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SHORT COMMUNICATION

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Pubertal dependent effects of cadmium on episodic prolactin secretion in male rats

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Abstract This work was undertaken to assess if exposure to cadmium related to puberty may affect the episodic pattern of prolactin. Male rats were submitted to cadmium exposure, from day 30 to 60 or from day 60 to 90 of life respectively, at a dose of 50 ppm in the drinking water. Control age-matched rats received cadmium-free water. Prepubertal cadmium administration decreased mean serum prolactin levels and the absolute amplitude of the prolactin pulses. Subchronic exposure to cadmium of adult rats decreased mean serum prolactin levels, the absolute amplitude of the prolactin pulses and their duration, and the mean half-life of the hormone. These results suggest that subchronic cadmium exposure changes the secretory pattern of prolactin in adult male rats in a puberty-dependent way.

Key words Cadmium · Prolactin pulsatility · Puberty

Introduction

Cadmium exposure affects reproductive function (Laskey and Phelps 1991; Paksy et al. 1992; Piasek and Laskey 1994) and developing organisms are more likely than others to be damaged by cadmium (Clarkson et al. 1985). Interactions between cadmium and pituitary hormones, such as prolactin and gonadotrophins, were reported (Zylber-Haran et al. 1982; Paksy et al. 1989). Cadmium exposure was reported to decrease (Lorenson et al. 1983; Winstel and Callahan 1992; Lafuente et al. 1997) or increase prolactin release (Paksy et al. 1989). Most results were obtained using single samples and employing a wide variety of doses and ways of administration of cadmium (Zylber-Haran et al. 1982; Paksy et al. 1989; Piasek and Laskey 1994; Lafuente et al. 1996a). We undertook the present study considering that prolactin, similar to other pituitary hormones, is released in an episodic fashion (Shin and Chi 1979; Lopez et al. 1989; Lafuente et al. 1992, 1993, 1994, 1996a, b). Two questions posed were whether (1) subchronic cadmium exposure affects the episodic pattern of prolactin and (2) cadmium effects are puberty-dependent.

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Materials and methods

Animals and treatment

Prepubertal (30 days old) and adult (60 days old) male Sprague-Dawley rats obtained from Animal Production of Santiago University, Spain were used in all experiments. Rats were maintained in a room with a controlled photoperiod (14 h light/10 h darkness) and temperature (22 ± 2 °C), and were supplied with rats 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 of 2 mg/kg body weight, as water consumption is ~20 ml per day and was not modified by cadmium addition). The dose was selected according to previous work from the literature (Zylber-haran et al. 1982; Paksy et al. 1989; Laskey and Phelps 1991; Piasek and Laskey 1994; Lafuente et al. 1998). This dose modified the pulsatile Leutinizing hormone (LH) secretion in male rats (Lafuente et al. 1998), which may lead to changes in prolactin release, as both secretory mechanisms are closely related (Lafuente et al. 1992).

Similar doses of cadmium induced prominent swelling and thickening of the basement membane of the glomerulus (Imai et al. 1995) and inhibited testicular microsomal Na^+, K^+-ATPase activity and increased hydroxyl free radical formation (Shen and Sangiah 1995). Also the incidence of prostatic proliferative lesions increased (Waalkes and Rehm 1992). Male rats were submitted to 50 ppm of cadmium in the drinking water from day 30 to 60 of life, and adults from day 60 to 90. Age-matched rats received cadmium-free water as controls. The number of animals per group was 6.

Cannula implantation

Forty hours before the day of the experiment, animals were anaesthetized with 2.5% tribromoethanol in saline (1 ml/100 g body

weight) and atrial cannulas were implanted following procedures previously described (Lafuente et al. 1992, 1993, 1994).

Experimental design and blood collection

On the day of experiment, conscious and freely moving rats of each group were continuously bled and the samples were collected every 7 min as previously described (Lafuente et al. 1992, 1993, 1994). The studies were conducted in accordance with the principles and procedures outlined in the National Institutes of Health (NIH) guide for the Care and Use of Laboratory Animals (National Research Council 1996).

Prolactin measurements

Prolactin levels were determined by a specific double-antibody radioimmunoassay. The reagents were kindly supplied by the National Hormone and Pituitary Programme (NHPP, Rockville, Md., USA) and Dr A. Parlow (Harbor UCLA Medical Center, Torrance, Calif., USA).

Data analysis

To identify and characterize prolactin pulses appearing in the hormonal profile of each rat, a computer program (Ultra-analysis) described by Van Cauter (1990) and reviewed by Richard et al. (1990), was used. The mean hormone levels, absolute and relative amplitudes of prolactin peaks, their frequency and pulse duration and mean half-life of the hormone were calculated.

Statistics

Statistical analysis of results was performed using Student's *t*-test. The results were considered significant at $P \leq 0.05$.

Results

Prolactin secretion in animals from the four experimental groups was episodic. A representative profile from one animal of each experimental group is shown in Fig. 1. Subchronic cadmium chloride administration, from day 30 to 60 of life, significantly decreased mean serum prolactin levels and the absolute amplitude of the prolactin pulses (Table 1; $P \leq 0.01$, $P \leq 0.05$ respectively). Cadmium chloride treatment markedly increased the relative pulse amplitude of prolactin peaks (Table 1, $P \leq 0.05$). However, no changes were observed in any other parameter studied (Table 1).

Subchronic cadmium chloride administration, from day 60 to 90 of life significantly decreased mean serum prolactin levels, the absolute amplitude of prolactin pulses and their duration (Table 1; $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ respectively), and the mean half-life of the hormone (Table 1, $P \leq 0.05$). The pulse frequency was not changed by subchronic cadmium administration (Table 1).

Discussion

The observed decline in serum prolactin levels from 1030 to 1130 hours seemed to be a consequence of the existence of circadian variations of the hormone (Lafuente et al. 1996a,b). Cadmium administration decreased the absolute amplitude of prolactin peaks in both 60- and 90-day-old male rats. This change may explain the decrease in mean prolactin levels as was previously shown using a single sample protocol (Lorenson et al. 1983; Lafuente et al. 1996a). This effect is further supported by the reduction in mean half-life of the hormone and the duration of the prolactin peaks in older animals. These later changes were not observed in 60-day-old male rats, thus suggesting the existence of puberty-dependent effects of cadmium on prolactin secretion. More parameters of prolactin pulsatility are changed in older animals

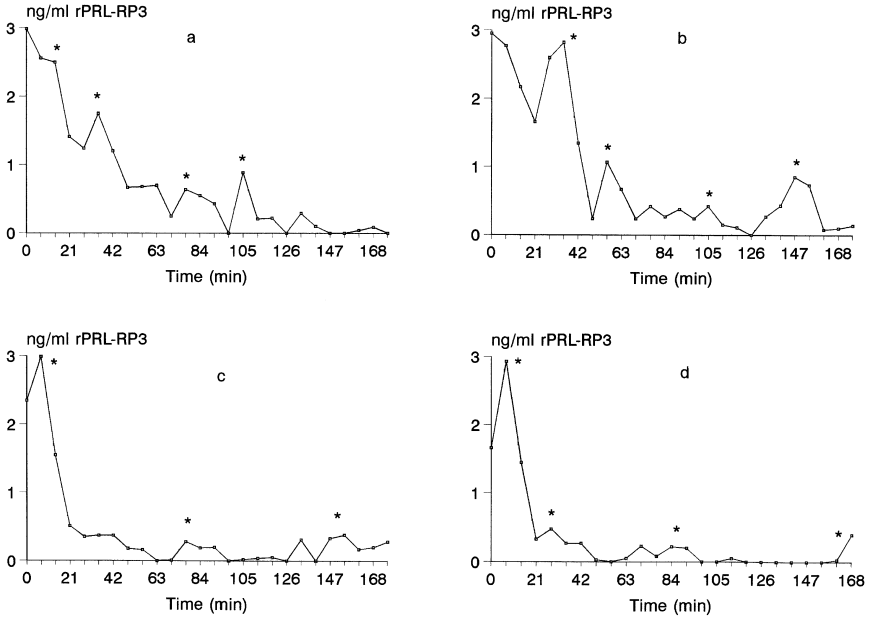

Fig. 1 Basal individual episodic prolactin (PRL-RP3) patterns in **a**, **c** 60- and **b**, **d** 90-day-old male rats treated with cadmium-free water (**a**, **b**) or with cadmium chloride (**c**, **d**) at a dose of 50 ppm in the drinking water (during 1 month) from day 30 or 60 of life respectively. *Asterisks* indicate the presence of a prolactin pulse

Table 1 Mean serum prolactin levels, absolute and relative pulse amplitude, frequency and duration of the pulses, and mean half-life of prolactin, in 60- or 90-day-old male rats treated with cadmium-free water or with cadmium chloride at a dose of 50 ppm in the drinking water, for 1 month, beginning from day 30 or 60 of life respectively

Group		PRL-PR3 (ng/ml)	Absolute amplitude	Relative amplitude	Frequency (pulse/3h)	Duration (min)	Half-life (min)
Prepubertal treatment	Day 60 Control	0.60 ± 0.10	0.26 ± 0.03	0.75 ± 0.14	3.57 ± 0.36	35.70 ± 5.79	09.01 ± 0.60
	Day 60 $CdCl_2$	0.20 ± 0.02**	0.16 ± 0.02*	1.58 ± 0.40*	3.55 ± 0.50	29.75 ± 2.16	12.15 ± 1.54**
Postpubertal treatment	Day 90 Control	0.88 ± 0.11	0.30 ± 0.04	2.35 ± 0.83*	4.12 ± 0.44	31.50 ± 2.19	10.37 ± 2.55
	Day 90 $CdCl_2$	0.49 ± 0.10$^{\&}$	0.15 ± 0.03$^{\&\&}$	1.70 ± 0.42	5.00 ± 0.56	20.56 ± 1.68$^{\&\&\&}$	5.33 ± 0.71$^{\&}$

The relative pulse amplitude was calculated as the quotient between absolute pulse amplitude and preceding nadir value. Values are expressed as mean ± SEM. Number of animals per group = 6
$^{*}P \leq 0.05$; $^{**}P \leq 0.01$ vs Day 60 control
$^{\&}P \leq 0.05$; $^{\&\&}P \leq 0.01$; $^{\&\&\&}P \leq 0.001$ vs Day 90 control

suggesting that the effects of cadmium are of a lesser extent than in postpubertal rats. The results reported in this work were obtained with an assumed cadmium dose of 2 mg/kg per day, similar to that used in previous studies reported in the literature (Zylber-haran et al. 1982; Das et al. 1993). It must be noted, however, that higher doses of cadmium (10 or 15 mg/kg) may increase serum prolactin levels in female rats (Paksy et al. 1989).

The present observations may be the result of a multiple interactive mechanism of cadmium with regulatory factors involved in prolactin secretion at the hypothalamic-hypophyseal axis (Das et al. 1993; Andersson et al. 1997; Lorenson et al. 1983; Cooper et al. 1987; Winstel and Callahan 1992). Cadmium may act directly on the lactotrophs, via an interaction with a disulphide group of the amino-terminal of the prolactin molecule, which is sensitive to divalent metals (Lorenson et al. 1983). However, previous studies have shown a normal response of prolactin to thyrotrophin-releasing hormone (TRH) in cadmium treated rats (Lafuente and Esquifino 1998), indicating that in vivo cadmium exposure does not compete with calcium at the hypophyseal level (Waalkes and Poirier 1984; Milos et al 1994), as was suggested from in vitro studies (Winstel and Callahan 1992). All these data suggest that cadmium effects on prolactin secretion may be exerted mainly at the hypothalamic level.

Changes in episodic prolactin secretion after cadmium administration may interfere with other functions, such as the immune response (Esquifino et al. 1991, 1996; Reber 1993; Arce et al. 1997) or gonadal function (Del Pozo and Bownell 1979), which are regulated by this hormone. In addition, cadmium may exert a puberty-dependent effect on testosterone secretion, which may differentially affect prolactin pulsatility, as testosterone is involved in the modulation of this pulsatile pattern (Grosser and Robaire 1988). In conclusion, our results confirm that subchronic cadmium administration inhibits the episodic release of prolactin. Moreover, a puberty-dependent effect of cadmium on prolactin secretion may be operative, as in other experimental situations (Esquifino et al. 1987, 1997).

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References

Andersson H, Petersson-Grave K, Lindqvist E, Luthman J, Oskarsson A, Olson L (1997) Low-level cadmium exposure of lactating rats causes alterations in brain serotonin levels in the offspring. Neurotoxicol Teratol 19: 105–115

Arce A, Castrillón PO, Cardinallei DP, Esquifino Al (1997) Age-dependent effect of pituitary transplant on immune response in rat spleen. Modulatory effect of cyclosporine. Biol Signals 6: 11–20

Clarkson TW, Nordberg GF, Sager PR (1985) Reproductive and developmental toxicity of metals. Scand J Work Environ Health 11: 145–154

Cooper RL, Goldman JM, Rehnberg GL, McElroy WK, Hen JF (1987) Effects of metal cations on pituitary hormone secretion in vitro. J Biochem Toxicol 2: 241–249

Das KP, Das PC, Dasgupta S, Dey CC (1993) Serotoninergic-cholinergic neurotransmitters' function in brain during cadmium exposure in protein restricted rat. Biol Trace Elem 36: 119–127

Del Pozo E, Bownell J (1979) Prolactin I. Mechanisms of control, peripheral actions and modifications by drugs. Horm Res 10: 143–172

Esquifino AI, Villanúa MA, Agrasal C (1987) Effect of neonatal melatonin administration on sexual development in the rat. J Steroid Biochem 27: 1089–1093

Esquifino AI, Villanua MA, Szary A, You J, Bartke A (1991) Ectopic pituitary transplants restore immunocompetence in Ames dwarf mice. Acta Endocrinol 125: 67–72

Esquifino AI, González ME, Lafuente A (1996) Possible interactions of cyclosporine and hyperprolactinemia modulating the episodic secretion of prolactin. Proc. Soc. Exp Biol Med 213: 206–211

Esquifino AI, Arce A, Villanúa MA, Cardinali DP (1997) Twenty-four hours rhythms of serum prolactin, growth hormone and luteinizing hormone levels, and of mediobasal hypothalamic dopamine and serotonin metabolism, in rats neonatally administered with melatonin. J Pineal Res 22: 52–58

Grosser, PM, Robaire B (1988) Espisodic prolactin secretion in adult male rat is affected by orchidectomy and testosterone replacement. Endocrinology 123: 2132–2139

Imai T, Omoto M, Seki K, Harada T (1995) The effects of long-term intake of restricted calcium, vitamin D, and vitamin E and cadmium-added diets on various organs and bones of mice: a histological and the roentgenological study. Nippon Eiseigaku Zasshi 50: 660–682

Lafuente A, Esquifino AI (1998) Cadmium does not inhibit pulsatile prolactin secretion through TRH. BioMetals 11: 235–241

Lafuente A, Marcó J, Esquifino AI (1992) Effects of hyperprolactinemia on prolactin and LH pulsatile pattern in female rats. Rev. Esp. Fisiol 48: 291–296

Lafuente A, Marcó J, Esquifino AI (1993) Pulsatile prolactin secretory patterns throughout the oestrous cycle in the rat. J Endocrinol 137: 43–47

Lafuente A, Marcó J, Esquifino AI (1994) Physiological roles of thyrotrophin-releasing hormone and vasoactive intestinal peptide on the pulsatile secretory pattern of prolactin in pituitary-grafted female rats. J Endocrinol 142: 581–186

Lafuente A, González E, Mouteira RC, Esquifino AI (1996a) Effects of a single dose of cadmium on the episodic secretion of prolactin. In: Collery P, Corbella J, Domingo JL, Etienne JC, Llobet JM (eds) Metal ions in biology and medicine, vol 4. John Libbey Eurotext, Paris, pp 462–464

Lafuente A, Salgado A, García-Bonacho M, Esquifino AI (1996b) Effects of cyclosporine treatment on prolactin pulsatility in chronic hyperprolactinemic male rats. J Neuroimmunol 65: 41–47

Lafuente A, Blanco A, Márquez N, Álvarez-Demanuel E, Esquifino AI (1997) Effects of acute and subchronic cadmium administration on pituitary hormone secretion in adult male rats. J Physiol Biochem 53: 265–270

Lafuente A, Marquez N, Piquero S, Esquifino AI (1998) Cadmium affects the episodic luteinizing hormone secretion in male rats: possible age-dependent effects. Toxicol Lett 104: 27–33

Laskey JW, Phelps PV (1991) Effect of cadmium and other metal cations on "in vitro" Leydig cell testosterone production. Toxicol Appl Pharmacol 108: 296–306

López FJ, Domínguez JR, Sánchez-Franco F, Negro-Villar A (1989) Role of dopamine and vasoactive intestinal peptide in the control of pulsatile prolactin secretion. Endocrinology 124: 527–535

Lorenson MY, Robson DL, Jacobs LS (1983) Divalent cation inhibition of hormone release from isolated adenohypophysial secretory granules. J Biol Chem 258: 8618–8622

Milos M, Comte M, Schaer JJ, Cox JA (1989) Evidence for capital and six auxiliary cation-binding sites on calmodulin: divalent cation interactions monitored by direct binding and microcalorimetry. J Inorg Biochem 36: 11–25

National Research Council (1996) Guide for the care and use of laboratory animals. Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, National Academy of Sciences (USA), National Institutes of Health

Paksy K, Varga B, Horvath E, Tatrai E, Ungvary G (1989) Acute effects of cadmium on preovulatory serum FSH, LH and prolactin levels and on ovulation and ovarian hormone secretion in oestrus rats. Reprod Toxicol 3: 241–247

Paksy K, Varga B, Lazar P (1992) Cadmium interferes with steriod biosynthesis in rat granulosa and luteal cells in vitro. Biometals 5: 245–250

Piasek M, Laskey JW (1994) Acute cadmium exposure and ovarian steroidogenesis in cycling and pregnant rats. Reprod Toxicol 8: 495–507

Reber PM (1993) Prolactin and immunomodulation. Am J Med 95: 637–644

Richard JL, Bringer J, Daurós JP, Parer-Richard C, Jaffiol C, Mirouze J (1990) Methodes d'analyse de la pulsatilité hormonale. Ann Endocrinol 51: 181–193

Shen Y, Sangiah S (1995) NA^+, K^+-ATPase, glutatione, and hydroxyl free radicals in cadmium chloride-induced testicular toxicity in mice. Arch Environ Contam Toxicol 29: 174–179

Shin SH, Chi HJ (1979) Unsuppressed prolactin secretion in the male rats is pulsatile. Neuroendocrinology 28: 73–81

Van Cauter E (1990) Diurnal and ultradian rhythms in human endocrine function: a minireview. Hormone Res 34: 45–53

Waalkes MP, Poirier LA (1984) In vitro cadmium-DNA interactions: cooperativity of cadmium binding and competitive antagonism by calcium, magnesium and zinc. Toxicol Appl Pharamacol 75: 539–546

Waalkes MP, Rehm S (1992) Carcinogenicity of oral cadmium in the male Wistar (WF/NCr) rat: effect of chronic dietary zinc deficiency. Fundam Appl Toxicol 19: 512–520

Winstel C, Callahan P (1992) Cadmium exposure inhibits the prolactin secretory response to thyrotrophin releasing hormone (TRH) in vitro. Toxicology 74: 9–17

Zylber-Haran EA, Gershman H, Rosenmann E, Spitz IM (1982) Gonadotrophin, testosterone and prolactin interrelationships in cadmium-treated rats. J Endocrinol 92: 123–130