Effects of *In-Utero* Exposure to Cadmium on the Brain Biogenic Amine Levels and Tissue Metal Distribution in Rats

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Abstract: The effects of cadmium in pups born to rats exposed, during the gestation period, to low levels of Cd (4.2 and 8.4 $\mu\text{g Cd}^{2+}/\text{ml}$ drinking water) on the brain biogenic amines and tissue metal distribution were studied. A significant increase in 5-HT level in whole brain was observed at day 1 of age, while after 90 days of withdrawal of treatment, increases in striatal DA and cortical 5-HT were evident. Zn level registered an increase in brain but a decrease in liver. Cu level was reduced in all the three regions studied, viz., brain, liver and kidney, at day 1 of age while after 90 days of withdrawal of treatment the effect persisted in liver. Cd levels showed significant increase only at day 1 of age. Our current findings reveal that exposure to even low levels of Cd during the early developmental stages might result in long lasting biochemical alterations in the young ones and some of the changes may persist even upto the adult stage.

Key words: In utero cadmium—Rat—Brain biogenic amines—Tissue metal distribution

INTRODUCTION

The neurotoxic effects of cadmium (Cd) have been well reported.¹⁾ The risk of environmental contamination by Cd is of increasing concern due to its widespread industrial use. Human beings are exposed to Cd in food, water and air. The human body Cd content has been found to increase from less than 1 μg in the new born to about 15–20 mg in an adult in the UK and Sweden.²⁾

The importance of age with respect to the neurotoxicity of Cd has been well documented.³⁾ Cd penetrates the fetal blood brain barrier with more ease in rats and causes hemorrhagic lesions in the brain. These facts and the prolonged biological half-life of Cd enhance its environmental hazard, especially in the young and immature organism. The present investigation was undertaken to study the effect of low level *in utero* exposure to Cd, on the brain biogenic amine levels and tissue

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metal distribution in rats.

MATERIALS AND METHODS

30 uniparous, timed-pregnant rats of Wistar-derived strain, weighing 200–225 g were drawn from our laboratory animal facility. They were randomly divided into groups of 10 each and were housed individually in acrylic cages with animal bedding. The room temperature was maintained between 22–23°C with artificial lighting provided on a 12 hr on/off cycle (on at 0600 hrs). The animals were provided with pellet diet (Hind Lever Laboratory Animal Feeds, India) and tap water *ad libitum*.

The experimental groups were exposed to either 4.2 or 8.4 $\mu\text{g Cd}^{2+}/\text{ml}$ (as cadmium acetate) drinking water throughout the gestation period. The diet and water consumption rates were measured on alternate days. All the animals were allowed to deliver normally.

Brain Biogenic Amines:

Batches of 6 male rats were sacrificed and weighed brain tissues were homogenized in a Potter-Elvehjem homogenizer with a teflon pestle using cold acidified n-butanol. The biogenic amines were extracted and estimated according to the procedure of Sadavongvivad.⁴⁾

The recovery experiments were done simultaneously. Recoveries for the different standards were $94 \pm 4\%$ for dopamine (DA) $85 \pm 6\%$ for Norepinephrine (NE) and $92 \pm 3\%$ for 5-Hydroxytryptamine (5-HT). Fluorescence was measured in an Aminco SPF-500 spectrofluorometer. The biogenic amine estimations were made in the whole brains of weaned pups (at day 21 of age) and in the brain regions, viz., cerebral cortex, cerebellum, pons-medulla, corpus striatum and rest of brain at day 90 of postnatal life.

Metal Estimation:

The distribution of Cd, Zn, Cu, Fe and Ca in the brain, liver and kidney were estimated by the wet-ash method,⁵⁾ using Perkin-Elmer Model 5000 Atomic Absorption Spectrophotometer at days 1, 21 and 90 of postnatal life.

Statistical Analysis:

The data were analysed by the one way ANOVA across groups, after ascertaining the homogeneity of variance and normality assumptions of the data. If the overall F ratio was found to be significant, post-hoc analyses were provided by computation of the Newman-Keul's Statistic. The significance levels were ascertained at $p < 0.05$ and $p < 0.01$.

RESULTS

Consummatory Behavior—Dams:

The diet and water consumption in the Cd-exposed pregnant mothers did not differ significantly from the control groups. The water consumption in both the Cd-exposed dams—during the gestation period was 32–48 ml/day/rat and did not significantly differ from each other.

Brain Biogenic Amines:

The effect of the *in utero* exposure to Cd on the whole brain levels of NE, DA and 5-HT in 21 day old pups are shown in Table 1. ANOVA indicated main effects of treatment only for the 5-HT levels ($F_{2, 15} = 5.846$, $p < 0.05$). Post-hoc analysis revealed a significant increase in the 5-HT levels in both the Cd exposed groups ($p < 0.05$). In the 90 days old rats (Table 2) significant increases in the striatal DA ($p < 0.01$) and cortical 5-HT ($p < 0.01$) levels were observed.

Table 1. Brain levels of biogenic amines in 21 day old rats exposed to Cd *in utero*

Group	Brain biogenic amine levels ($\mu\text{g/g}$ fresh weight) w.b.		
	NE	DA	5-HT
Control	0.483 ± 0.061	0.629 ± 0.060	0.611 ± 0.058
Cd _{4.2}	0.492 ± 0.047	0.557 ± 0.050	$0.751 \pm 0.023^*$
Cd _{8.4}	0.443 ± 0.020	0.517 ± 0.021	$0.776 \pm 0.022^*$

Values are the mean $\pm$ S.E. of 6 pups (littermate).

* $p < 0.05$ compared to controls.

w.b.: Whole brain.

Table 2. Brain regional levels of biogenic amines in 90 day old rats exposed to Cd *in utero*

Parameter	Group	Brain regions				
		Cerebral cortex	Corpus striatum	Cerebellum	Pons-medulla	Rest of brain
NE ($\mu\text{g/g}$)	Control	0.525 ± 0.037	0.374 ± 0.022	0.689 ± 0.059	0.751 ± 0.057	0.452 ± 0.042
	Cd _{8.4}	0.627 ± 0.045 (119)	0.432 ± 0.033 (115)	0.706 ± 0.069 (102)	0.892 ± 0.073 (118)	0.427 ± 0.023 (94)
DA ($\mu\text{g/g}$)	Control	0.789 ± 0.071	6.148 ± 0.711	0.252 ± 0.023	1.780 ± 0.156	1.924 ± 0.203
	Cd _{8.4}	0.677 ± 0.055 (86)	$10.847 \pm 0.977^{**}$ (176)	0.311 ± 0.038 (123)	1.810 ± 0.205 (101)	1.731 ± 0.169 (90)
5-HT ($\mu\text{g/g}$)	Control	0.958 ± 0.104	1.308 ± 0.163	0.369 ± 0.051	1.540 ± 0.131	1.745 ± 0.138
	Cd _{8.4}	$1.404 \pm 0.132^{**}$ (146)	1.406 ± 0.205 (107)	0.273 ± 0.034 (74)	1.488 ± 0.124 (97)	1.694 ± 0.159 (97)

Values are the mean $\pm$ S.E. of 6 rats.

** $p < 0.01$ compared to controls; Values in parentheses indicate the percentage compared to controls.

Tissue Metal Distribution:

Table 3 shows the effects of the *in utero* Cd-exposure on the tissue metal distribution in one day old pups. Statistical analysis showed that a significant increase in Cd level was observed in the liver and kidney ($p < 0.05$) without any accumulation in the brain. Zn levels registered a significant increase in the brain ($p < 0.05$) but a decrease in the liver ($p < 0.05$). While the Cu levels were reduced in all the three tissues ($p < 0.05$), the Fe level was decreased only in kidney ($p < 0.05$). These changes were observed only in 8.4 $\mu\text{g/ml}$ group.

In the 21 day old pups, the liver Zn and Fe levels were significantly decreased ($p < 0.05$ and $p < 0.01$, respectively) in the 8.4 $\mu\text{g/ml}$ Cd-exposed group and a decrease in the Cu level ($p < 0.05$) was observed in the 4.2 $\mu\text{g/ml}$ Cd-exposed group (Table 4).

In the 90 day old rats, no significant alterations in any of the metals were observed except a decrease in the liver Cu ($p < 0.01$) in the 8.4 $\mu\text{g/ml}$ Cd-exposed group (Table 5).

Table 3. Tissue metal distribution in 1 day old rats exposed to cadmium *in utero*

Tissue	Group	Metal contents ($\mu\text{g/g}$ fresh weight)				
		Cd	Zn	Cu	Fe	Ca
Brain	Control	0.12 ± 0.05	11.74 ± 1.09	0.82 ± 0.09	16.92 ± 2.48	13.52 ± 1.15
	Cd _{4.2}	0.14 ± 0.06	10.63 ± 0.90	0.90 ± 0.12	17.21 ± 1.87	12.48 ± 1.05
	Cd _{8.4}	0.18 ± 0.05	$15.20 \pm 1.05^*$	$0.60 \pm 0.04^*$	15.45 ± 1.64	13.84 ± 0.88
Liver	Control	0.22 ± 0.02	49.32 ± 3.57	18.39 ± 0.98	217 ± 15.11	15.52 ± 1.22
	Cd _{4.2}	0.25 ± 0.03	45.27 ± 4.83	17.33 ± 1.54	188 ± 18.78	16.33 ± 1.18
	Cd _{8.4}	$0.33 \pm 0.04^*$	$37.32 \pm 3.62^*$	$16.44 \pm 1.13^*$	168 ± 19.33	17.65 ± 2.02
Kidney	Control	0.57 ± 0.04	15.26 ± 1.46	1.33 ± 0.12	23.55 ± 1.77	22.76 ± 2.52
	Cd _{4.2}	0.61 ± 0.05	12.61 ± 1.12	1.44 ± 0.24	19.76 ± 1.94	25.28 ± 1.98
	Cd _{8.4}	$0.72 \pm 0.05^*$	14.35 ± 1.43	$0.97 \pm 0.13^*$	$17.81 \pm 1.88^*$	23.66 ± 2.34

Values are the mean $\pm$ S.E. of 6 pups (littermate).

* $p < 0.05$; Compared to controls.

Table 4. Tissue metal distribution in 21 day old rats exposed to cadmium *in utero*

Tissue	Group	Metal contents ($\mu\text{g/g}$ fresh weight)				
		Cd	Zn	Cu	Fe	Ca
Brain	Control	0.14 ± 0.06	18.09 ± 2.12	0.93 ± 0.20	24.62 ± 1.81	15.25 ± 1.22
	Cd _{4.2}	0.16 ± 0.05	18.18 ± 1.37	1.02 ± 0.15	26.74 ± 2.74	14.34 ± 1.55
	Cd _{8.4}	0.17 ± 0.04	20.12 ± 1.86	1.03 ± 0.17	25.88 ± 3.32	16.78 ± 1.39
Liver	Control	0.30 ± 0.03	51.27 ± 4.75	8.32 ± 0.53	237 ± 18.01	17.27 ± 1.43
	Cd _{4.2}	0.42 ± 0.05	41.37 ± 3.80	$5.97 \pm 0.58^*$	183 ± 10.43	18.31 ± 2.66
	Cd _{8.4}	0.39 ± 0.04	$36.69 \pm 2.54^*$	6.87 ± 0.38	$165 \pm 11.12^{**}$	17.88 ± 2.08
Kidney	Control	0.61 ± 0.07	21.30 ± 2.11	10.17 ± 0.92	27.36 ± 1.89	27.19 ± 2.53
	Cd _{4.2}	0.66 ± 0.08	24.55 ± 1.97	8.25 ± 0.55	29.22 ± 1.99	24.35 ± 1.66

Values are the mean $\pm$ S.E. of 6 pups (littermate).

* $p < 0.05$; ** $p < 0.01$; Compared to controls.

Table 5. Tissue metal distribution in 90 day old rats exposed to cadmium *in utero*

Tissue	Group	Metal contents ($\mu\text{g/g}$ fresh weight)				
		Cd	Zn	Cu	Fe	Ca
Brain	Control	0.13 ± 0.04	20.56 ± 2.59	1.54 ± 0.23	21.34 ± 2.71	15.85 ± 1.64
	Cd _{4.2}	0.14 ± 0.03	21.43 ± 1.95	1.23 ± 0.18	20.66 ± 2.03	17.21 ± 1.59
	Cd _{8.4}	0.16 ± 0.04	19.35 ± 2.68	1.10 ± 0.28	18.81 ± 1.22	16.87 ± 1.83
Liver	Control	0.33 ± 0.05	38.74 ± 2.78	8.95 ± 0.54	237 ± 18.11	19.80 ± 1.63
	Cd _{4.2}	0.38 ± 0.04	32.28 ± 2.64	6.90 ± 0.57	243 ± 25.3	17.86 ± 1.31
	Cd _{8.4}	0.40 ± 0.05	36.55 ± 2.45	$5.71 \pm 0.50^{**}$	213 ± 18.9	19.67 ± 1.95
Kidney	Control	0.58 ± 0.14	20.17 ± 2.33	11.14 ± 1.09	27.04 ± 1.63	24.17 ± 2.50
	Cd _{4.2}	0.69 ± 0.16	19.22 ± 1.87	10.06 ± 2.05	28.67 ± 1.88	26.88 ± 3.24
	Cd _{8.4}	0.63 ± 0.08	19.36 ± 1.86	12.28 ± 1.33	25.17 ± 2.06	28.18 ± 2.43

Values are the mean $\pm$ S.E. of 6 rats.

** $p < 0.01$; Compared to control.

DISCUSSION

The data presented in the present communication show that *in utero* exposure to a very low level of Cd produces marked alterations in the brain biogenic amine levels and tissue distribution of Zn, Cu and Fe and some of these changes persist even at the older age after withdrawal of the treatment. These changes were observed despite a low level accumulation of Cd in the different tissues.

The current findings on the effect of Cd-exposure on the whole brain 5-HT level in one day old pups are in accordance with the earlier report⁽⁶⁾ where an increase in 5-HT level in different brain regions was observed following Cd administration through intraventricular cannula in adult rats. A noteworthy feature of the present findings is that the low level *in utero* Cd-exposure during gestation period caused persistent effects even at 90 days after the withdrawal of exposure, in the form of elevated striatal dopamine and cortical 5-HT. Earlier report⁽⁷⁾ indicated an increase in the striatal DA by Cd exposure which returned to normal after withdrawal of treatment but the 5-HT level remained lowered even after withdrawal of treatment. One of the major reasons for the contradictory results may be the fact that in our case the rats were exposed to the metal during the early developmental stage when there is differentiation and rapid cell multiplication.

In spite of the very low dose and short duration of exposure, a low level Cd transfer in the foetal liver and kidney was observed in the present study. Earlier reports have indicated similar changes at much higher doses.^{8,9,10)} The low accumulation of Cd in the foetal tissues may be explained by the mode of administration (oral) of this toxicant, in which a very small amount is retained by the dam and by the effective clearance of Cd from the maternal circulation followed by the incorporation of this metal in the target organs as metallothionein.¹⁰⁾

The decreased Zn level in the liver in the present experiment is in contrast to the findings of Bunn and Matrone,¹¹⁾ and Schroeder and Nason¹²⁾ but are in agreement with that of Sowa and Steibert.¹⁰⁾ An interesting finding in this study is the

increase in Zn level of brain by Cd exposure. There is a possibility of redistribution of Zn from foetal liver to other organs following Cd exposure.¹³⁾

Some investigators^{14,15)} have reported the decrease of whole body Cu and Fe in rat pups following Cd administration and others¹⁰⁾ have reported decrease in Cu level in foetal liver, kidney, placenta and brain. In the present experiment also, there was a significant decrease in Cu level in all organs studied and the changes persisted in the liver even after 90 days of withdrawal of exposure, although there was no significant difference in Cd level compared to the controls at 90 days. These findings indicate that Cd can cause profound changes in essential metal metabolism even at low level exposure conditions.

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