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Neurochemical Changes in Developing Rat Brain After Pre- and Postnatal Cadmium Exposure

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Cadmium (Cd), a common environmental pollutant, is known to produce toxic effects in a number of tissues including tissues of the nervous system (Friberg et al. 1974). Cadmium exposure has been reported to cause anosmia in refinery and smelter employees (Adams and Crabtree 1961) and behavioural changes in children (Pihl 1979). Numerous studies have demonstrated behavioural aberrations and morphological and biochemical changes in the central and peripheral nervous systems in Cd-exposed experimental animals (Ali et al. 1986; Babitch 1988). It has been suggested that age of the animal plays a vital role in determining neurotoxicity and that newborn animals are more susceptible to the neurotoxic effects of Cd compared to adults (Gabbiani et al. 1967). Gestational exposure to Cd has been reported to induce fetal growth retardation in rats (Ahokas et al. 1980). Since Cd is known to pass through the placenta and to also be excreted in the mother's milk (Lucis et al. 1972), the developmental patterns of certain neurochemicals in rat pups after Cd exposure during the gestation and lactation period were studied to explore the possible mechanism of Cd-induced neurotoxicity in growing animals. Therefore, the present study examines the developmental profiles of DNA, RNA, proteins, DNA synthesis, thymidine kinase activity, and concentration of Cd and zinc in the rat brain at postnatal ages of 7, 14 and 21 days.

MATERIALS AND METHODS

^{3}H-thymidine (22,600 mci/mmole) was purchased from Bhabha Atomic Research Centre, Bombay, India. Discs of DEAE-cellulose paper (Whatman DE-81) were purchased from Whatman International Ltd., Maidstone, England. DNA, RNA, bovine serum albumin and ATP were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Other chemicals and solvents used were of analytical grade (BDH), Bombay, India).

Female albino rats of the Druckery strain (180-120 g) in oestrus were mated with males and the pregnancy was confirmed by the presence of sperm in vaginal smears (day 0). Twenty pregnant rats were housed individually in cages under standard laboratory conditions (light/dark schedule 12/12 hr, 21 $\pm$ 1°C) with free access to rat pellet diet (Hindustan Lever Laboratory Animal Feed, Calcutta, India). They were divided into two groups of 10 rats each. Rats in the experimental group were exposed to 50 ppm

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Cd as cadmium acetate through drinking water available ad libitum from 0 day of pregnancy to 21 days after parturition. Another group of pregnant rats were kept as controls. After delivery, the pups were adjusted to 8 pups per litter and were kept up to 21 days. The daily intake of water was recorded to determine the dose of Cd ingested by the rats during gestational and lactational periods. Pups at 7, 14 and 21 days of age were selected randomly from mothers of control and experimental groups for various investigations.

Five pups, selected randomly among different litters from each group were injected intraperitoneally with a single dose of 0.5 μCi ^{3}H-thymidine/g body weight (specific activity, 22,600 mci/mmole) and were euthanized by decapitation after 2 hours. Brains were removed and placed on ice, rinsed and homogenized in 9 volumes of physiological saline. An aliquot was kept for protein estimation by the method of Lowry et al. (1951) and the rest of the homogenate was processed for the isolation and estimation of nucleic acids according to the method of Schneider (1957). After removal of the lipids from the acid-insoluble portion, the pellet was suspended in the required volume of 1 N KOH and incubated for 20 hours at 37°C. A 0.5 ml aliquot was analysed for radioactivity in an LKB liquid scintillation counter after neutralizing with 1 N HCl. The remainder of the aliquot was used for the estimation of DNA and RNA concentrations.

An additional 10 pups from each group were euthanized by decapitation. Brains were removed immediately and rinsed with cold physiological saline. Five brains were processed for determination of thymidine kinase activity and five were used for Cd and Zn analysis. The enzyme activity was determined in the supernatant fraction according to the method of Prasad et al. (1974) using ^{3}H-thymidine as substrate. Activity was expressed as nmoles of thymidine monophosphate formed/hour/mg protein. Brain tissue was wet digested in a mixture of concentrated HNO_3-$HClO_4$ (6:1). The residue was dissolved in 0.1 N HNO_3 and brought up to desired volume with double distilled deionized water as described by Berman (1980). Cadmium and zinc concentrations were measured on an atomic absorption spectrophotometer (Perkin Elmer Model 5000). Blank and spiked samples were also processed and analysed simultaneously. The data were analysed by two-way ANOVA (treatment x days) after ascertaining the homogeneity of variance and normality assumptions for the computation of LSD.

RESULTS AND DISCUSSION

The average cadmium intake in pregnant rats as calculated by the consumption of water was 5.0-6.3 mg/d/kg body weight. During the lactation period, the average intake upto 7 days after parturition was 7.38 mg/d/kg, from 7-14 days it was 7.71 mg/d/kg and thereafter it was 8.0 mg/d/kg body weight. The pattern of body and brain growth for control and Cd-exposed pups is shown in Fig. 1. The body weight of Cd-exposed and control pups increased significantly upto 21 days after birth. Cd-exposure was found to significantly reduce the body weight of pups at 7 days (16.42$\pm$0.45 vs. 14.08$\pm$0.33), 14 days (29.14$\pm$2.24 vs. 21.74$\pm$1.47) and 21 days (38.00$\pm$2.80 vs. 28.32$\pm$1.22) of age compared to corresponding controls. Similarly Cd-exposure also produced significant decrease in the brain weights at all the time intervals (7d: 0.922$\pm$0.015 vs. 0.777$\pm$0.01; 14d: 1.348$\pm$0.013 vs. 1.171$\pm$0.008; 21d: 1.614$\pm$0.007 vs. 1.429$\pm$0.023). It has been reported that maternal exposure to Cd throughout gestation results in fetal growth retardation in

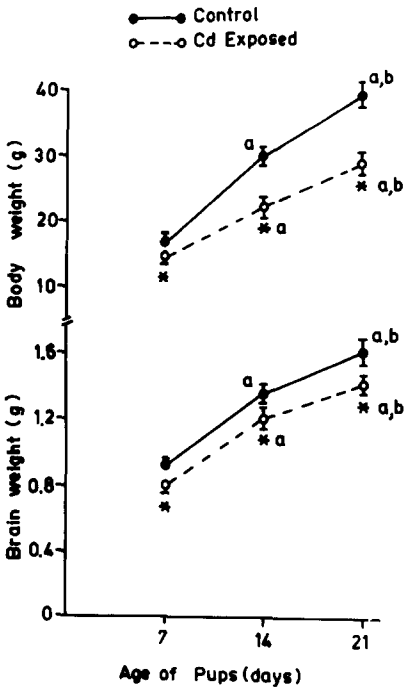

Figure 1. Effect of gestational and lactational cadmium exposure (50 ppm) on brain and body weight of the pups at 7 to 21 days.

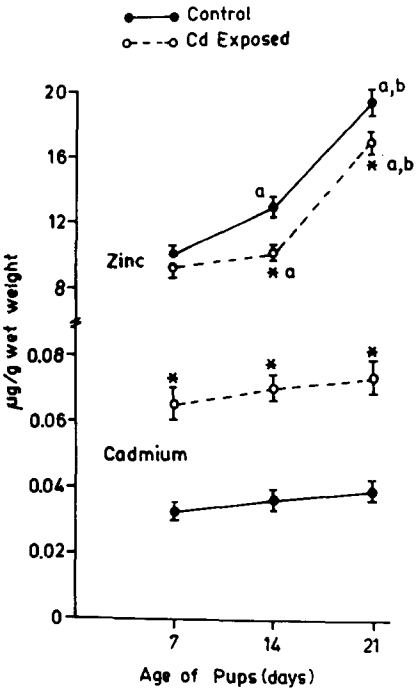

Figure 2. Effect of gestational and lactational cadmium exposure (50 ppm) on cadmium and zinc concentration in the brain of pups at 7 to 21 days.

All values represent the mean ± SE of 5 determinations.
*Significantly different when compared with their corresponding controls ($p < 0.05$).
a,b=Significantly different when compared with 7 and 14 days among the same groups (two-way ANOVA).

laboratory animals (Webster 1978; Ahokas et al. 1980). Maternal Cd exposure during gestation and lactation resulted in a significant accumulation of Cd in the brain of pups at 7 days after birth. Thereafter brain Cd concentrations remained almost identical at 14 and 21 days of age (Fig. 2). Cd exposure also resulted in a significant decrease in brain concentration of zinc in 14- and 21-day old pups.

The concentration of DNA, the activity of its synthesis-related enzyme thymidine kinase, and the concentrations of RNA and protein in Cd-exposed and control pups are presented in Table 1. Synthesis of DNA was reduced significantly in the brain of Cd-exposed pups as evident by a 11% reduction in ^{3}H-thymidine incorporation at 7 days and 67% and 53% reduction at 14 and 21 days of age, respectively. The activity of thymidine kinase was also reduced in 7, 14 and 21 day old Cd-exposed pups in comparison to controls. The concentrations of DNA, RNA and protein were found to be increased significantly from 7 to 21 days in both groups in an identical manner.

Table 1. Effect of pre-and postnatal cadmium exposure on DNA synthesis, thymidine kinase activity, protein and nucleic acid concentrations in rat pups at postnatal days 7 to 21

Parameters	Group	Age of pups (days)		
		7	14	21
[^{3}H]-Thymidine incorporation	Control	1650±69	29095±1175[a]	22404±917[a]
into DNA (cpm/mg DNA)	Cd-exposed	1471±22* (-11%)	9461±998*[a] (-67%)	10344±1652*[a] (-53%)
Thymidine kinase	Control	0.581±0.021	0.736±0.031[a]	0.775±0.045[a]
(nmoles/mg protein/hr)	Cd-exposed	0.482±0.010* (-17%)	0.625±0.009*[a] (-15%)	0.554±0.020* (-28%)
DNA	Control	1.063±0.010	1.236±0.044[a]	1.352±0.034[a]
(mg/g wet tissue)	Cd-exposed	0.966±0.025* (-9%)	1.043±0.054*[a] (-15%)	1.150±0.022*[a] (-14%)
RNA	Control	1.925±0.034	2.437±0.097[a]	2.925±0.060[ab]
(mg/g wet tissue)	Cd-exposed	1.785±0.049	2.282±0.042[a]	2.774±0.036[ab]
Protein	Control	38.326±0.626	42.304±1.460[a]	49.934±1.018[ab]
(mg/g wet tissue)	Cd-exposed	36.694±0.782	40.544±0.447[a]	46.996±0.483[ab]

The values represent the mean ± S.E., n = 5
*Significantly different when compared with their corresponding controls ($p < 0.05$)
a,b = Significantly different when compared with 7 and 14 days of postnatal age respectively, among the same control and Cd-treated group ($p < 0.05$; two-way ANOVA)
Values in parentheses denote percent change from control.

Cadmium has also been shown to suppress ^{3}H-thymidine incorporation into regenerating liver, testes and kidneys of suckling mice (Cihak and Inou 1979). A slight but significant reduction in the brain weight of Cd-exposed pups at 7, 14 and 21 days of postnatal age may be a consequence of depression in cell multiplication due to decreased DNA synthesis. However, a possibility of cell degeneration due to the continuous presence of the heavy metal in the brain may also be a contributory factor for such changes.

It is interesting to note that maxium accumulation of Cd in the brain of Cd-exposed pups occurred at 7 days of age, while maximum changes in DNA synthesis were observed at 14 and 21 days of age. Maximum changes in DNA synthesis and in the activity of thymidine kinase coincide with zinc deficiency in the brain of Cd-exposed pups. Fetal zinc deficiency has also been reported after maternal Cd-exposure (Daston 1982). Therefore, the zinc deficiency may also be one of the factors responsible for causing a decrease in the activity of this enzyme and ^{3}H-thymidine incorporation into DNA in the brain of Cd-exposed pups. The absence of zinc deficiency at 7 days of age in our study might be due to the increased transport of zinc and subsequent sequestration of Cd, in the early milk of nursing dams, in order to meet the fetal requirements (Lucis et al. 1972).

It has been reported that depressed zinc-dependent thymidine kinase activity results in an overall decrease in DNA synthesis in zinc deficient embryos (Dreosti and Hurley 1975). Webb and Samarawickrama (1981) have also reported the inhibition of DNA synthesis associated with the marked decrease in the activity of embryonic thymidine kinase. Cadmium interfere with nucleic acid synthesis in a variety of ways. Many enzymes involved in nucleic acid synthesis contain zinc as an essential component (Jacobson and Turner 1980) and Cd may replace Zn in these enzymes. Low level of Cd can bind directly to DNA and if (Webb 1979) this binding occurs in specific regions, the potential for interference with DNA functions becomes possible which leads to increased chromosomal breaks (Bauchinger et al. 1976). It may, therefore, be concluded that Cd-exposure during pre-and postnatal periods causes significant reduction in DNA synthesis and thymidine kinase activity in developing brain which may be directly related to Cd toxicity. However, the possibility of zinc deficiency playing a role in aggravating the effect of Cd on DNA synthesis cannot be ruled out.

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