperitoneal injections of histochrome in a dose of 0.1 mg/kg before and after lead citrate challenge attenuated the manifestations of oxidative stress and prevented the changes in some morphological and histochemical parameters of the brain, developing under the effect of lead exposure.

Key Words: *lead; brain; progeny; free radical oxidation; antioxidants*

Progressive deterioration of ecological situation necessitates studies of the negative consequences of exposure to toxicants present in the air, water, foodstuffs, on the status of the nervous system, particularly during the early ontogenesis. Lead occupies an important place among agents which can disorder brain function and development [2,9,11, 12,15]. We previously described delayed effects of prenatal exposure to lead nitrate for the brain [8]. These consequences manifested in 40-day-old rats by signs of intense neuronal death, reduced activity of NADH dehydrogenase in neurons of different cortical compartments, pronounced activation of free radical oxidation (FRO). Published reports [10, 14] and our results [8] indicate that the development of oxidative stress is an important mechanism of toxic effect of lead. The efficiency of exogenous antioxidants in minimization of the aftereffects of long-term exposure to lead was described [13].

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The aim of this study was experimental evaluation of Histochrome (drug created at the Pacific Institute of Organic Biochemistry, Far-Eastern Department of Russian Academy of Sciences, Vladivostok) based on echinochrome, a quinoid pigment of sea invertebrate, capable of binding LPO initiators in inert complexes [4,6].

MATERIALS AND METHODS

Three groups of animals were formed: 1) progeny of 6 intact rat females (n=37); 2) progeny of 7 females receiving a single dose (200 mg/kg) of lead nitrate (0.9-1.1 ml of 4% solution) through a gastric tube on days 17-18 of gestation (n=60); and 3) progeny of 3 rat females treated with 1% histochrome solution (1 ml) containing 0.01 g echinochrome (2,3,5,7,8-pentahydroxy-6-ethylnaphthalinedion-1,4) and 0.045 g sodium carbonate (n=26). The drug diluted in saline (1:50) was twice injected intraperitoneally in a dose of 0.1 mg/kg to pregnant females: 1 day before and on the next day after poisoning. The choice of the single dose (0.1 mg/kg) was based on published data indicating that echinochrome injected intraperitoneally in doses of

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0.08-0.16 mg/kg corrected the manifestations of oxidative and nitrosative stresses [5]. All animals were kept in the same vivarium with free access to water and fodder. At the age of 40 days, the animals were decapitated. The right hemisphere was dissected strictly perpendicularly to the log and upper surface. The anterior parietal lobe was fixed in Carnoy fluid and embedded in paraffin; 7-μ sections were then stained with methylene blue. Neurons were counted at ×40 of the objective in 5 standard visual fields in the neocortical layers II and V. Pathologically changed neurons were counted under an objective ×20 in 10 standard visual fields in cortical layers II-III and V-VI. Cryostate sections (20 μ) were sliced from the hemispheric parietal lobe of the righy hemisphere; reactions for NADH and NADPH dehydrogenases (NADH-D and NADPH-D) reflecting predominantly the intensity of mitochondrial and extramitochondrial oxidative processes, respectively [7], were carried out. Activities of these reactions were measured on a Mekos-C device as described previously [8]. Free radical oxidation was studied in homogenates of the cortex, white matter of the left hemisphere, and brain stem by the chemiluminescent method (CLM). Chemiluminescence of the brain was registered on an LS 50B fluorescent spectrometer (Perkin Elmer). Standardization of the signal and mathematical processing of CLM curves were carried out using Finlab software installed in the spectrometer. Spontaneous and Fe^{2+}-induced CLM was evaluated as described previously [3]. The following parameters were evaluated: photosum over 1 min of spontaneous CLM (S_{sp}) — this value correlates with the intensity of free radical processes; fast flash peak (h) of induced CLM, indicating the content of lipid hydroperoxides; photosum (S_{ind} 1) over 4 min after "fast" flash, reflecting the rate of lipid peroxide radicals formation. The kinetics of CLM initiated by H_2O_2 in the presence of luminol [1] was analyzed by 2 parameters: maximum fluorescence indicating potential capacity of the biological object to peroxidation and photosum over 2 min CLM (S_{ind} 2) indicating activity of the antioxidant antiradical defense. The intensity of CLM (mV) was calculated per gram wet tissue and expressed in arbitrary units.

The parameters of higher nervous activity were evaluated in an elevated plus-maze (EPM) by recording (by means of Rat Test Version 1.0 original software) the duration and number of "elementary" behavioral acts (rearing, peeping down, sniffing, stay in open and closed arms, grooming) of 30-day-old pups. Each animal was observed over 3 min. The data were statistically processed using Statistica 6.0 software, the differences were considered significant at $p<0.05$.

RESULTS

Analysis of CLM of homogenates of the cortex, white matter, and brain stem of group 2 animals showed an increase (3.2 times) of the cerebrocortical S_{sp} value reflecting FRO intensity. Increased concentrations of lipid hydroperoxides (h amplitude increased 4-fold) and accelerated formation of peroxide radicals (S_{ind} 1 increased 3.4 times) were recorded. This was paralleled by attenuation of antioxidant antiradical defense and reduction of lipid peroxidation resistance: S_{ind} 2 and H values increased 3.2 and 2.8 times, respectively. Analysis of the white matter homogenate CLM showed increased S_{sp} (2.8 times), h (3.5 times), S_{ind} 1 (2.7 times), H and S_{ind} 2 (2.2 times). The values in brain stem homogenates increased 2.9, 3.6, 2.8, 2.4, and 2.3 times, respectively (Table 1). These changes in free radical status indicate oxidative stress [1].

Morphometry showed significant reduction in the number of neurons in the standard visual field in neocortical layers II and V by 10.8 and 17.6%, respectively, an increase in the number of pathologically changed neurons in a visual field by 39.5% in cortical layers II-III and by 44.8% in layers V-VI. The thickness of the cerebral cortex and layer I was lower in experimental animals in comparison with intact ones, though this difference was statistically negligible (Table 1). Histochemical analysis showed significant decrease in NADH-D activity in the neocortical neurons and of NADPH-D activity in the neocortical and hippocampal neurons (Table 1). Characteristics of higher nervous activity in group 2 animals indicated prolongation and increased number of movements, excursions to open and closed arms, peeping down, which meant that lead nitrate modified the behavior of experimental animals increasing their motor activity (Table 2). Hence, metabolic shifts, changes in the cerebrocortical morphology and behavior of animals were observed after a long period following prenatal exposure to lead. These data are in line with published reports indicating frequent detection of symptoms of motor dysregulation, excessive excitability and irritation in children living under conditions of lead pollution of the environment [2,9] and with the data indicating that intrauterine exposure of experimental animals to lead caused their hyperactivity [15].

The majority of the studied CLM parameters in group 3 differed significantly from the norm, their values being intermediate between the values in

TABLE 1. Parameters of CLM and Morphometrical and Histochemical Characteristics of the Brain in the Progeny of Females Exposed to lead Nitrate and Lead Nitrate in Combination with Histochrome (*M*±*m*)

Parameter	Group 1	Group 2	Group 3
Hemispheric cortex			
CLM intensity, arb. units			
S_{sp}	0.083±0.005	0.264±0.009*	0.093±0.008**
h	0.654±0.028	2.647±0.091*	1.316±0.066*,**
S_{ind} 1	0.787±0.040	2.723±0.121*	1.373±0.070*,**
H	1.275±0.043	4.087±0.119*	2.133±0.122*,**
S_{ind} 2	2.437±0.085	6.847±0.292*	3.479±0.277*,**
Hemispheric white matter			
S_{sp}	0.108±0.006	0.306±0.008*	0.188±0.014*,**
h	0.667±0.036	2.328±0.053*	1.168±0.057*,**
S_{ind} 1	0.987±0.062	2.695±0.079*	1.732±0.153*,**
H	1.587±0.043	3.507±0.092*	2.895±0.074*,**
S_{ind} 2	2.944±0.090	6.520±0.223*	5.046±0.273*,**
Brain stem			
S_{sp}	0.093±0.005	0.275±0.009*	0.189±0.014*,**
h	0.751±0.037	2.726±0.087*	1.519±0.069*,**
S_{ind} 1	1.229±0.067	3.513±0.091*	2.559±0.176*,**
H	1.394±0.043	3.405±0.069*	3.066±0.088*,**
S_{ind} 2	2.654±0.083	6.191±0.270*	5.130±0.177*,**
Thickness, μ of cortex	1536.0±25.9	1477.0±15.7	1576.0±13.5**
layer I	133.0±2.7	126.0±2.8	129.0±2.9
Number of neurons in visual field			
layer II	18.5±0.3	16.5±0.29*	16.5±0.19*
layer V	10.8±0.3	8.9±0.23*	8.4±0.16*
Number of dying/pathologically changed neocortical neurons in a visual field			
in layers II-III	0.490±0.106	0.810±0.112*	0.550±0.087
in layers V-VI	0.750±0.113	1.360±0.155*	0.930±0.129**
NADH-D activity in neurons of			
hippocampus	0.693±0.041	0.617±0.029	0.696±0.036
neocortex	0.655±0.033	0.571±0.025*	0.606±0.035
NADPH-D activity in neurons of			
hippocampus	0.860±0.024	0.783±0.023*	0.837±0.031
neocortex	0.750±0.029	0.666±0.025*	0.643±0.024*

Note. Here and in Table 2: $p<0.05$ *vs.* *group 1, **group 2.

groups 1 and 2 (Table 1). The CLM values for homogenates of the brain cortex, white matter, and brain stem in group 3 differed significantly from all parameters in group 2. For example, the S_{sp} of cortex homogenates was 2.8 times lower and did not differ from the value in intact control; the concentration of hydroperoxides (h) and formation of peroxide radicals (S_{ind} 1) decreased 2-fold. H amplitude decreased by 48%, S_{ind} 2 value by 49%, this reflecting increasing resistance to peroxidation and improvement of antioxidant antiradical defense almost 2-fold. The S_{sp} value in white matter homogenates decreased by 38.5%, content of lipid hydroperoxides (h) decreased 2-fold, S_{ind} 1 value decreased by 35.7%, maximum fluorescence (H) by 17.4%, and S_{ind} 2 value decreased by 22.6%. In the brain stem, S_{sp} decreased by 31.3%, h by 44.3%, S_{ind} 1 by 27.1%, H by 9.9%, and S_{ind} 2 by 17.1%. These results indicate an appreciable reduction of FRO intensity under the effect of the drug, though the

TABLE 2. Behavior of 30-Day-Old Progeny of Females, Exposed to Lead Nitrate and Lead Nitrate+Histochrome, in EPM

Parameter		Group 1	Group 2	Group 3
Time of, sec				
	peeping down	2.4±0.4	5.4...ŕ±0.76*	3.10±0.51**
	rearing	8.4±1.1	7.9±0.7	8.00±1.05
	grooming	14.80±1.97	11.50±1.16	8.0±1.5
	sniffing	157.00±1.99	159.40±1.66	167.90±1.59*,**
	movements	69.70±4.13	84.0±4.1*	110.8±4.8*,**
	in open arms	22.2±2.8	41.9±4.4*	32.7±4.2
	in closed arms	155.60±2.77	135.9±4.5*	145.90±4.24
Number of				
	peeping down	2.40±0.39	5.3±0.6*	3.20±0.54**
	rearing	7.10±0.73	7.70±0.55	8.10±0.87
	grooming	4.20±0.35	3.90±0.37	3.20±0.31
	sniffing	5.00±0.35	5.00±0.37	4.30±0.36
	movements	8.90±0.54	11.70±0.61*	11.40±0.93*
	excursions to open arms	1.70±0.19	2.50±0.24*	2.50±0.29
	excursions to closed arms	2.50±0.19	3.20±0.24*	3.30±0.29*

parameters did not reach the levels characteristic of intact animals (Table 1).

The thickness of the cerebral cortex and its layer I in group 3 did not differ from that in intact animals. The number of pathologically changed neurons in cortical layers II-III and V-VI was significantly lower and did not differ from that in the progeny of intact females (Table 1). On the other hand, the number of neurons in a standard visual field in neocortical layers II and V remained significantly lowered and did not differ from the corresponding values in group 2. Activity of NADH-D in neocortical neurons did not differ from the corresponding parameter in group 1. Activity of NADPH-D in the hippocampal neurons also remained at the level typical of intact animals, while in the neocortex this value was significantly lower than intact animals and did not differ from that in group 2 (Table 1). Study of animal behavior in EPM in group 3 showed that the time and duration of peeping down were significantly lower than in group 2 rats and virtually did not differ from the parameters characteristic of intact animals. The rest studied parameters, similarly as in group 2, differed from the control (Table 2).

Hence, histochrome treatment of pregnant rats after single dose of lead nitrate reduced the consequences of toxic exposure in the brain of their progeny, suppressing the development of oxidative stress, but not preventing it completely. It seems that this effect of the drug was responsible for less pronounced shifts in the studied morphological and histochemical parameters developing under the effect of exposure to lead nitrate.

Insufficient protective effect of the drug used according to our protocol for some morphological and histochemical parameters can be explained by persisting high FRO level in brain tissues and/or the fact that the effect of lead on the brain is not confined to FRO activation. However, the results indicate that histochrome reduces the delayed negative effect of prenatal exposure to lead and exhibits a pronounced antioxidant effect, manifesting after an appreciable period after the treatment.

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