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Effects of acute and subchronic cadmium administration on pituitary hormone secretion in rat

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Cadmium administration is known to be followed by deleterious effects on the endocrine system although its action mechanism is not well understood. The purpose of this study was to determine, *in vivo*, the effects of acute and/or subchronic cadmium chloride administration (6 mg/kg or 4 mg/kg/day during 14 days) on prolactin, luteinizing hormone (LH), follicle stimulating hormone (FSH), growth hormone (GH) and thyroid stimulating hormone (TSH) secretion in adult male rats. Six h after a single injection of $CdCl_2$, a diminution of plasma levels of prolactin (4.52 ± 0.53 vs. 16.2 ± 2.7 ng/mL, $p < 0.01$), GH (3.39 ± 0.43 vs. 6.71 ± 1.30, ng/mL, $p < 0,01$), TSH (2.76 ± 0.64 vs. 7.65 ± 1.15 ng/mL, $p < 0.01$) and LH (4.1 ± 1.3 vs. 5.18 ± 0.28, $p < 0.05$) was observed, where as plasma FSH levels did not change. On the other hand, subchronic cadmium chloride administration for 14 days, increased plasma levels of GH (13.39 ± 2.74 vs. 6.71 ± 1.3 ng/mL, $p < 0.05$), TSH (27.8 ± 4.42 vs. 6.65 ± 1.15 ng/mL, $p < 0.001$), LH (11.1 ± 1.3 vs. 5.18 ± 0.28 ng/mL, $p < 0.001$) and FSH (53.16 ± 3.66 vs. 12.51 ± 1.45, $p < 0.001$), whereas plasma prolactin levels decreased (6.86 ± 1.38 vs. 16.2 ± 2.7 ng/mL, $p < 0.01$). In animals subchronically treated with $CdCl_2$, body weight gain was lower than in control rats ($p < 0.001$). The present findings suggest that acute and subchronic cadmium administration modify pituitary hormone secretion differentially and specifically.

Key words: Cadmium, Prolactin, GH, TSH, FSH, LH.

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Cadmium exposure could interfere with various physiological processes (5, 13, 14). However, field and laboratory studies indicate that bioaccumulation of metals, including cadmium, occurs in primary and secondary consumers of the food web (1, 8), so that increased cadmium uptake in the said food web creates a health risk for both humans and animals, although the target organs for cytotoxic effects of these metals remain poorly understood.

Among the cytotoxic effects described for cadmium, the endocrine system has been recently studied, although only scant evidences have been obtained. Cadmium inhibits (10) or increases (2) *in vitro* basal pituitary release of prolactin. However, *in vitro* KCl-stimulated prolactin secretion was not modified by cadmium exposure (2). Other hormones like GH have also been studied. In this context cadmium did affect *in vitro* release of GH from bovine pituitary glands (10). On the other hand *in vivo* administration of cadmium (0.3 mg/l00 g bw) for two weeks to adult male rats significantly reduced serotonin and acetylcholine contents in various discrete regions of the brain (4). All these data indicate that cadmium may exert direct effects on the pituitary gland, or indirect effects through changes in hypothalamic neurotransmitters (12, 17) involved in the regulation of pituitary hormone secretion.

In spite of the update data, the effects of cadmium on pituitary hormone secretion are not well understood. This work was designed to analyze the effectsof cadmium administration on circulating plasma prolactin, GH, TSH, FSH and LH levels in adult male rats.

Materials and Methods

Animals and experimental design.– Adult male Sprague-Dawley rats weighing 250-260 g were used in all experiments. They were housed with controlled photo-period (14 h L/10 h D; lights on from 07.00 to 21.00 h) and temperature (22 ± 2 °C), and with rat chow and water available *ad libitum*.

Acclimatized animals were divided into three groups of eight animals each. One group was injected daily with cadmium chloride ($CdCl_2$ dissolved in 0.9 % NaCl solution) at a dose of 4 mg/kg b. w./day, sc, for a period of 14 days. A second group was treated with a single ip injection of $CdCl_2$ at a dose of 6 mg/kg b. w. The third group of animals was injected with either one or 14 injections of 0.9 % NaCl solution without cadmium, used as controls. The $CdCl_2$ or NaCl solutions were injected at 08.00 hour.

Rats were killed by decapitation at 14:00 h to avoid the diurnal secretion pattern of pituitary hormones, and trunk blood was collected in tubes containing EDTA (60 g/l). Plasmas were obtained after centrifuging the samples at 1,500 *g* for 15 min at 4 °C and were kept frozen at -20 °C until analyzed.

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

Hormones measurements.– Prolactin, GH, TSH, FSH and LH were determined by specific double-antibody radioimmunoassay systems. The reagents were kindly supplied by the National Hormone and Pituitary Program (NHPP, Rockville, MD, USA). Prolactin values are expressed in terms of NIADD rat PRL RP-3 reference preparation, GH in terms of NIADD rat GH RP-2, TSH in terms of NIADD rat TSH RP-3, FSH in terms of NIADD rat FSH RP-2, and LH in terms of NIADD rat LH RP-3 reference preparation. Minimum sensitivity of each assay was 5, 2, 40, 20 or 0.5 pg/tube

for prolactin, GH, TSH, FSH or LH respectively. Samples were analyzed within the same assay to avoid interassay variations. The intra-assay coefficients of variation were 7.4 %, 8.1 %, 6.6 %, 9.5 % and 7.3 % for prolactin, GH, TSH, FSH and LH, respectively.

Data analysis.– Comparison of values was done by Student's *t* test. The results were considered significant at $p < 0.05$. All values represent the mean ± S.E.M.

Results

Acute administration of $CdCl_2$ (6 mg/kg), decreased plasma levels of prolactin, GH, TSH and LH 6 h after (table I) as compared to the values found in the control group, while plasma concentration of FSH did not change.

The $CdCl_2$ administration (4 mg/kg/day), for 14 days, decreased plasma prolactin levels, but increased plasma concentrations of the other hormones. The body weight gain was lower than in control animals ($p > 0.001$: 262.73 ± 7.27 vs 331.43 ± 5.08).

Discussion

The preceding results indicate that cadmium accumulation plays an important role for cytotoxic effects of this metal at the hypothalamic-pituitary axis, as indicated by the differential effects on pituitary hormone secretion exherted by acute versus subchronic cadmium treatments.

The decrease in prolactin plasma levels shown after acute or chronic administration of cadmium chloride agreed with previous data from *in vitro* and *in vivo* studies (10, 13, 15). However, in long term studies (19), cadmium increased plasma prolactin levels from the 12th day of treatment. In addition, cadmium treatment significantly decreased TRH-stimulated *in vitro* prolactin release from the anterior pituitary gland (18). Thus, cadmium could interfere with the TRH receptors at the lactotrophs, similarly as did other toxic substances (11). However, the inhibitory effect of cadmium on prolactin secretion could be explained by an increase in the dopaminergic tone (7), thus leading to a reduction in prolactin secretion. These data may be also supported by the decrease in serotonin content at the hypothalamus after cadmium treatment, as was previously reported (4).

Acute cadmium administration decreased plasma GH levels in agreement with *in vitro* studies on GH and prolactin release from bovine adenohypophysis (13). Cadmium may interact at the pituitary level, within the somatotroph cells being a possible mechanism for this divalent metal to modify GH release from the pituitary. However, hypothalamic effects of cadmium changing somatostatin release cannot be discarded.

Table I. *Pituitary plasma hormone levels (ng/mL) in adult rats treated with CdCl2 (6 mg/Kg b. w. for acute administration, or 4 mg/Kg b. w. for 14 days) or vehicle.*
Values are expressed as mean ± SEM; n = 8 rats in each group.

Group	r-RPL-RP3	r-GH-RP2	r-TSH-RP3	r-FSH-RP2	r-LH-RP3
Control	16.2 ± 2.70	6.71 ± 1.30	7.65 ± 1.15	12.51 ± 1.45	5.18 ± 0.28
Acute	5.52 ± 0.53**	2.39 ± 0.43*	2.76 ± 0.64**	10.04 ± 0.31	4.10 ± 0.16*
14 days	6.86 ± 1.38**	13.39 ± 2.74*	27.8 ± 4.4***	53.2 ± 3.6***	11.1 ± 1.3***

*$p < 0.05$; **$p < 0.01$ and ***$p < 0.001$ vs. the control group.

An increase in plasma GH levels during the subchronic cadmium administration was surprisingly observed, which may be explained by taking into account the changes in acetylcholine described by DAS *et al.* (4) at the hypothalamic level, as this neurotransmitter is exerting an important role in the regulatory mechanism of GH secretion (9). Furthermore, high levels of GH may explain the reduction in body weight gain observed at the end of the chronic treatment with cadmium as a consequence of the well-known regulatory effects of this hormone on lipid metabolism (3).

The effects of cadmium administration on TSH are similar to those described for GH, although the comparison with previous observations cannot be made as there is, in our knowledge, no information available. As it was indicated for GH a direct effect of the metal at the hypothalamus modifying TRH release may not be excluded, as interferences of cadmium with the effects of this neuropeptide at the hypophyseal level, have been shown in previous *in vitro* studies (18).

Acute cadmium chloride administration decreased plasma LH but not FSH levels. The effects on LH do agree with those observed by PAKSY *et al.* (15). However, the reduction in FSH, which is not observed in this study, may be due to the higher dose of cadmium used by these authors as compared to the one used in this work. These effects are compatible with an impaired gonadal function (16). However, subchronic $CdCl_2$ administration for 14 days, increased instead of decreased gonadotropin secretion, in agreement with the data described by ZYLBER-HARAN *et al.* (19). Chronic increases in gonadotropin secretion are also compatible with an impaired gonadal function (16).

In summary, cadmium exposure interferes with the normal regulatory mechanisms involved in pituitary hormone secretion. Data obtained in the acute experiments point to direct effects at the pituitary level, although indirect effects mediated by the hypothalamus cannot be discarded, as it has previously been suggested (4).

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A. LAFUENTE, A. BLANCO, N. MÁRQUEZ, E. ÁLVAREZ-DEMANUEL y A.I. ESQUIFINO. *Efecto de la administración aguda y subcrónica de cadmio sobre la secreción de la hormona pituitaria en rata.* J. Physiol. Biochem. (Rev. esp. Fisiol.,), 53, 3, 265-270, 1997.

La administración de cadmio induce efectos deletéreos sobre el sistema endocrino a través de mecanismos no bien entendidos. El presente trabajo pretende analizar en un primer paso si la administración de cadmio modifica la secreción hipofisaria de forma generalizada o si ejerce efectos específicos sobre cada hormona. Para ello se administra una sola dosis de cloruro de cadmio (6 mg/Kg) o una dosis de 4 mg/Kg, durante 14 días, a ratas macho adultas. Se analizan los cambios en los niveles circulantes de prolactina, hormona luteinizante (LH), hormona folículo estimulante (FSH), hormona del crecimiento (GH) y hormona tireotropa (TSH). Seis horas despues de la administración de cadmio, las concentraciones plasmáticas de prolactina (5,52 ± 0,53 vs. 16,2 ± 2,7 ng/mL, $p < 0,01$), GH (2,39 ± 0,43 vs. 6,71 ± 1,3 ng/mL, $p < 0,05$), TSH (2,76 ± 0,64 vs. 7,65 ng/mL, $p < 0,01$), y LH (4,1 ± 0,16 vs. 5,18 ± 0,28 ng/mL, $p < 0,05$) disminuyen y no se modifica la de FSH. Sin embargo, la administración de cadmio durante 14 días, incrementa la concentración plasmática de GH

(13,39 ± 2,74 vs. 6,71 ± 1,3 ng/mL, $p < 0,05$), TSH (27,8 ± 4,4 vs. 7,65 ± 1,15 ng/mL, $p < 0.001$), LH (11,1 ± 0,31 vs. 5,18 ± 0,28 ng/mL, $p < 0,001$) y FSH (53,16 ± 3,6 ng/mL, $p < 0.01$), mientras que las de prolactina continúan disminuidas. El incremento de peso en los animales tratados con cadmio durante 14 días es menor que el de los controles ($p < 0.001$). Estos datos sugieren que la administración de cadmio induce cambios específicos sobre las distintas hormonas estudiadas y que las modificaciones también dependen del tiempo de exposición al metal.

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