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Cadmium effects on dopamine turnover and plasma levels of prolactin, GH and ACTH

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This paper analyzes possible dopamine (DA) mediated cadmium effects on plasma levels of prolactin, growing hormone (GH) and adrenocorticotropic hormone (ACTH), and if these changes are related to metal accumulation. For that purpose, adult male rats were treated with 50 mg/L of $CdCl_2$ in the drinking water for one month. Plasma levels of prolactin, ACTH and GH were measured by specific double antibody radioimmunoassays. DA was measured by high performance liquid chromatography using electrochemical detection. Cadmium content in the tissues was measured by atomic absorption spectometry with graphite furnace. Analysis was performed by using a T-Student test. Metal exposure increased DA content (34.79±3.06 *vs.* 18.2±2.88 pg/mg protein) and decreased its turnover (0.40±0.07 *vs.* 0.75±0.06) in posterior hypothalamus. Cadmium also decreased DA turnover in median eminence (0.48±0.15 *vs.* 1.50±0.63). Plasma levels of prolactin and GH decreased (2.4±0.11 *vs.* 3.1±0.15 ng/mL and 5.37±0.05 *vs.* 9.87±1.8 ng/mL respectively), while those of ACTH increased (2.73±0.14 *vs.* 1.7±0.16 ng/mL). Cadmium concentration increased in both hypothalamus (4.88±0.34 *vs.* 0.72±0.2 µg/g) and pituitary (22.82±4.57 *vs.* 5.02±1.25 µg/g) after the metal exposure. These results suggest that cadmium effects on the secretion of these hormones are not mediated by dopamine and might be correlated to the metal accumulation at pituitary level.

Key words: Cadmium, Dopamine turnover, Prolactin, GH, ACTH.

Metals are among the few compounds that have been shown to accumulate in the hypothalamus and the pituitary (17-19), and the exposure to this metal is associated with changes in the activity of the endocrine system (12-19, 22, 25). It was previously reported in rats that cadmium differentially modified both basal pro-

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lactin and adrenocorticotropin (ACTH) hormone secretions as well as their ultradian patterns of secretion (12-15, 17, 22, 25).

Cadmium changes the level of dopamine (DA) within the brain, since the areas studied are not involved in the modulation of pituitary hormone secretion (1, 2, 6, 8, 11, 23). HRDINA *et al.* (11) described changes in the DA content in brain stem and CHANDRA *et al.* (6) in half brains after cadmium exposure. NATION *et al.* (23) also demonstrated variations in dopamine content in the striatum and hypocampus of rats exposed to cadmium alone, or in combination with lead. Besides, ANDERSSON *et al.* (1) showed differences in dopamine, and their metabolic rates (DA/3,4-dihydoxyfenyl acetic acid, DOPAC) in the frontal cortex, nucleus accumbens, olfatory tubercule, and the striatum of rats exposed to cadmium.

Considering that dopamine inhibits prolactin, GH and ACTH secretions (9, 10, 20), cadmium may modify secretion of these pituitary hormones through changes in dopamine metabolism at the hypothalamus.

The aim of the present work was designed to answer the following questions: a) if cadmium accumulation in the hypothalamus and the pituitary affects the secretory mechanisms of prolactin, GH and ACTH and b) if cadmium effects on plasma levels of prolactin GH and ACTH are mediated by changes in dopamine metabolism at the hypothalamus.

Materials and Methods

Animals and treatment.– Adult male Sprague-Dawley rats (Animal Production of Santiago University, Spain) were used in all experiments. They were maintained in a room with a controlled photo-period (14h light/10h darkness) and temperature (22 ± 2 °C), and were supplied with rat's chow (Panlab, Barcelona, Spain) and water *ad libitum*. Cadmium was given at a concentration of 50 ppm of cadmium chloride ($CdCl_2$) in the drinking water (calculated daily dose per rat: 2 mg/Kg body weight). The dose was selected according to previous works from literature (13, 25). This dose modified the pulsatile LH and prolactin secretion in male rats (15, 16).

Two groups of 16 adult animals were used. Group 1 was submitted to 50 ppm of $CdCl_2$ in the drinking water for one month and Group 2 received cadmium-free water and was used as control. At the end of cadmium exposure all animals were killed by decapitation at 14:00h to avoid the diurnal secretion pattern of dopamine turnover and pituitary hormones. Care was taken to avoid any major stress and the decapitation procedure was completed within 10-20 seconds. Trunk blood was collected in tubes containing EDTA (60 g/L) and plasma was obtained after centrifugation at 1,500 g for 15 min at 4 °C, kept frozen at –20 °C until prolactin, GH and ACTH was measured. The hypothalamus and pituitary were immediately removed and frozen at –80 °C until further assayed. The hypothalamus from 8 animals of each group were used to measure cadmium accumulation and, the other hypothalamus were used to measure dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) contents at the median eminence and anterior, mediobasal and posterior hypothalamus. Also the body weight gain of all the animals was recorded.

The studies were conducted in accord with the principles and procedures outlined in the NIH guide for the Care and Use of Laboratory Animals (24).

Measurements.– Hypothalamus were quickly dissected out according to previous works from the group (9). Amine contents were analyzed by high performance liquid chromatography as previously described (9).

Cadmium concentration was determined in the hypothalamus and pituitary gland of individual animals. Tissue cadmium concentrations were determined by graphite furnace atomic absorption spectrophotometry after microwave digestion (GFAAS) (21).

Plasma levels of prolactin, GH and ACTH were determined by specific double-antibody radioimmunoassay (12-19). The reagents were kindly supplied by the National Hormone and Pituitary Program (NHPP, Rockville, MD, USA) and Dr. A. Parlow (Harbor UCLA, Medical Centre, Torrance, CA). 125I was obtained from ICN (Irvine, CA, USA).

Statistical analysis.– It was performed employing a Student t-test (SPSS for Windows 98). The level for statistical significance was $P<0.05$. All values represent the mean ± S.E.M.

Results

Cadmium intake significantly decreased plasma levels of prolactin and GH, while those of ACTH increased (Table I).

After cadmium exposure DA content increased in posterior hypothalamus (Table II) as compared to the control group. Besides, dopamine turnover, measured as DOPAC/DA ratio, diminished in the median eminence and posterior hypothalamus (Table II).

Metal exposure increased cadmium both in the hypothalamus (4.88±0.34 *vs.* 0.72±0.2 µg/g in control; $P<0.001$) and in the pituitary gland (22.8±4.57 *vs.* 5.02 ± 1.25 µg/g in control group; $P<0.001$). Besides body weight gain decreased in those animals exposed to cadmium (140±12.4 g in control group *vs.* 110±9 g in cadmium treated animals; $P<0.05$).

Discussion

Ongoing results indicate that cadmium exposure differentially affects pituitary hormone secretion. These effects could be explained by the specific cadmium accumulation at the pituitary level. However, these effects are not correlated with dopamine metabolism at the hypothalamic level. A disruption of the regulatory mechanisms of the hypothalamic-pituitary axis activity emerges (17-19).

After cadmium exposure, DA turnover decreased in both median eminence and posterior hypothalamus. This inhibitory effect on DA turnover may be expected considering that this effect was previously shown in other brain areas [i.e. striatum, (8)]. However, the absence of changes on DA content observed in the majority of the brain areas studied, agrees with the data obtained by NATION *et al.* (23) in the frontal cortex and striatum of male rats after cadmium administration at a dose of 100 mg/L in the drinking water, during 60 days. Similar results have been also shown in the whole brain by ARITO *et al.* (2).

The decrease in plasma prolactin levels observed after cadmium exposure, agrees with previous data gathered in adult rats after acute or shorter exposures to cadmium (12, 14). Cadmium effectively depressed prolactin in both *in vivo* and *in vitro* studies (14, 15, 22). Such inhibitory effect could be explained by the decreased amplitude of prolactin peaks reported elsewhere (13). Cadmium may act directly on the lactotrophs, through an interaction

Table I.- *Plasma levels (ng/mL) of prolactin (PRL), GH and ACTH in adult male rats treated with cadmium-free water (control) or with cadmium chloride (50 mg/L in the drinking water, for one month).*

Group	rPRL-RP3	rGH-RP3	rACTH-RP3
Control	3.10 ± 0.15	9.87 ± 1.80	1.70 ± 0.16
Cd-treated	2.40 ± 0.11**	5.37 ± 0.05**	2.73 ± 0.14***

Values are expressed as mean ± S.E.M. (n=8 in each group). **P<0.01, ***P<0.001 vs. control group.

Table II.- *Effect of cadmium administration (50 mg/L cadmium chloride in the drinking water for one month) on dopamine (DA) content (pg/mg protein) and DOPAC/DA ratio in different regions of rat hypothalamus.*

Values are mean ± S.E.M. (n=8). *P<0.05; ***P<0.001 vs. control.

	DA		DOPAC/DA	
	Control	Treated	Control	Treated
Med. Eminence	33.70 ± 12	29.61 ± 11.1	1.50 ± 0.63	0.48 ± 0.15*
Ant. Hyp.	12.2 ± 2.99	10.6 7± 2.96	0.50 ± 0.20	0.30 ± 0.12
Med. Hyp.	18.06 ± 5.90	17.56 ± 2.76	1 ± 0.2	0.74 ± 0.11
Post. Hyp.	18.12 ± 2.88	34.79 ± 3.06***	0.75 ± 0.06	0.40 ± 0.07*

with the prolactin molecule, that is sensitive to divalent metals, as was shown *in vitro* (22). However, previous studies *in vivo* indicated a normal response of prolactin to the thyrotropin releasing hormone (TRH) in cadmium-treated rats (13), thus indicating a differential intracellular kinetics of cadmium *in vivo* as compared to the *in vitro* studies using cultures of pituitary cells (22). The effects of the metal on DA at the median eminence would be suggestive of increased rather than decreased plasma prolactin levels, considering the well known inhbitory role of DA on prolactin (9).

The changes in plasma GH levels after cadmium exposure may be due to a direct interaction of the metal at the pituitary level similar to that described for prolactin or at the hypothalamic level diminishing acetylcholine secretion (7), which is a positive neuromodulator for GH secretion (5). The changes in DA at the median eminence may account for an increase in plasma levels of GH rather than a reduction. Also, the reduction of body weight-gain may be explained by the reduction in plasma GH levels described in this and in previous papers (3).

Cadmium exposure increased plasma ACTH levels, although previous data (12) using other doses of the metal and shorter lengths of metal exposure showed opposite effects. Thus indicating that cadmium effects are dependent on the dose of metal selected and on the duration of the treatment. Other neuromodulators not analyzed in this study may account for the changes in ACTH shown in this study as DA changes at the median eminence may account for decreases rather than increases in plasma ACTH levels.

The marked changes in DA content and its turnover in posterior hypothalamus may be related to changes in the autonomic nervous system reactivity as this

area modulate the activity of this system (4). Whether or not other functions related to posterior hypothalamus are changed deserves further investigation.

Cadmium was accumulated in the hypothalamus and the pituitary after its exposure, which agrees to data previously reported (17, 18). Cadmium content at pituitary level seems to correlate with the observed changes in the plasma levels of prolactin GH and ACTH. However, the amount of the metal found at the hypothalamus does not affect dopamine content or its turnover in the hypophysiotropic area of the hypothalamus.

In summary, this data show an inhibitory effect of cadmium on prolactin and GH secretion but a stimulatory one on ACTH. Besides, these changes could be explained by the accumulation of cadmium at the pituitary level, but not by the changes in dopamine turnover at the hypothalamic level.

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A. LAFUENTE, N. MÁRQUEZ, D. PAZO y A. I. ESQUIFINO. *Efectos del cadmio sobre la tasa de metabolización de dopamina y los niveles plasmáticos de prolactina, GH y ACTH.* J. Physiol. Biochem., **57** (3), 231-236, 2001.

Se estudia el posible efecto del cadmio sobre los niveles plasmáticos de prolactina, hormona del crecimiento (GH) y adrenocorticotropina (ACTH) mediados por la dopamina (DA), y si los cambios están relacionados con la acumulación del metal. Para ello, se han tratado ratas macho adultas con 50 mg/L de $CdCl_2$ en el agua de bebida durante un mes. Las hormonas se miden por radioinmunoanálisis específicos. La DA y su metabolito DOPAC se miden por cromatografía líquida de alta resolución. El contenido de cadmio en el hipotálamo y la hipófisis se mide mediante cromatografía de absorción atómica con cámara de grafito. La exposición al metal aumenta el contenido de DA (34,79±3,06 *vs.* 18,2±2,88 pg/mg proteina) y disminuye su tasa de recambio (0,40±0,07 *vs.* 0,75±0,06) en el hipotálamo posterior. También disminuye la tasa de recambio de DA en la eminencia media (0,48±0,15 *vs.* 1,50±0,63). Los niveles plasmáticos de prolactina y GH disminuyen (2,4±0,11 *vs.* 3,1±0,15 ng/mL y 5,37±0,05 *vs.* 9,87±1,8 ng/mL, respectivamente), mientras que aumentan los de ACTH (2,73±0,14 *vs.* 1,7±0,16 ng/mL). La concentración de cadmio aumenta en el hipotálamo (4,88±0,34 *vs.* 0,72±0,2 µg/g) e hipófisis (22,82±4,57 *vs.* 5,02±1,25 µg/g) tras la exposición al metal. Estos resultados sugieren que los efectos del cadmio sobre la secreción de estas hormonas no son a través de la dopamina, aunque se correlacionan con la acumulación del metal a nivel hipofisario.

Palabras clave: Cadmio, Dopamina, Prolactina, GH, ACTH

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