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Are cadmium effects on plasma gonadotropins, prolactin, ACTH, GH and TSH levels, dose-dependent?

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Abstract

It is well established that cadmium affects plasma levels of the pituitary hormones studied. However, whether the effects of the metal are dose dependent needs to be clarify. This work was designed to evaluate the possible changes in plasma levels of gonadotropins, prolactin, ACTH, GH and TSH after oral cadmium exposure in adult male rats. Plasma levels of these hormones were measured in adult male rats exposed to cadmium chloride ($CdCl_2$) in the drinking water at the doses of 5, 10, 25, 50 or 100 ppm for one month. The lower dose of cadmium increased plasma prolactin levels and higher doses of the metal (25 or 50 ppm) decreased them. There was a continuous increase of plasma ACTH levels from the lower to 25 ppm dose of $CdCl_2$ and decreased them after to basal values with the highest dose. Plasma GH levels were increased with the dose of cadmium of 10 ppm, although the doses of 5, 25 and 50 ppm decreased them. Plasma LH levels were only reduced with the dose of 50 ppm of $CdCl_2$, whereas those of FSH increased. Plasma TSH levels were increased with the doses of 5, 25 and 100 ppm of $CdCl_2$. Cadmium concentration increased in pituitary with the doses of 125, 50 and 100 ppm of $CdCl_2$. These data suggest that cadmium differentially affects the secretory mechanisms of the pituitary hormones studied depending on the dose used. The effects of the metal on prolactin and ACTH are dose-dependent.

Introduction

Exposure to cadmium is associated with a disruption in the activity of the endocrine system in both male and female rats (Zylber-Haran *et al.* 1982; Lorenson *et al.* 1983; Laskey & Phelps 1991; Winstel & Callahan 1992; Piasek & Laskey 1994; Lafuente & Esquifino 1999; Lafuente *et al.* 1997, 1998a, b, 1999a, b). Cadmium administration brought about a number of gonadal (Laskey & Phelps 1991; Piasek & Laskey 1994), adrenal (Anca *et al.* 1982; Hidalgo & Armario 1987) and immune alterations (Descotes 1992; Teocharis *et al.* 1994).

Our group is trying to characterise the effects of cadmium on hypophyseal function. Previous studies have shown that acute cadmium administration differentially modified prolactin and adrenocorticotropin hormone (ACTH) ultradian secretory patterns of secretion (Lafuente *et al.* 1998a, b). Subchronic exposure differentially modified both plasma prolactin levels and its ultradian secretory pattern according with the route of metal exposure (oral vs. subcutaneously administration of the metal, Esquifino *et al.* 1998; Lafuente *et al.* 1999a, 2000b). Furthermore the effects of the metal on prolactin secretion resulted to be age dependent with either of the routes of administration of cadmium used (Esquifino *et al.* 1998; Lafuente *et al.* 1999a, 2000b). The data obtained are in partial agreement with findings published by other authors (Zylber-Haran *et al.* 1982; Lorenson *et al.* 1983). Plasma levels of other pituitary hormones (i.e., gonadotropins, GH, ACTH or TSH) were differntially

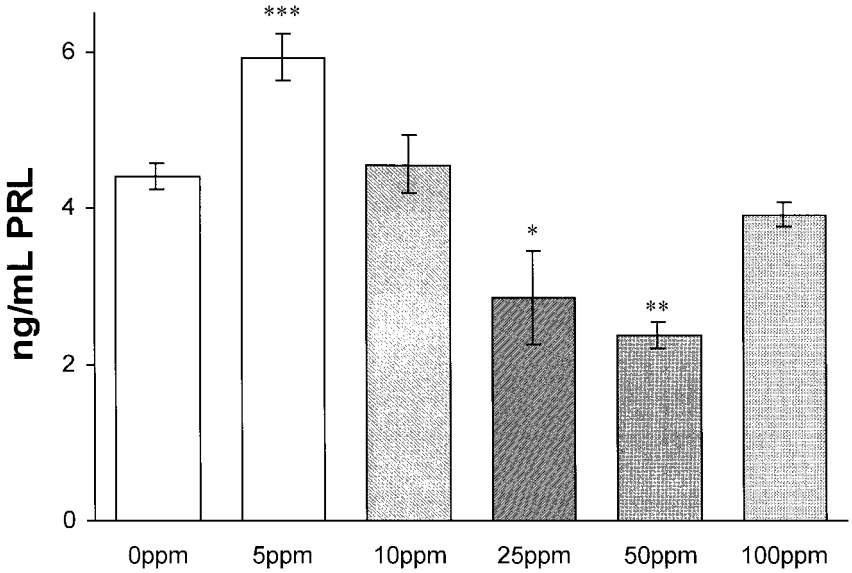

Fig. 1. Plasma prolactin levels in adult male rats treated for one month with cadmium-free water or with cadmium chloride at a dose of 5, 10, 25, 50 or 100 ppm in the drinking water. The values are expressed as mean ± S.E.M. ($n = 10$ in each group). F = 7.16, $P \leq 0.001$. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ vs. control group.

modified by the route of metal exposure and age of the animals as happened for prolactin (Lafuente & Esquifino 1999). All data indicate the necessity for dose response studies to understand the effects of cadmium on the endocrine system.

In female rats, a dose response study using sc. administration of cadmium showed an impairment in the preovulatory surges of prolactin and gonadotropin (Paksy *et al.* 1989). However, these studies were not carried out with regard to animals submitted to cadmium exposure in the drinking water.

According with the above mentioned data, this work was designed to analyse a possible dose-dependent effect of cadmium on plasma levels of prolactin, gonadotropins, ACTH, GH and TSH.

Material and methods

Animals and experimental designs

Experiments were carried out in adult male Sprague-Dawley rats (250–300 g), kept under controlled conditions of light (light between 07.00 and 21.00 h daily) and temperature (22 ± 2 °C). Food and water were available ad libitum. Six groups of 10 animals were used. Five groups were treated for one month with cadmium chloride ($CdCl_2$) at a dose of 5, 10, 25, 50 and 100 ppm, respectively, of $CdCl_2$ in the drinking water. The sixth group received cadmium-free water to use it as control. The lower dose of cadmium administered to the animals in this work, is approximately 300 times higher than Provisional Tolerable Weekly Intake (PTWI) of this heavy metal (WHO 1993).

At the end of the treatment, animals were killed by decapitation at 12:00 h to avoid the diurnal secretion pattern of the pituitary hormones analysed, according with unpublished data of the group. Care was taken to avoid any major stress before sacrifice and the decapitation procedure was completed within 5–7 sec. Plasma from the heparinized trunk blood was obtained after centrifuging the samples at 1, 500 × *g* for 15 min. Samples were kept frozen at −20 °C until analysed.

The studies were conducted in accordance with the principles and procedures outlined in the NIH guide for the Care and Use of the Laboratory Animals (National Research Council 1996).

Pituitary hormone measurements

Plasma levels of gonadotropins, prolactin, ACTH, GH and TSH were measured by specific radioimmunoassay methodology using the reagents generously provided by the National Hormone and Pituitary Program (NHPP, Rockville, MD) and Dr A. Parlow (Harbor, UCLA Medical Center, CA), previously described (Lafuente *et al.* 1997, 2000b, 2001). The detection limit of the assay was 0.048 ng/ml for prolactin

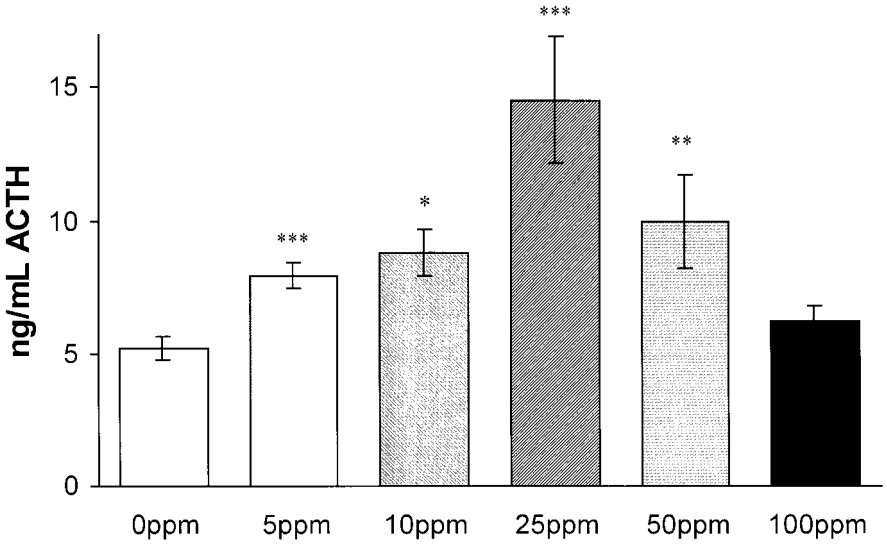

Fig. 2. Plasma ACTH levels in adult male rats treated for one month with cadmium-free water or with cadmium chloride at a dose of 5, 10, 25, 50 or 100 ppm in the drinking water. The values are expressed as mean ± S.E.M. ($n = 10$ in each group). F = 9.79, $P \leq 0.001$. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ vs. control group.

and GH; 0.049 ng/ml for LH; 0.097 ng/ml for FSH; 0.196 ng/ml for TSH and 0.390 ng/ml for ACTH.

Cadmium determination

Cadmium concentration was determined in the pituitary gland of individual animals. Tissue cadmium concentrations were determined by graphite furnace atomic absorption spectrophotometry after microwave digestion (GFAAS) (López-Artíguez *et al.* 1993). The samples were mineralized in a Parr 4780 microwave acid digestion bomb and a Samsung M-745 microwave oven. The mineralization step was performed by treating dried homogenized whole pituitary with 3.0 ml of ultrapure nitric acid and 1 ml of distilled water. The mineralization was complete after two digestions at 450 W for 2 min, 20 s each. For cadmium determination, an atomic absorption spectrophotometer (Perkin-Elmer, Varian Spectra 250 plus) with Zeeman background correction was used. Accuracy was obtained by calibration against aqueous standards. Accuracy has been obtained by calibration against aqueous standards. For the aqueous standards control, we have checked that the absorbance measures correspond with the technical characteristics of the device, allowing a deviation of 5%. That is to say, the RSD (relative standard deviation) is inferior to the 5% for the samples and for the patterns. Every ten samples a reslope was made. The lowest level of sensitivity was 1.6 ng/g. Samples of the whole experiment were analyzed in the same assay to avoid interassay variations; the intra-assay coefficient of variation was 4.2%.

Statistical analysis

Results were analyzed by one way ANOVA followed by a Tukey test for multiple comparations. The results were considered significant at $P \leq 0.05$. All values represent the mean ± S.E.M.

Results

Figure 1 shows plasma prolactin levels in animals exposed to various doses of cadmium in the drinking water for one month (F = 7.16, $P \leq 0.001$). The lower dose of cadmium (5 ppm of $CdCl_2$) increased plasma prolactin levels ($P \leq 0.001$; 5.93 ± 0.3 vs. 4.41 ± 0.16 ng/ml in the control group). However, the circulating levels of this hormone decreased in the animals treated with 25 ppm of $CdCl_2$ ($P \leq 0.05$; 2.85 ± 0.6 vs. 4.41 ± 0.16 ng/ml in the control group) and in those treated with 50 ppm of $CdCl_2$ ($P \leq 0.01$; 2.37 ± 0.17 vs. 4.41 ± 0.16 ng/ml in the control group). The highest dose of cadmium used did not modify plasma prolactin levels, as compared to the values found in the control group (Figure 1; 3.92 ± 0.16 vs. 4.41 ± 0.16 ng/ml in the control group).

Oral cadmium exposure modified plasma ACTH levels (Figure 2, F = 9.79, $P \leq 0.001$). Specifically,

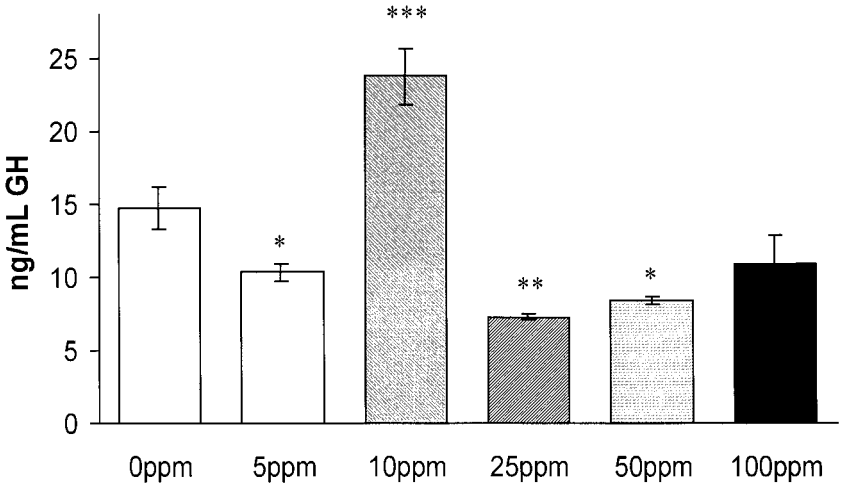

Fig. 3. Plasma GH levels in adult male rats treated for one month with cadmium-free water or with cadmium chloride at a dose of 5, 10, 25, 50 or 100 ppm in the drinking water. The values are expressed as mean ± S.E.M. ($n = 10$ in each group). F = 16.62, $P \leq 0.001$. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ vs. control group.

after cadmium treatment, plasma levels of this hormone increased with the dose of 5 ppm of $CdCl_2$ in the drinking water ($P \leq 0.001$; 7.92 ± 0.48 vs. 5.17 ± 0.44 ng/ml in the control group), 10 ppm of $CdCl_2$ ($P \leq 0.05$; 8.81 ± 0.86 vs. 5.17 ± 0.44 ng/ml in the control group), 25 ppm of $CdCl_2$ ($P \leq 0.001$; 14.53 ± 2.4 vs. 5.17 ± 0.44 ng/ml in the control group) or 50 ppm of $CdCl_2$ ($P \leq 0.01$; 9.96 ± 1.17 vs. 5.17 ± 0.44 ng/ml in the control group). In the animals treated with the dose of 100 ppm, the plasma levels of this hormone were not changed (Figure 2; 6.21 ± 0.62 vs. 5.17 ± 0.44 ng/ml in the control group).

Plasma GH levels were affected with either used dose of cadmium, with the exemption of the highest dose of 100 ppm of $CdCl_2$ (Figure 3, F = 16.62, $P \leq 0.001$). Plasma GH levels increased with the dose of cadmium of 10 ppm of $CdCl_2$ (Figure 3; $P \leq 0.001$; 23.74 ± 1.87 vs. 14.71 ± 1.47 ng/ml in the control group), whereas the doses of 5, 25 and 50 ppm of $CdCd_2$ decreased them (Figure 3; $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.05$, respectively; the values found were 10.33 ± 0.61, 7.29 ± 0.2 and 8.43 ± 0.24 ng/ml, respectively, vs. 14.71 ± 1.47 ng/ml in the control group). The highest dose of cadmium was not able to modify plasma levels of GH, although a tendency to lower values was observed as compared to controls (Figure 3; 10.86 ± 1.96 vs. 14.71 ± 1.47 ng/ml in the control group).

Figures 4 and 5 show plasma levels of LH and FSH in control and cadmium exposed groups (F = 2.45, $P \leq 0.05$ and F = 7.60, $P \leq 0.001$, respectively). Plasma LH concentrations were only reduced with the dose of 50 ppm of $CdCl_2$ (Figure 4; $P \leq 0.05$; 0.27 ± 00.02 vs. 0.46 ± 0.03 ng/ml in the control group), whereas those of FSH were increased with the same dose of cadmium (Figure 5; $P \leq 0.05$; 7.78 ± 0.64 vs. 5.99 ± 0.55 ng/ml in the control group).

Plasma TSH levels were modified by cadmium exposure (Figure 6, F = 6.49, $P \leq 0.001$). Concretely, plasma TSH levels increased with the doses of 5 ppm ($P \leq 0.05$; 2.25 ± 0.12 vs. 1.6 ± 0.14 ng/ml in the control group), 25 ppm ($P \leq 0.001$; 3.1 ± 0.4 vs. 1.6 ± 0.14 ng/ml in the control group) and 100 ppm of $CdCl_2$ in the drinking water (Figure 6; $P \leq 0.01$; 2.3 ± 0.07 vs. 1.6 ± 0.14 ng/ml in the control group). The doses of 10 and 50 ppm of $CdCl_2$ were not able to modify plasma levels of TSH, (Figure 6; the values found were 1.99 ± 0.15 and 2.01 ± 0.10 ng/ml, respectively, vs. 1.6 ± 0.14 ng/ml in the control group).

As shown in Figure 7, cadmium content in the pituitary gland increased in those animals treated with $CdCl_2$ (F = 64.69, $P \leq 0.001$). The concentration of the metal in this gland increased with doses of 25, 50 and 100 ppm of $CdCl_2$ in the drinking water ($P \leq 0.05$, $P \leq 0.01$ or $P \leq 0.001$, respectively, from 3.95 ± 0.36 μg/g in the control group to 17.6 ± 0.98, 22.83 ± 3.5 and 73.1 ± 7.1 in the groups treated with 25, 50 or 100 ppm of $CdCl_2$).

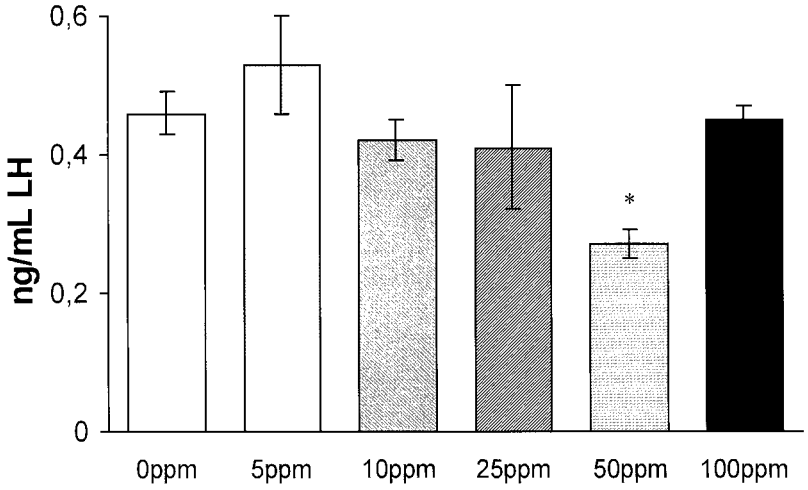

Fig. 4. Plasma LH levels in adult male rats treated for one month with cadmium-free water or with cadmium chloride at a dose of 5, 10, 25, 50 or 100 ppm in the drinking water. The values are expressed as mean ± S.E.M. ($n = 10$ in each group). $F = 2.45$, $P \leq 0.05$. $^*P \leq 0.05$ vs. control group.

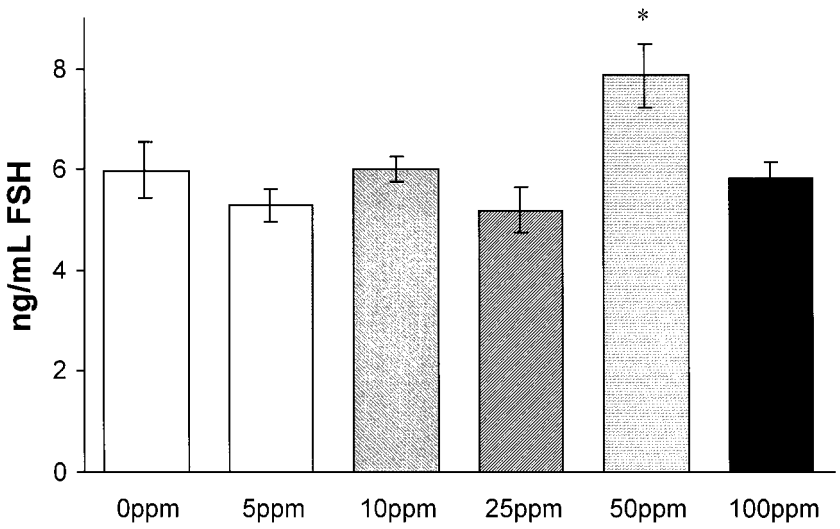

Fig. 5. Plasma FSH levels in adult male rats treated for one month with cadmium-free water or with cadmium chloride at a dose of 5, 10, 25, 50 or 100 ppm in the drinking water. The values are expressed as mean ± S.E.M. ($n = 10$ in each group). $F = 7.60$, $P \leq 0.001$. $^*P \leq 0.05$ vs. control group.

Discussion

Ongoing results suggest that cadmium differentially affects the secretory patterns of prolactin, gonadotropins, ACTH, GH and TSH as demonstrated by the changes in circulating values of these pituitary hormones. The effects of the metal were dose-dependent for prolactin and ACTH, although this property was not observed for any other studied hormone.

A biphasic effect of cadmium on plasma prolactin levels is described. The lower dose of cadmium used in this work increased plasma prolactin levels whereas no changes or decreased plasma levels of the hormone were found with the other doses of the metal used. The data obtained with the dose of 25 or 50 ppm agrees with previous data shown in adult rats after acute or shorter exposures to the metal (Lafuente & Esquifino 1996, 1998a; Lafuente *et al.* 1997). Previous studies from the literature also described this inhibitory effect on prolactin secretion in both *in vivo* and *in vitro* studies (Lorenson *et al.* 1983; Nomiyama 1986; Paksy *et al.* 1989). Such inhibitory effect could be ex-

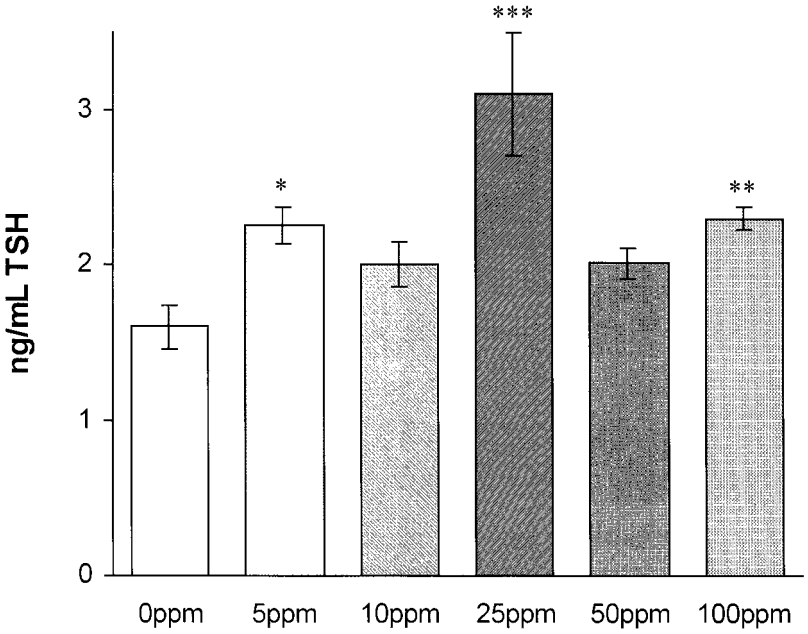

Fig. 6. Plasma TSH levels in adult male rats treated for one month with cadmium-free water or with cadmium chloride at a dose of 5, 10, 25, 50 or 100 ppm in the drinking water. The values are expressed as mean ± S.E.M. ($n = 10$ in each group). F = 6.49, $P \leq 0.001$. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ vs. control group.

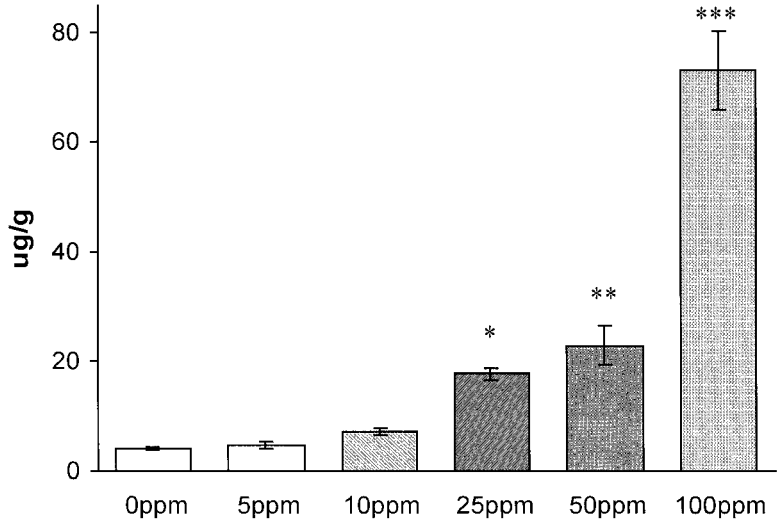

Fig. 7. Cadmium concentration in the pituitary gland in adult male rats treated for one month with cadmium-free water or with cadmium chloride at a dose of 5, 10, 25, 50 or 100 ppm in the drinking water. The values are expressed as mean ± S.E.M. ($n = 10$ in each group). F = 64.69, $P \leq 0.001$. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ vs. control group.

plained by the decreased amplitude of prolactin peaks reported elsewhere (Lafuente *et al.* 1999a; Lafuente & Esquifino, 1998a). Cadmium may act directly on the lactotrophs, through an interaction with the prolactin molecule, that is sensitive to divalent metals, as was shown *in vitro* (Lorenson *et al.* 1983), thus inhibiting its secretion. However, previous *in vivo* studies indicated a normal response of prolactin to TRH in cadmium-treated rats (Lafuente *et al.* 1998a), thus indicating a differential intracellular kinetics of cadmium *in vivo* as compared to the *in vitro* situation in pituitary cells (Waalkes & Poirier 1984; Milos *et al.* 1989; Winstel & Callahan 1992). The data obtained with the lower dose of cadmium agree with previ-

ous reports from *in vitro* studies from the literature (Cooper *et al.* 1987) and may be related to the inhibition of the immune response described after low doses of the metal (Krzystyniak *et al.* 1987), as subchronic elevation of prolactin may inhibit this system (Arce *et al.* 1997).

Cadmium exposure during the adulthood, increased plasma levels of ACTH with any of the doses used with the exemption of the highest dose of the metal used that was not able to modify the levels of this hormone. These changes may be mediated at least in part by the decrease in the dopamine content at the hypothalamic level previously described with the dose of 25 and 50 ppm of cadmium (Márquez 1999), as ACTH release is tonically inhibited by the dopaminergic pathways (Hagan *et al.* 1996). However, other neurotransmitters (not measured until now) may also be involved. The changes in ACTH may also affect the immune response as this hormone is a key parameter to determine the degree of activity of this system (Sanders 1997). A dose-response effect of the metal on ACTH emerges.

A biphasic effect of cadmium on GH secretion is observed with the dose of 10 ppm, the one that increased plasma levels of the hormone. This effect may be expected considering previous works from the laboratory that show that the length of treatment as well as the dose of the metal used differentially affect plasma GH levels (Lafuente *et al.* 1997; Lafuente & Esquifino 1999). On the other hand, the effects of the metal on GH secretion may be mediated by changes in the hypothalamic neuromodulators implicated in the regulation of its secretion, as the metal did not affect GH secretion as described in *in vitro* studies (Lorenson *et al.* 1983).

Plasma LH and FSH levels were differentially affected by the dose of 50 ppm of cadmium chloride, and the changes observed agree with previous works from our laboratory (Lafuente *et al.* 2001). These changes did not correlate with norepinephrine content nor serotonin metabolism at the hypothalamic level as these neurotransmitters were unchanged after metal exposure as was shown elsewhere (Lafuente *et al.* 2001). Data indicates that the effect of cadmium on LH or FSH was not dose-dependent.

The increase in plasma levels of TSH with some of the doses of cadmium used in this study agrees with previous reports of the laboratory in which animals were exposed to cadmium for shorter periods of time and using different doses of cadmium (Lafuente *et al.* 1997). This increase in plasma TSH levels, observed in this study explains the reduction in body weight gain, described in other studies of the group (Lafuente *et al.* 2000a). The mechanism may be similar to that described for hyperthyroidism (Haisenleder *et al.* 1986).

However, the reduction in plasma TSH levels after acute administration of cadmium chloride together with the increases in plasma levels of the hormone after subchronic exposure to the metal did suggest a time of exposure-dependent effect of cadmium on TSH secretion. After subchronic administration, the accumulation of cadmium at the hypothalamic or pituitary level (Lafuente *et al.* 2000b, 2001) may interfere at the hypothalamus and/or the pituitary level to change pituitary hormone secretion in a different way. The changes in plasma TSH levels after subchronic exposure to different doses of the metal indicated that cadmium did not exhibit a dose-response effect on TSH.

The increase of cadmium concentration at pituitary gland found in treated animals, was not correlated to the changes observed in plasma levels of the pituitary hormones.

In conclusion, cadmium exposure differentially affects circulating concentrations of prolactin, ACTH, GH, LH, FSH and TSH. The effects of cadmium on prolactin and ACTH seemed to be dose dependent whereas for GH, TSH, FSH and LH the effects were not dose-dependent.

Acknowledgements

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