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INORGANIC COMPOUNDS

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Alternate cadmium exposure differentially affects the content of gamma-aminobutyric acid (GABA) and taurine within the hypothalamus, median eminence, striatum and prefrontal cortex of male rats

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Abstract This work examines changes of gamma aminobutyric acid (GABA) and taurine contents in the hypothalamus, striatum and prefrontal cortex of the rat after an alternate schedule of cadmium administration. Age-associated changes were also evaluated, of those before puberty and after adult age. In control rats GABA content decreased with age in the median eminence and in anterior, mediobasal and posterior hypothalamus, prefrontal cortex and the striatum. Taurine content showed similar results with the exception of mediobasal hypothalamus and striatum, where no changes were detected. In pubertal rats treated with cadmium from 30 to 60 days of life, GABA content significantly decreased in all brain regions except in the striatum. When cadmium was administered from day 60 to 90 of life, GABA content was significantly changed in prefrontal cortex only compared with the age matched controls. Taurine content showed similar results in pubertal rats, with the exception of the median eminence and the mediobasal hypothalamus, neither of which showed a change. However, when cadmium was administered to rats from day 60 to 90 of life, taurine content only changed in prefrontal cortex compared with the age matched controls. These results suggest that cadmium differentially affects GABA and taurine contents within the hypothalamus, median eminence, striatum and prefrontal cortex as a function of age.

Keywords Cadmium · GABA · Taurine

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Introduction

Cadmium intake is likely to increase in the future being present in compounds used in agriculture and also in the environment via its agricultural application (Piscator 1985). As the half-life of cadmium in the living organism is 15–20 years, a cumulative effect of the metal within the tissues has to be considered. 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 and Phelps 1991; Winstel and Callahan 1992; Piasek and Laskey 1994; Lafuente and Esquifino 1998a, 1998b; Lafuente et al. 1996, 1997, 1999a, 1999b). Cadmium administration caused a number of gonadal (Laskey and Phelps 1991; Piasek and Laskey 1994), adrenal (Anca et al. 1982; Hidalgo and Armario 1987; Mgbonyebi et al. 1993) and immune alterations (Descotes 1992; Teocharis et al. 1994).

Heavy metal effects on the central nervous system (CNS) have been demonstrated. Cadmium exposure increases (Singhal et al. 1976; Chandra et al. 1985; Gutierrez-Reyes et al. 1998), decreases (Shrivastava and Sathyanesan 1988; Rajanna et al. 1990; Antonio et al. 1998) or does not affect (Nation et al. 1989) dopamine (DA) content in different brain areas or in whole brain. Whereas the effects of lead on amino acid metabolism were previously described (Shailesh et al. 1990; Lasley et al. 1999), the effects of cadmium were not yet tested, although there is some evidence of an inhibitory effect of the metal on gamma-aminobutyric acid (GABA) transport in the synaptosomal membranes (Wong et al. 1981).

Previous research at our laboratory has shown that alternate subcutaneous administration of cadmium changes the ultradian secretory pattern of prolactin (Esquifino et al. 1998; Lafuente and Esquifino 1998b) and differentially changes plasma levels of adrenocorticotrophic hormone (ACTH), luteinizing hormone (LH), follicle stimulating hormone (FSH), thyrotrophin (TSH) and growth hormone (GH) (Lafuente et al. 1997).

Moreover, we have shown that cadmium affects dopamine, norepinephrine and serotonin contents and metabolism within various hypothalamic areas (Lafuente and Esquifino 1999; Lafuente et al. 2000a). These changes do not, however, explain the modifications of the secretory patterns of the pituitary hormones analysed (Esquifino et al. 1998; Lafuente and Esquifino 1998b; Lafuente et al. 1999a, 1999b). We speculated other neuromodulators which were not measured in those studies may account for the observed changes. Among them, the amino acids may play a key role in the modulation of pituitary hormone secretion, as was previously suggested (Casanueva et al. 1984; Feleder et al. 1996).

Among excitatory amino acids, glutamate modulates the secretion rate of all pituitary hormones (Brann 1995), whereas there is only scant evidence on the role of aspartate and glutamine on pituitary hormone secretion (Carbone et al. 1992). However, the role of inhibitory amino acids on pituitary hormone secretion is better known (Casanueva et al. 1984; Feleder et al. 1996). Changes in aminoacidergic metabolism associated with cadmium accumulation at the hypothalamus may explain the modification in the secretory capacity of the pituitary cells.

The exposure to heavy metals (i.e. cadmium) is not constant in humans and normally it is taken up by inhalation. In rats the doses of the metal are controlled and given in the drinking water (Nation et al. 1989; Antonio et al. 1999; Lafuente et al. 1999a, 1999b). Alternate sc. schedules of cadmium administration would reproduce in animals the exposure associated to humans and also change pituitary hormone secretion (Esquifino et al. 1998; Lafuente et al. 2000a, 1999b). In fact, the content of cadmium in the hypothalamus described by our group (Márquez et al. 1999; Lafuente et al. 2000b) is similar to that found in liver or kidney in humans (Ellis et al. 1979). However, it needs to be proven whether or not the final accumulation of cadmium in the tissues plays a major role in metal toxicity (Paksy et al. 1990; Márquez et al. 1998). To our knowledge, cadmium exposure was not associated with possible changes in GABA or taurine concentration within the hypothalamus or other brain areas. Those data prompted us to examine in some detail the possible changes in GABA and taurine concentrations at the level of the hypothalamus, striatum and prefrontal cortex, after an alternate schedule of cadmium administration. Age-associated changes were also evaluated by comparing prepubertal and adult exposed male rats.

Materials and methods

Animals and treatment

Male rats of the Sprague-Dawley strain kept under controlled conditions of light (lights on from 0700 to 2100 hours) and temperature (22 ± 2°C) and having access to food and water ad libitum were used. After weaning, four animals/cage were kept in the same room for the duration of study. Four groups of eight animals were used. 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 (adult age). Groups 2 and 4 were treated s.c. from day 30 to 60 or from day 60 to 90 respectively, with cadmium chloride ($CdCl_2$; 0.5 and 1.0 mg/body wt.) every 4th day in an alternate schedule, starting from the smaller dose as follow: 0.5 mg of $CdCl_2$/body wt. on the 1st day of the treatment and 1.0 mg of $CdCl_2$/body wt. on the 4th day. The 8th day of treatment the dose of 0.5 mg $CdCl_2$/body wt. was repeated, and so on. The latest dose of cadmium was given 48 h before death. Groups 1 and 3 received via s.c. route, 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 to previous works from the literature (Zylber-Haran et al. 1982; Paksy et al. 1989; Laskey and Phelps 1991; Piasek and Laskey 1994) and from our laboratory (Esqifino et al. 1998; Lafuente et al. 2000a, 1999b). At the 60th day of life, groups 1 and 2, and at 90th day of age, groups 3 and 4 were killed by decapitation at 1400 hours. Care was taken to avoid any major stress before death and the decapitation procedure was completed within 5–7 s. The studies were conducted according to 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).

Tissue preparation

After thawing, the median eminence, anterior, mediobasal and posterior hypothalamus, prefrontal cortex and striatum blocks were dissected out in accordance with pervious works from the laboratory (Esquifino et al. 1995) and immediately homogenized in cold 2.0 M acetic acid (1–4°C), heated for 5 min in a 100°C water bath and centrifuged at 11,000 *g* for 10 min, at 4°C. The supernatant was removed and kept frozen at –80°C until amino acid determination. To avoid variations due to post-mortem enzyme-mediated modifications of amino acid concentrations, the time employed to process every tissue sample was always the same. Before heating a small aliquot of the tissue homogenates were obtained and used to determine protein content by the Bradford method.

Amino acid measurements

GABA and taurine were separated and analysed by high-performance liquid chromatography (HPLC), with fluorescence detection after precolumn derivatization with O-phthaldialdehyde (OPA). An aliquot of the tissue supernatant containing homoserine as internal standard was neutralized with NaOH (4.0 M) and reacted at room temperature with OPA reagent (4.0 mM OPA, 10% methanol, 2.56 mM 2-mercaptoethanol, in 1.6 M potassium borate buffer, pH 9.5) for 1 min. Afterwards, the reaction was stopped by addition of acetic acid (0.5, v/v). Samples were immediately loaded onto a Rheodyne (model 7725i) injector system with a 2.0 µL loop sample to reach a C-18 reverse-column (4.6 mm i.d. × 150 mm, Nucleosil 5) eluted with a mobile phase consisting of 0.1 M sodium acetate buffer (pH 5.5) containing 35% methanol, at a flow rate of 1 mL/min, and pressure of 140 bar. The column was subsequently washed with the same buffer containing 70% methanol and re-equilibrated with the elution buffer before re-use.

The HPLC system consisted of a solvent delivery system coupled to a filter fluorometer (excitation 340 nm, emission 455 mm). This procedure allows for a clear separation and resolution of all the amino acids measured in this study as previously described (Duvilanski et al. 1998). GABA and taurine contents in each tissue were calculated from the chromatographic peak areas by using standard curves and the internal standard. The linearity of the detector response for the amino acids studied was verified within the concentration ranges found in extracts of the brain regions.

Statistical analysis

Amino acid concentrations were expressed as pg/μg protein, and analysed employing a one-way analysis of variance (ANOVA; SPSS for Windows 98), for studying the differences between pubertal and adult control groups and between treated and control groups of the same age. In addition, a two-way ANOVA was used for studying the interaction between the age of the animals and the cadmium treatment. The level of statistical significance was $p \leq 0.05$ for each analysis.

Results

In adult control rats GABA content decreased in median eminence ($F=7.06$; $P \leq 0.05$), and in anterior ($F=12.80$; $P \leq 0.001$), mediobasal ($F=2.86$; $P \leq 0.05$) and posterior ($F=18.25$; $P \leq 0.001$) hypothalamus, in prefrontal cortex ($F=10.87$; $P \leq 0.01$) and in the striatum ($F=5.42$; $P \leq 0.01$) compared with the values found in pubertal controls (Figs. 1, 2; Table 1). In pubertal rats treated with cadmium from 30 to 60 days of life, GABA content decreased in the median eminence ($F=11.81$; $P \leq 0.01$), and in anterior hypothalamus ($F=11.71$; $P \leq 0.01$), mediobasal hypothalamus ($F=4.46$; $P \leq 0.05$), posterior hypothalamus ($F=96.36$; $P \leq 0.001$) and in prefrontal cortex ($F=8.66$; $P \leq 0.01$), compared with the values found in the age-matched controls (Figs. 1, 2; Table 1). When cadmium was administered from day 60 to 90 of life, GABA content only changed in prefrontal cortex (Table 1; $F=8.68$; $P \leq 0.05$) compared with the age matched controls.

In adult control rats (90 days old), taurine content decreased in the median eminence ($F=5.62$; $P \leq 0.01$), and in anterior ($F=32.22$; $P \leq 0.001$) and posterior hypothalamus ($F=5.34$; $P \leq 0.05$) and in prefrontal cortex ($F=8.19$; $P \leq 0.01$) compared with the values found in pubertal controls (60 days old; Figs. 3, 4; Table 1). However, taurine content was not changed in mediobasal hypothalamus or in striatum as a function of age.

In pubertal rats treated with cadmium from 30 to 60 days of life, taurine content decreased in anterior ($F=25.88$; $P \leq 0.01$) and in posterior hypothalamus ($F=5.34$; $P \leq 0.05$), in prefrontal cortex ($F=9.56$; $P \leq 0.01$) and in the striatum ($F=3.88$; $P \leq 0.05$) compared with the values found in the age-matched controls

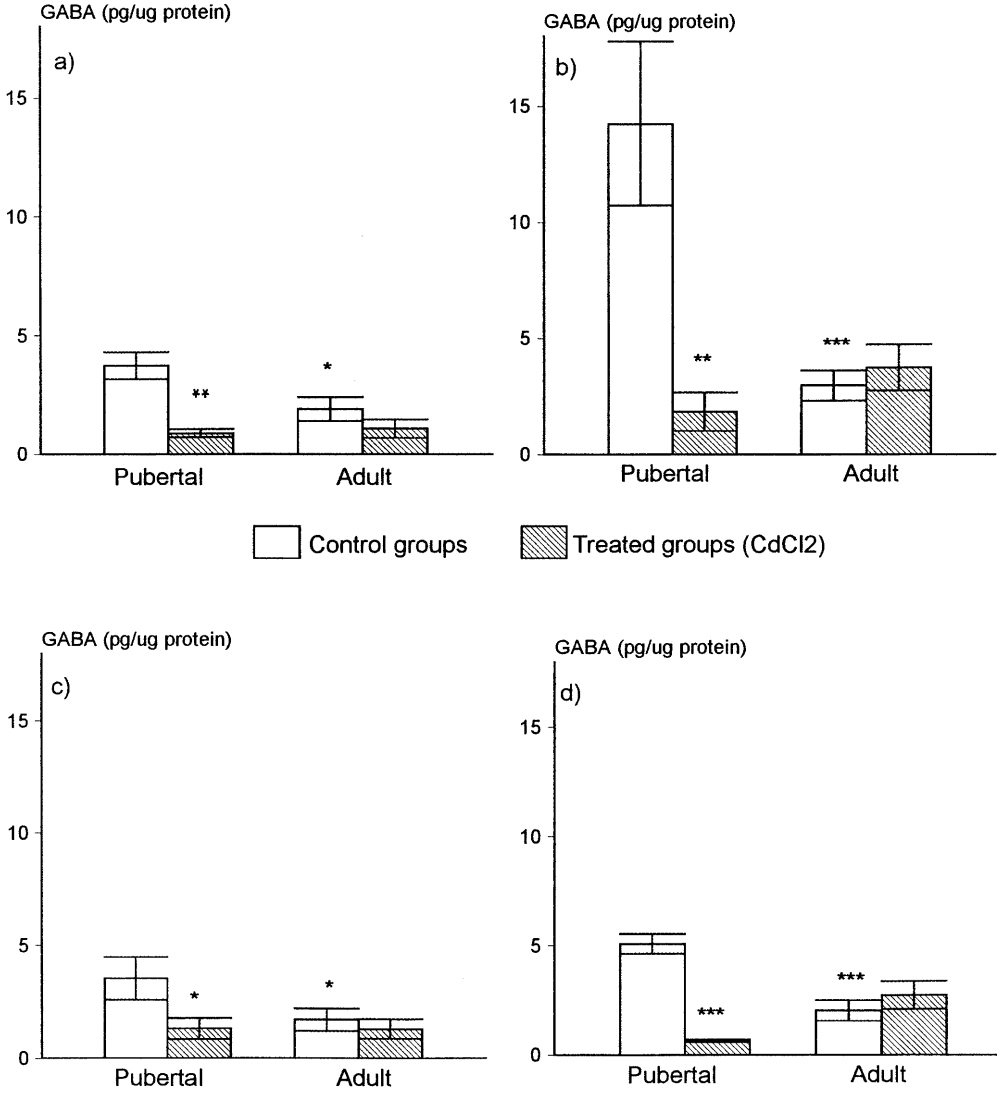

Fig. 1 Gamma-aminobutyric acid (GABA) content in **a** median eminence, and in **b** anterior, **c** mediobasal and **d** posterior hypothalamus, in pubertal and adult male rats treated with s.c. injections of saline every 4 days, or with cadmium chloride (0.5 or 1 mg/kg body wt.) every 4 days in an alternate schedule, during 1 month. Values are expressed as mean ± SEM ($n=8$ in each group). $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$ vs pubertal control group

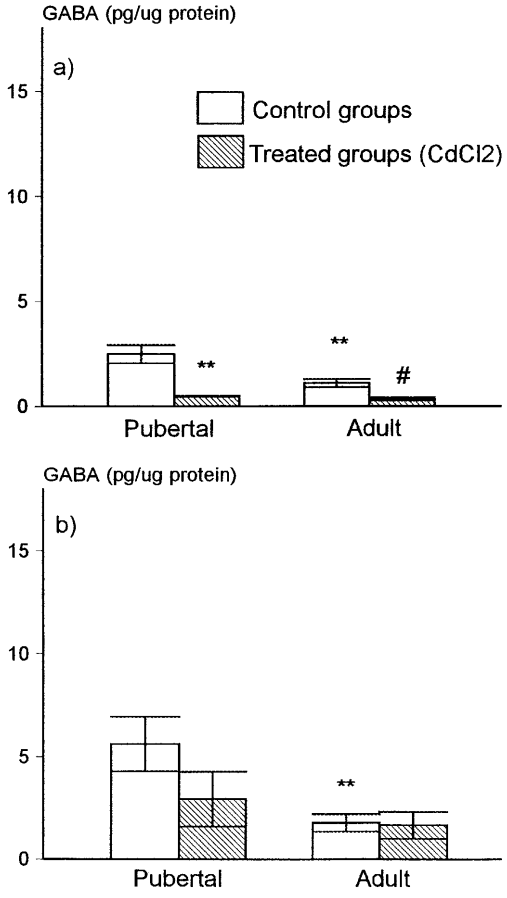

Fig. 2 GABA content in **a** prefrontal cortex and **b** striatum, in pubertal and adult male rats treated with s.c. injections of saline every 4 days, or with cadmium chloride (0.5 or 1 mg/kg body wt.) every 4 days in an alternate schedule, during 1 month. Values are expressed as mean ± SEM ($n = 8$ in each group). $^{**}P \leq 0.01$ vs pubertal control group; $^{\#}P \leq 0.05$ vs adult control group

(Figs. 3, 4; Table 1). No significant changes were observed in median eminence or in the mediobasal hypothalamus. However, when cadmium was administered from day 60 to 90 of life, taurine content only changed in prefrontal cortex (Fig. 4; Table 1; $F = 6.13$; $P \leq 0.05$) compared with the age matched controls. There was an interaction between the age of the animals during the cadmium treatment and the metal exposure for GABA content in anterior ($F = 14.56$; $P \leq 0.001$) and posterior ($F = 18.46$; $P \leq 0.001$) hypothalamus (Table 1), and in prefrontal cortex ($F = 5.53$; $P \leq 0.05$). An interaction between age and cadmium was also found for taurine content in anterior ($F = 34.05$; $P \leq 0.001$) and posterior hypothalamus ($F = 8.58$; $P \leq 0.01$; Table 1).

Discussion

The present study shows that cadmium differentially modified GABA and taurine contents as function of age, during the metal exposure, in the brain areas studied. GABA and taurine have been related to a great variety of physiological functions, which include neuroendocrine regulation, immune activity and behaviour (Devoino et al. 1992; Arias et al. 1994; Stapleton et al. 1994; Feleder et al. 1996). In this work, associated changes of these amino acids with cadmium accumulation were analysed within the brain.

The reduction in GABA content in pubertal animals, in the anterior and mediobasal hypothalamus, median eminence and striatum may be associated with increased plasma prolactin levels, as this amino acid is an inhibitory input for prolactin secretion (Locatelli et al. 1979). However, in previous studies, we have demonstrated that plasma prolactin levels were reduced after cadmium ex-

Table 1 Gamma-aminobutyric acid (GABA) and taurine content in median eminence, anterior, mediobasal and posterior hypothalamus, prefrontal cortex and striatum in pubertal and adult male rats treated with s.c. injections of saline every 4 days, or with cadmium chloride (0.5 or 1 mg/kg body wt.) every 4 days in an alternate schedule, during 1 month. Values are expressed as mean ± SEM ($n = 8$ in each group). $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$ vs pubertal control group (60 days of age); $^{\#}P \leq 0.05$ vs adult control group (90 days of age)

	Age during the treatment	Experimental group	GABA (pg/μg protein)	Taurine (pg/μg protein)
Median eminence	30–60 days	Control	3.74 ± 0.56	3.93 ± 0.64
		Treated	0.89 ± 1.06**	1.88 ± 0.78
	60–90 days	Control	1.91 ± 0.50*	1.41 ± 0.38**
		Treated	1.08 ± 0.39	1.12 ± 0.22
Anterior hypothalamus	30–60 days	Control	14.2 ± 3.53	9.10 ± 1.54
		Treated	1.84 ± 0.83**	1.54 ± 0.62**
	60–90 days	Control	2.97 ± 0.65***	1.45 ± 0.25***
		Treated	3.74 ± 0.99	2.24 ± 0.70
Mediobasal hypothalamus	30–60 days	Control	3.54 ± 0.94	1.87 ± 0.43
		Treated	1.32 ± 0.47*	0.95 ± 0.55
	60–90 days	Control	1.71 ± 0.50*	0.99 ± 0.24
		Treated	1.30 ± 0.44	0.77 ± 0.23
Posterior hypothalamus	30–60 days	Control	5.07 ± 0.45	5.21 ± 1.80
		Treated	0.65 ± 0.05***	0.49 ± 0.04*
	60–90 days	Control	2.04 ± 0.47***	1.14 ± 0.22*
		Treated	2.74 ± 0.64	2.25 ± 0.81
Prefrontal cortex	30–60 days	Control	2.49 ± 0.44	9.82 ± 1.61
		Treated	0.48 ± 0.02**	2.41 ± 0.58**
	60–90 days	Control	1.10 ± 0.20**	4.30 ± 0.91**
		Treated	0.34 ± 0.07$^{\#}$	1.47 ± 0.20$^{\#}$
Striatum	30–60 days	Control	5.61 ± 1.32	2.24 ± 0.34
		Treated	2.92 ± 1.34	1.15 ± 0.41*
	60–90 days	Control	1.76 ± 0.42**	1.30 ± 0.30
		Treated	1.65 ± 0.66	1.43 ± 0.42

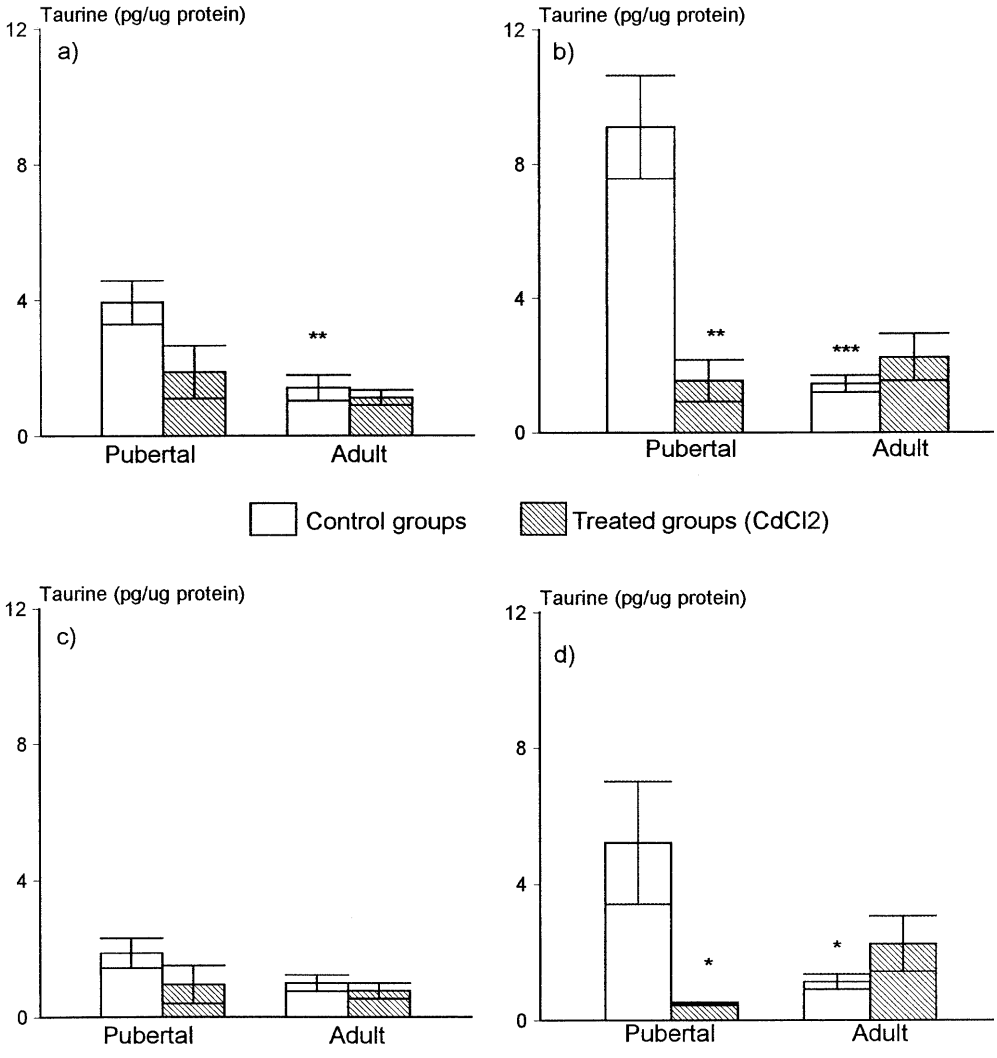

Fig. 3 Taurine content in **a** median eminence, and in **b** anterior, **c** mediobasal and **d** posterior hypothalamus, in pubertal and adult male rats treated with sc. injections of saline every 4 days, or with, cadmium chloride (0.5 or 1 mg/kg body wt.) every 4 days in an alternate schedule, during 1 month. Values are expressed as mean ± SEM ($n = 8$ in each group). $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ vs pubertal control group

posure, using the same schedule or after oral administration of the metal (Lafuente et al. 1996, 1999a; Lafuente and Esquifino 1998b), thus indicating that cadmium effects on prolactin would not be directly correlated with the changes in GABA shown in this study. A similar argument can be used for other pituitary hormones like LH or ACTH (Lafuente and Esquifino 1998a; Lafuente et al. 1999b). These data, together with dopamine changes described in previous work from the laboratory (Lafuente et al. 2000b), may indicate that metal exposure is associated with a disruption of the mechanisms that regulate the activity of the hypothalamic-pituitary axis.

In addition, pubertal cadmium exposure reduced GABA content in posterior hypothalamus and prefrontal cortex, thus indicating that other physiological functions, such as appetite or osmolarity regulated by this amino acid (Richard and Bourque 1995) would be changed. The data on GABA content, shown in this study, are similar to those described by Wong et al. (1981) in central nerve ending particles. The changes in posterior hypothalamus may be related to the activity of the autonomic nervous system, as this neurotransmitter was found in the nervous endings of autonomic neurons (Wong et al. 1981). The changes in prefrontal cortex may be correlated with behavioral alterations, as this region is involved in the modulation of behaviour among other functions not well established.

Providing that taurine contents, in the brain areas studied, are directly correlated with the amino acid pool involved in pituitary hormone secretion, the reduction in taurine content, after pubertal exposure to the metal in the hypothalamic areas may be directly correlated with pituitary hormone secretion as was previously shown (Arias et al. 1994, 1995). However, the modifications in this amino acid, within these brain regions after cadmium exposure, were not directly correlated with the changes in plasma levels of pituitary hormones, as described in previous work from our laboratory (Lafuente and Esquifino 1998a, 1998b, 1999; Lafuente et al. 1999a, 1999b) or from the literature (Zylber-Haran et al. 1982; Lorenson et al. 1983; Paksy et al. 1989; Winstel and Callahan 1992). The data may also indicate the existence of a disrupter effect of cadmium in the activity of the hypothalamic-pituitary axis.

Besides pubertal cadmium exposure giving reduced taurine content in posterior hypothalamus and pre-

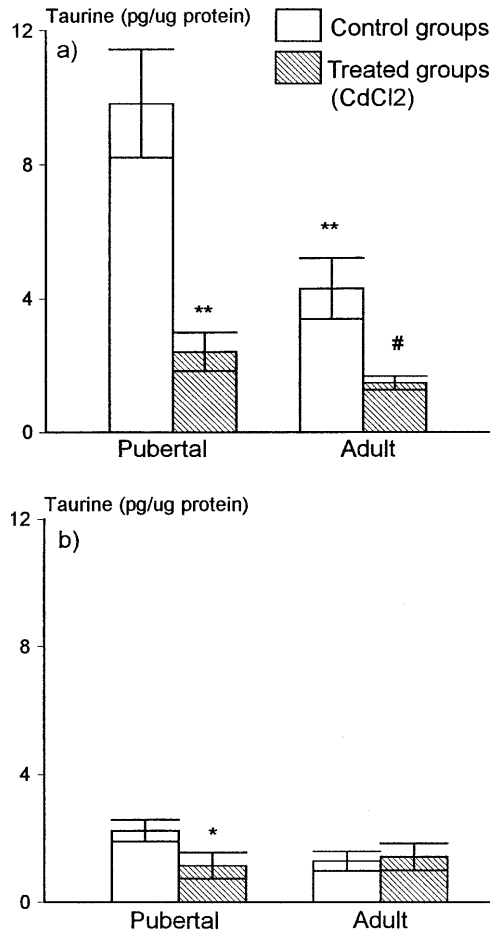

Fig. 4 Taurine content in **a** prefrontal cortex and **b** striatum, in pubertal and adult male rats treated with s.c. injections of saline every 4 days, or with cadmium chloride (0.5 or 1 mg/kg body wt.) every 4 days in an alternate schedule, during 1 month. The values are expressed as mean ± SEM ($n = 8$ in each group). $^{*}P \leq 0.05$, $^{**}P \leq 0.01$ vs pubertal control group; $\#P \leq 0.05$ vs adult control group

frontal cortex, other physiological functions are indicated, as the immune response regulated by this amino acid could be changed (Duvilanski et al. 1998). The changes in posterior hypothalamus may be related to the activity of the autonomic nervous system, although the relationship of taurine with the autonomic nervous system has to be established. The changes in prefrontal cortex may be correlated with behavioral changes, as this region is involved in the modulation of behaviour.

In adult animals GABA concentrations were not changed in the regions mentioned above after the exposure to the metal. Hence normal levels of this amino acid together with the previously described reduction in dopamine turnover (Lafuente et al. 2000b) indicate differential age-dependent effects of cadmium on the hypothalamus and also suggest that these changes are not directly correlated to the observed modification in pituitary hormone secretion as happened in pubertal animals. Similarly for GABA, taurine content was not changed in the hypothalamic areas involved in pituitary hormone secretion. As taurine is established as a major regulatory input for LH, the absence of changes in taurine may correlate with the absence of changes in plasma LH levels described in other studies from our group measuring this hormone (Pérez 1999). An age-dependent effect of cadmium on taurine content at the hypothalamic level was found. However, minor changes of taurine content were detected when adult animals were exposed to the metal. It has also to be considered that taurine is involved in the central response to injury (Stapleton et al. 1994; Duvilanski et al. 1998), which may be changed by cadmium exposure. Further studies will be needed to clarify the role of cadmium induced changes in taurine content within the brain in male rats.

In conclusion, cadmium exposure differentially affects GABA and taurine content within the hypothalamus and other brain areas as a function of age. However, cadmium accumulation (Márquez et al. 1998) does not apparently correlate with the changes in the amino acid studied. Considering the data of this work together with previous data from the laboratory using the same schedule of metal exposure (Esquifino et al. 1998; Perez 1999; Lafuente and Esquifino 1999; Lafuente et al. 2000a, 2000b), a disruption in the mechanism that regulates the activity of the hypothalamic pituitary axis is proposed. Whether or not the changes in GABA and taurine contents described in this study reflect cell death or gliosis within the brain regions, needs further investigation.

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