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# Effects of subchronic alternating cadmium exposure on dopamine turnover and plasma levels of prolactin, GH and ACTH

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**Abstract**

This study was undertaken to analyze if the effects of subchronic alternating cadmium exposure on pituitary hormone secretion are mediated by changes in dopamine turnover in an age dependent way or are directly correlated to cadmium accumulation at the hypothalamic-pituitary axis. Male rats were treated sc. from day 30 to 60 (prepubertal period) or from day 60 to 90 (adult age) of life, with cadmium chloride ($CdCl_2$) at a dose of 0.5 and 1.0 mg $kg^{-1}$ bw, every 4th day in an alternate schedule, starting with the smaller dose. Dopamine (DA) turnover, expressed as the ratio of acid 3,3-dihidroxifenil acetic (DOPAC)/DA in various hypothalamic areas, the plasma levels of prolactin, growth hormone (GH) and adrenocorticotropic hormone (ACTH), and cadmium accumulation in the hypothalamus and pituitary were studied. Prepubertal cadmium exposure decreased DA content in all hypothalamic areas studied, although its turnover was not modified. A decrease in plasma ACTH levels with no changes in plasma prolactin and GH levels were found. Cadmium did not accumulate in pituitary while it increased in the hypothalamus. Metal exposure during adulthood decreased DA content in mediobasal and posterior hypothalamus, and its turnover in posterior hypothalamus and median eminence. It decreased plasma prolactin and ACTH levels but not those of GH. Cadmium concentration increased in both hypothalamus and pituitary. These results suggest that cadmium exposure produces age dependent changes on the secretory mechanisms of the pituitary hormones studied, related to the selective accumulation of the metal at both hypothalamic and hypophyseal level changes. However the effects of the metal are not mediated by dopamine.

## Introduction

Cadmium is present in the environment. It is feasible that cadmium intake by humans and animals will increase in the future due to the increasing application of cadmium derivatives in agriculture. Other sources of cadmium such as emissions from smelters, waste incineration, etc., must also be considered. Moreover, acid rain tends to lower soil pH thus increasing cadmium uptake by crops (Piscator 1985). All these considerations points to an increase in cadmium concentration in the tissues of a living organism. Its presence in the tissues is over 20–30 years, thus suggesting that its accumulation is critical for its toxicity.

Exposure to cadmium is associated with changes in the activity of the endocrine system in male and female animals (Zylber-Haran *et al.* 1982, Lorenson *et al.* 1983; Laskey & Phelps 1991; Winstel & Callahan 1992; Piasek & Laskey 1994; Lafuente *et al.* 1996, 1997, 1998a, b, 1999). Cadmium administration brought about a number of gonadal (Laskey & Phelps 1991; Piasek & Laskey 1994); adrenal (Anca *et al.* 1982, Hidalgo & Armario 1987, Mgbonyebi *et al.* 1993) and immune alterations (Descotes 1992, Teocharis *et al.* 1994). As shown previously in rats, cadmium accumulation differentially modified prolactin and adrenocorticotropin hormone (ACTH) ultradian secretory patterns of secretion in males (Lafuente

*et al.* 1996, 1998a,b). Basal hormone levels were also affected (Lafuente *et al.* 1997), the data being in partial agreement with findings published by other groups in male animals (Zylber-Haran *et al.* 1982; Lorenson *et al.* 1983).

On the other hand, dopamine (DA) inhibits prolactin, growth hormone and adrenocorticotropine hormone secretion (Lopez *et al.* 1989; Hagan *et al.* 1996; Esquifino *et al.* 1997). In addition, cadmium exposure increases (Singhal *et al.* 1976; Chandra *et al.* 1985; Gutierrez-Reyes *et al.* 1998), decreases (Shrivastava & Sathyanesan 1988; Rajanna *et al.* 1990), or increases DA content in mesencephalon. It also decreases in metencephalon (Antonio *et al.* 1998) or not modifies (Nation *et al.* 1989) dopamine content in the brain.

Besides, the neuroendocrine system exhibits an age dependent evolution that may interfere with cadmium exposure as has been previously shown using other experimental approaches to analyze pituitary hormone secretory patterns (Villanua *et al.* 1989).

The exposure to the heavy metals (i.e. cadmium) is not constant in humans and normally it is in the atmosphere. On the other hand, in rats the doses of the metal are controlled and given in the drinking water. However, it is the final accumulation of cadmium in the tissues that plays a major role in metal toxicity (Paksy *et al.* 1990; Marquez *et al.* 1998). In the case of the hypothalamic-pituitary axis, such accumulation had not been related to hormonal changes. This prompted us to examine in certain detail the association of changes in pituitary hormone secretion with cadmium accumulation at the hypothalamic-pituitary axis as a function of age. Also we are interested in knowing if these alterations in pituitary hormones are mediated by changes in dopamine turnover at hypothalamic level.

## Materials and methods

### *Animals and treatment*

Male rats of the Sprague-Dawley strain kept under controlled conditions of light (lights on from 07.00 to 21.00 h) and temperature (22±2 °C) and having access to food and water '*ad libitum*' were used. After weaning, 4 animals/cage were kept in the same room for the period studied. Four groups of 16 animals were used. The rats of the groups 1 and 2 were 30 day old rats at the beginning of the experiment (prepubertal age) and the animals of groups 3 and 4 were 60 day old rats at the beginning of the experiment (young adults). Groups 2 and 4 were treated sc. from day 30 to 60 or from day 60 to 90 respectively, with cadmium chloride ($CdCl_2$) (0.5 and 1 mg $kg^{-1}$ bw) every 4th day in an alternate schedule, starting from the smaller dose. That is to say, we have administered 0,5 mg of $CdCl_2$ $kg^{-1}$ b.w. on the first day of the treatment, and 1 mg of $CdCl_2$ $kg^{-1}$ bw on the fourth. Then, on the 8th day of treatment we repeated the dose of 0.5 mg of $CdCl_2$ $kg^{-1}$ bw, and so on. The latest dose of cadmium was given 48 h in advance to sacrifice. Groups 1 and 3 received sc., from day 30 to 60 or from day 60 to 90 respectively, 0.3 ml of saline every 4th day, to be used as controls. The dose of cadmium was selected according with previous works from literature (Zylberharan *et al.* 1982; Paksy *et al.* 1989; Laskey & Phelps 1991; Piasek & Laskey 1994).

At 60th day of life, groups 1 and 2, and at 90th day of age, groups 3 and 4 were killed by decapitation at 14:00 h to avoid the diurnal secretion pattern of dopamine turnover and pituitary hormones. Care was taken to avoid any major stress before sacrifice and the decapitation procedure was completed within 10–20 s. Trunk blood was collected in tubes containing EDTA (60 g $l^{-1}$) and plasmas were obtained after centrifugation the samples at 1500 *g* for 15 min at 4 °C, and were kept frozen at −20 °C until prolactin, growth hormone and adrenocorticotropine hormone were 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 hypothalamus from the eight remaining were used to measure dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) contents at the median eminence, and anterior, mediobasal and posterior hypothalamus.

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 (National Research Council 1996).

### *Amine measurements*

Hypothalamus were quickly dissected out according with previous works from the group (Esquifino *et al.* 1995). Tissue was weighed and homogenized in chilled (0–1 °C) 2 M acetic acid. After centrifugation (at 15,000 × *g* for 30 min, at 4 °C), the supernatants were analyzed by high performance liquid chromatography (HPLC), using electrochemical detection (Coulochem, 5100A,ESA; USA). A C-18

reverse phase column, eluted with a mobile phase (pH=4; 0.1 M sodium acetate, 0.1 M citric acid, 0.7 mM sodium octylsulphate and 0.57 mM EDTA containing 10% methanol, v/v), was employed. Flow rate was 1 ml $min^{-1}$, at a pressure of 2200 psi. Fixed potentials against $H_2/H^+$ reference electrode were: conditioning electrode $-0.4$ V; preoxidation electrode $+0.10$ V; working electrode $+0.35$ V. Amines concentrations were calculated from the chromatographic peak areas by using external standards. The linearity of the detector response for dopamine and DOPAC was tested within the concentration ranges found in supernatants of mediobasal hypothalamus (Esquifino *et al.* 1996).

The amount of protein from the hypothalamus regions was measured with the *Bradford* method. We have used as patron BSA (bovine serum albumine), and the optic density was measured with a spectrophotometer at a $\lambda = 295$ nm .

*Hormone measurement*

Prolactin, growth hormone and adrenocorticotropine hormone levels were determined by specific double-antibody radioimmunoassays, previously described made in our laboratory (Tresguerres & Esquifino 1981). 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). Hormones were iodinated by the lactoperoxidase technique as was previously described (Esquifino 1976). Prolactin values were expressed in terms of NIADD rat-PRL-RP-3 reference preparation, growth hormone in terms of NIADD rat-GH-RP-2 and adrenocorticotropine hormone in terms of NIADD rat-ACTH-RP-3 reference preparation. The antiserum and their dilution used are the following: anti-rat-PRL-S-9 (final dilution 1:437,000), anti-rat-GH-S-5 (final dilution 1:500,000) and for rat adrenocorticotropine hormone the antibody used was AFP6328031 (final dilution 1:600,000). The lowest level of sensitivity was 5, 2 or 1 pg/tube for prolactin, growth hormone or adrenocorticotropine hormone respectively. Samples were analyzed within the same assay to avoid interassay variations. The intra-assay coefficients of variation were 7.4%, 8.1% and 4,3% for prolactin, growth hormone and adrenocorticotropine hormone respectively.

*Cadmium determination*

Cadmium concentration was determined in the hypothalamus and pituitary gland of individual animals. Immediately after the sacrifice, the hypothalamus were dissected as in previous studies (Esquifino *et al.* 1995). 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 tissues (hypothalamus or 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. For the aqueous standards control, we have checked that the absorvance 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 has been made. The lowest level of sensitivity was 0.02 mg $ml^{-1}$. Samples of the whole experiment were analyzed in the same assay to avoid interassay variations; the intraassay coefficient of variation was 4%.

*Statistical analysis*

Statistical analysis of results was performed by using a two-way Anova followed by a multiple comparison Fisher Test (Statview from Macintosh). The results were considered significant at $P \leq 0.05$.

## Results

In older control rats dopamine content decreased in median eminence, and anterior hypothalamus as compared to the values found in younger controls ($P \leq 0.05$, Figures 1 and 2), while dopamine level was not changed in mediobasal or posterior hypothalamus (Figures 3 and 4). However, the ratio acid 3,3-dihydroxyfenyl acetic (DOPAC)/dopamine (DA) increased with age in median eminence and mediobasal hypothalamus ($P \leq 0.05$, Figures 1 and 3).

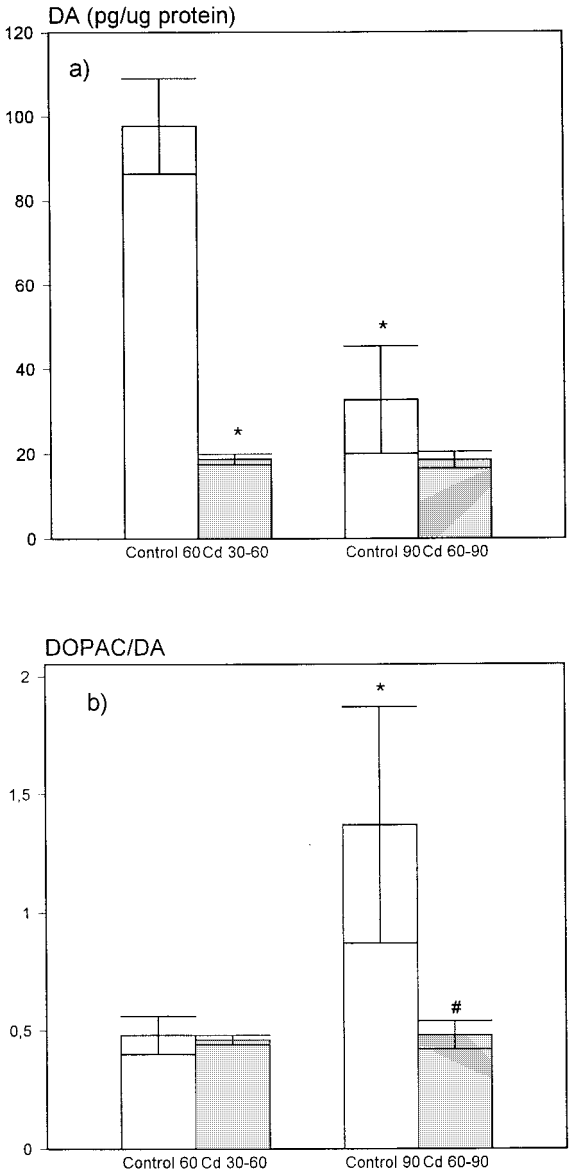


*Figure 1.* Dopamine content (a) and DOPAC/DA ratio (b) in median eminence in control and treated groups. Control rats were administered 0.3 ml of sc. saline every 4 days during one month, from day 30 or 60 of life. In cadmium exposed groups, $CdCl_2$ (dissolved in saline solution) was administered sc. (0.5 and 1 mg $kg^{-1}$ bw) every 4th day in an alternate schedule, starting from the smaller dose, from day 30 to 60 or from day 60 to 90. The latest dose of cadmium was given 48 h in advance to sacrifice. Values are expressed as mean ± S.E.M. ($n = 8$ in each group). $^{*}P \leq 0.05$ vs control at age of 60 days. $^{\#}P \leq 0.05$ vs. control at age of 90 days of life.

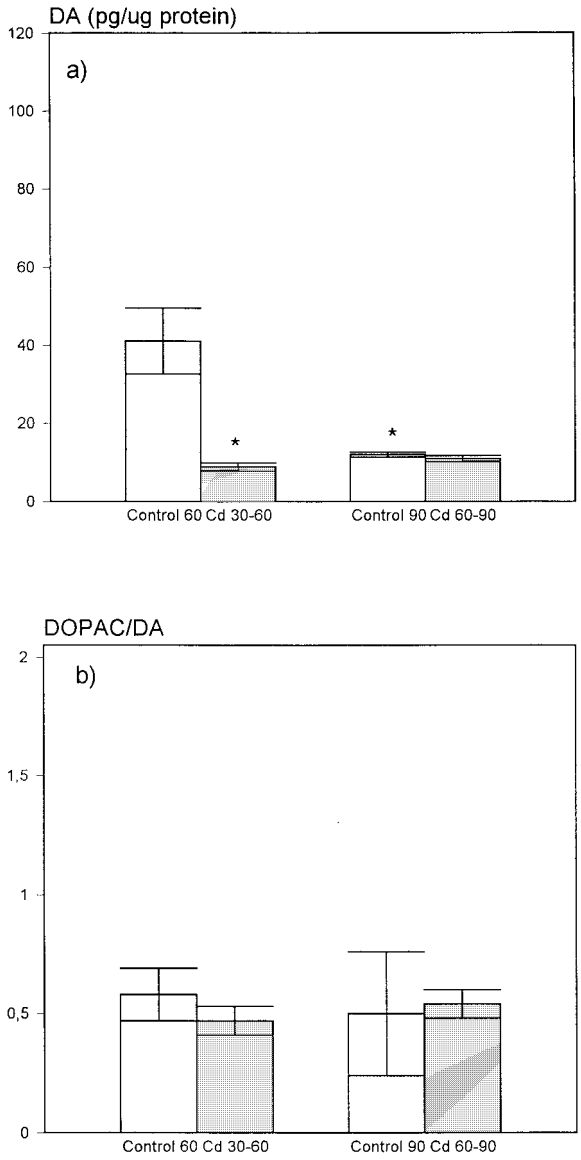


*Figure 2.* Dopamine content (a) and DOPAC/DA ratio (b) in anterior hypothalamus in control and treated groups. Control rats were administered 0.3 ml of sc. saline every 4 days during one month, from day 30 or 60 of life. In cadmium exposed groups, $CdCl_2$ (dissolved in saline solution) was administered sc. (0.5 and 1 mg $kg^{-1}$ bw) every 4th day in an alternate schedule, starting from the smaller dose, from day 30 to 60 or from day 60 to 90. The latest dose of cadmium was given 48 h in advance to sacrifice. Values are expressed as mean ± S.E.M. ($n = 8$ in each group). $^{*}P \leq 0.05$ vs control at age of 60 days of life.

In rats treated with $CdCl_2$ from 30 to 60 day of life, dopamine content decreased in all brain regions studied ($P \leq 0.05$ in anterior hypothalamus, Figures 1–4) as compared to the values found in its age-matched controls. Nevertheless, after prepubertal cadmium treatment, dopamine turnover, measured as DOPAC/DA ratio, was not modified in any brain area studied (Figures 1–4).

When cadmium was administered from day 60 to 90 of life, dopamine content decreased in mediobasal and posterior hypothalamus ($P \leq 0.05$ respectively, Figures 3 and 4) as compared to the values found in age-matched controls. In this group, dopamine turnover, measured as DOPAC/DA ratio, decreased in median eminence and in posterior hypothalamus ($P \leq 0.05$, Figures 1 and 4) as compared to its control group, while this ratio was not changed in anterior or in mediobasal hypothalamus (Figures 2 and 3).

As shown in Table 1, plasma concentration of prolactin of 90 day old control rats was higher, and that of adrenocorticotropine hormone was lower, than in younger animals ($P \leq 0.05$). Prepubertal cadmium exposure (from 30 to 60 days of life) diminished plasma adrenocorticotropine hormone levels ($P \leq 0.05$ vs. control group), while circulating levels of prolactin and growth hormone were not modified. However, in cadmium treated rats from 60 to 90 days of life plasma prolactin levels decreased while those of adrenocorticotropine hormone increased ($P \leq 0.05$, Table 1) and those of growth hormone were not changed, compared to its control group of age.

Table 2 summarizes the results on cadmium content in hypothalamus and pituitary gland of the groups of rats studied. After prepubertal exposure to $CdCl_2$, the concentration of metal did not change in the pituitary, while it increased in the hypothalamus ($P \leq 0.05$ vs control). In adult treated rats, the concentration of cadmium increased in both the pituitary gland and the hypothalamus ($P \leq 0.05$ vs. control group of the same age).

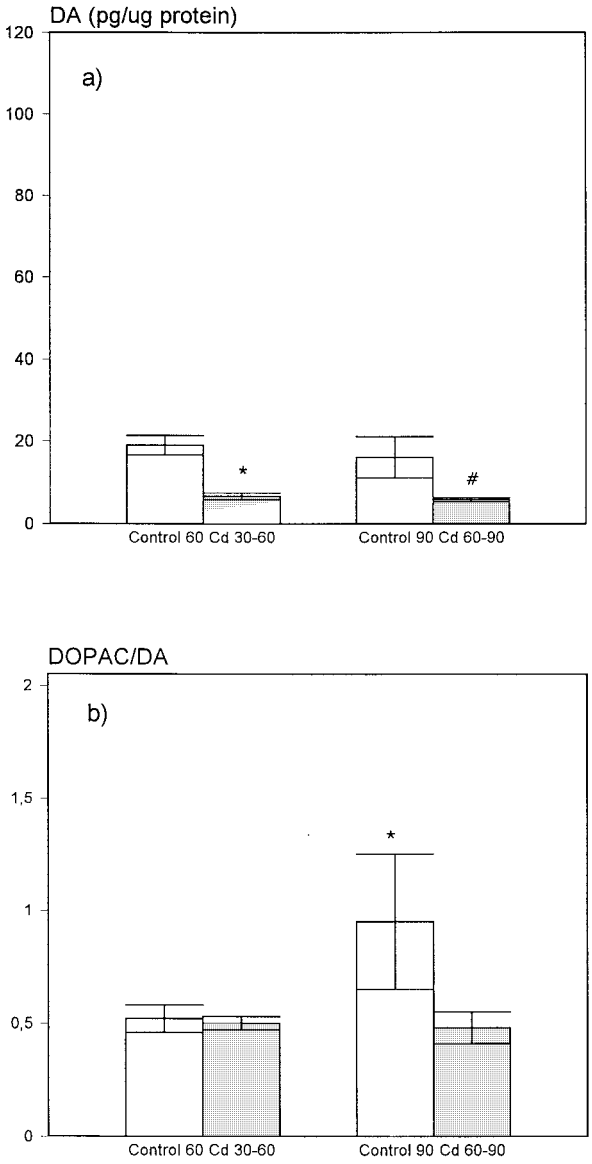


*Figure 3.* Dopamine content (a) and DOPAC/DA ratio (b) in mediobasal in control and treated groups. Control rats were administered 0.3 ml of sc. saline every 4 days during one month, from day 30 or 60 of life. In cadmium exposed groups, $CdCl_2$ (dissolved in saline solution) was administered sc. (0.5 and 1 mg $kg^{-1}$ bw) every 4th day in an alternate schedule, starting from the smaller dose, from day 30 to 60 or from day 60 to 90. The latest dose of cadmium was given 48 h in advance to sacrifice. Values are expressed as mean $\pm$ S.E.M. ($n = 8$ in each group). $^{*}P \leq 0.05$ vs control at age of 60 days. $^{\#}P \leq 0.05$ vs. control at age of 90 days of life.

## Discussion

Ongoing results indicate that cadmium exposure exerts its effects at both, the hypothalamus and the pituitary. These effects seemed to be age dependent and could be explained by the specific cadmium retention at the hypothalamic and/or pituitary level. A disruption of the regulatory mechanisms of the hypothalamic-pituitary axis emerges.

*Table 1.* Plasma levels of prolactin, GH and adrenocorticotropine hormone in control and cadmium chloride exposed groups. Control rats were administered 0.3 ml of sc. saline every 4 days during one month, from day 30 or 60 of life. In cadmium exposed groups, $CdCl_2$ (dissolved in saline solution) was administered sc. (0.5 and 1 mg $kg^{-1}$ bw) every 4th day in an alternate schedule, starting from the smaller dose, from day 30 to 60 or from day 60 to 90. The latest dose of cadmium was given 48 h in advance to sacrifice. Values are expressed as mean ± S.E.M. ($n = 8$ in each group).

| Age during the treatment | Treatment | rPRL-RP3 (ng $ml^{-1}$) | rGH-RP3 (ng $ml^{-1}$) | rACTH-RP3 (ng $ml^{-1}$) |
|---|---|---|---|---|
| 30–60 days | Control group | 0.44 ± 0.10 | 15.64 ± 2.39 | 2.60 ± 0.18 |
| | $CdCl_2$sc (0.5 and 1.0 mg $kg^{-1}$ bw) | 0.38 ± 0.09 | 10.67 ± 2.70 | 1.76 ± 0.17* |
| 60–90 days | Control group | 3.05 ± 0.15* | 10.04 ± 1.80 | 1.66 ± 0.16* |
| | $CdCl_2$sc (0.5 and 1.0 mg $kg^{-1}$ bw) | 1.90 ± 0.17# | 10.48 ± 2,04 | 2.63 ± 0.48# |

*P<0.05 vs. control group (60 days of age).
#P<0.05 vs. control group (90 days of age).

In this study age dependent changes in dopamine content and its turnover within the hypothalamus as well as changes in circulating plasma levels of the hormones studied were observed, in agreement with data previously described in the literature (Esquifino *et al.* 1995).

Generally, the data of the present study shows an inhibitory effect of cadmium exposure on dopamine at the hypothalamic level. This effect ties with the results found by Shrivastava & Sathyanesan (1988); Rajanna *et al.* (1990) and in mesencephalon by Antonio *et al.* (1998). Discrepancies with other authors (Singhal *et al.* 1976; Gutierrez-Reyes *et al.* 1998) could be due to differences in age of animals during cadmium exposure and treatment duration and brain area analyzed. Chandra *et al.* (1985) found that diabetes enhances the effect of cadmium elevating the levels of dopamine. Besides, dopamine turnover was not changed by prepubertal cadmium exposure, although in adult animals it decreased. This inhibitory effect on cadmium turnover also was shown in striatum by Desole *et al.* (1991).

The results obtained in the present work suggest that cadmium exposure differentially affects the secretion of the hormones here studied, thus for example, in adult animals after the metal administration from 60 to 90 day of life, plasma prolactin levels, those of adrenocorticotropine hormone increased or those of growth hormone were not changed. In addition, prepubertal metal exposure was only able to modify the plasma adrenocorticotropine hormone levels.

The decrease observed in plasma prolactin levels after cadmium exposure from 60 to 90 days of life, agrees with previous data gathered in adult male rats after acute or shorter exposures to the metal (Lafuente *et al.* 1996, 1997, 1998a). Cadmium effectively depressed prolactin in both in vivo and in vitro studies (Lorenson *et al.* 1983; Nomiyama 1986; Paksy *et al.* 1989). Such inhibitory effect could be explained by the decreased amplitude of prolactin peaks reported elsewhere (Lafuente *et al.* 1996, 1998b). 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 male rats (Lafuente *et al.* 1998b), 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).

Surprisingly, prepubertal cadmium treatment in younger rats was unable to change the circulating levels of prolactin. This fact agrees with that shown by Zylber-Haran (1982) on 19th, 75th or 185th day after acute metal administration, and could be explained by the higher sensibility to cadmium in adult than in younger male animals (Phelps & Laskey 1989, Wong & Klaassen 1990).

The unchanged plasma growth hormone levels found following cadmium exposure in both young and old rats could be due to changes in hypothalamic concentration of acetylcholine (Das *et al.* 1993) as this

*Table 2.* Cadmium levels at the hypothalamus and pituitary in control and cadmium chloride exposed groups. Control rats were administered 0.3 ml of sc. saline every 4 days during one month, from day 30 or 60 of life. In cadmium exposed groups, $CdCl_2$ (dissolved in saline solution) was administered sc. (0.5 and 1 mg $kg^{-1}$ bw) every 4th day in an alternate schedule, starting from the smaller dose, from day 30 to 60 or from day 60 to 90. The latest dose of cadmium was given 48 h in advance to sacrifice. Values are expressed as mean ± S.E.M. ($n = 8$ in each group).

| Age during the treatment | Treatment | Hypothalamus ($\mu$g/g) | Pituitary ($\mu$g/g) |
|---|---|---|---|
| 30–60 days | Control group | $0.33 \pm 0.04$ | $5.99 \pm 1.15$ |
| | $CdCl_2$sc (0.5 and 1.0 mg $kg^{-1}$ bw) | $2.40 \pm 0.36^{*}$ | $4.72 \pm 0.73$ |
| 60–90 days | Control group | $0.75 \pm 0.20$ | $04.80 \pm 1.25$ |
| | $CdCl_2$sc (0.5 and 1.0 mg $kg^{-1}$ bw) | $3.23 \pm 0.12^{\#}$ | $10.10 \pm 1.30^{\#}$ |

$^{*}P<0.05$ vs control (60 days of age).
$^{\#}P<0.05$ vs control (90 days of age).

neurotransmitter stimulates growth hormone secretion (Casanueva *et al.* 1986).

The inhibitory effect of cadmium on adrenocorticotropine hormone secretion upon administration of the xenobiotic in prepubertal period agrees with previous data from our Laboratory also in male rats (Lafuente & Esquifino 1998a), indicating that an acute cadmium administration decreased the mean values of adrenocorticotropine hormone during the bleeding period and the absolute amplitude of adrenocorticotropine hormone pulses. The reduction in absolute amplitude of adrenocorticotropine hormone peaks is by itself enough to explain the decrease in mean adrenocorticotropine hormone levels. Such an inhibitory effect of cadmium on adrenocorticotropine hormone release was also observed with a dose of cadmium chloride of 3 mg $kg^{-1}$ $day^{-1}$ for 8 days using a single sample protocol in male rats (Alvarez *et al.* 1996). However, cadmium exposure between 60 and 90 days of life, increased plasma levels of this hormone, that may be mediated, at least in part, by the decrease in dopamine content at the hypothalamic level, as adrenocorticotropine hormone release is tonically inhibited by dopaminergic pathways (Hagan *et al.* 1996).

Generally the changes observed in the plasma hormones levels (except for augmentation on circulating levels of adrenocorticotropine hormone in adult rats) can not be explained by the alterations found in dopamine content and its turnover. Indeed, there are data showing alterations on 'in vitro' pituitary hormone release using isolated adenohypophysial cells (Lorenson *et al.* 1983; Cooper *et al.* 1987).

Cadmium is accumulated in hypothalamus and the pituitary, like it had been previously reported (Clark *et al.* 1985; Paksy *et al.* 1990; Márquez *et al.* 1998). It is of interest to note that cadmium differentially accumulated in the tissues studied depending upon the age at which cadmium exposure began, and the metal retention was higher in adults than in prepubertal rats. This fact could explain the higher toxicity of the xenobiotic in older than in younger animals (Phelps & Laskey 1989; Wong & Klaassen 1990), that may correlate with the observed changes in the hormones studied. Then alterations showed in the present study on the hormone secretion were more important in older rats than in younger animals.

We have found a certain concentration of cadmium in the control animals. This could be explain as cadmium is already introduced into the food chain. The water we have used comes from the Faculty supply, and the food is special for rodents. But, we can't be sure that the water and the food don't content a certain amount of cadmium. I the same way that all the population is exposed to this metal though the food chain. Besides, this metal can also pass through the placenta, and it is accumulated through all the animal's life.

In summary, the results obtained suggest that the alterations induced by cadmium exposure in pituitary hormone secretion are dependent on the age at which the exposure to the metal occurs (prepubertal or postpubertal).These alterations could be partially

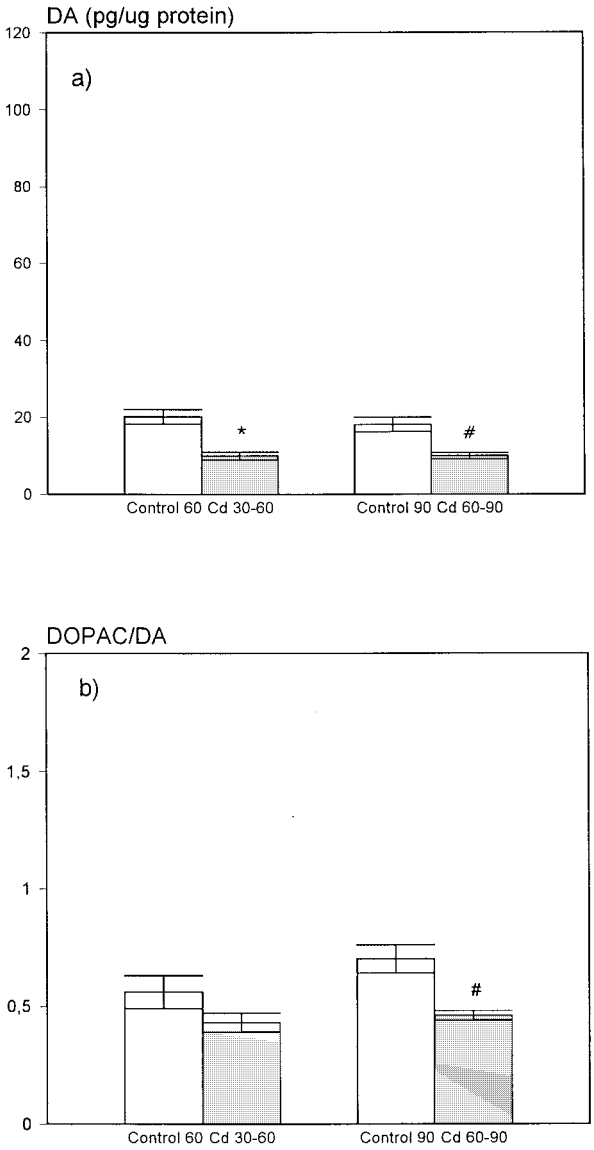


*Figure 4.* Dopamine content (a) and DOPAC/DA ratio (b) in posterior hypothalamus in control and treated groups. Control rats were administered 0.3 ml of sc. saline every 4 days during one month, from day 30 or 60 of life. In cadmium exposed groups, $CdCl_2$ (dissolved in saline solution) was administered sc. (0.5 and 1 mg $kg^{-1}$ bw) every 4th day in an alternate schedule, starting from the smaller dose, from day 30 to 60 or from day 60 to 90. The latest dose of cadmium was given 48 h in advance to sacrifice. Values are expressed as mean $\pm$ S.E.M. ($n = 8$ in each group). $^{*}P \leq 0.05$ vs control at age of 60 days. $^{\#}P \leq 0.05$ vs. control at age of 90 days of life.

explained by a differential accumulation of cadmium but not by changes in the dopamine turnover at the hypothalamic level.

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