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Possible role of glutamate, aspartate, glutamine, GABA or taurine on cadmium toxicity on the hypothalamic pituitary axis activity in adult male rats

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Abstract

This work was designed to evaluate the possible changes in glutamate, aspartate, glutamine, GABA and taurine within various hypothalamic areas the striatum and prefrontal cortex after oral cadmium exposure in adult male rats, and if these changes are related to pituitary hormone secretion. The contents of glutamine, glutamate, aspartate, GABA and taurine in the median eminence, anterior, mediobasal and posterior hypothalamus, and in prefrontal cortex in adult male rats exposed to 272.7 μmol l^{-1} of cadmium chloride ($CdCl_2$) in the drinking water for one month. Cadmium diminished the content of glutamine, glutamate and aspartate in anterior hypothalamus as compared to the values found in the untreated group. Besides, there is a decrease in the content of glutamate, aspartate and taurine in the prefrontal cortex. The amino acids studied did not change in median eminence, mediobasal and posterior hypothalamus or the striatum by cadmium treatment. Plasma prolactin and LH levels decreased in rats exposed to the metal. These results suggest that (1) cadmium differentially affects amino acid content within the brain region studied and (2) the inhibitory effect of cadmium on prolactin and LH secretion may be partially explained by a decrease in the content of both glutamate and aspartate in anterior hypothalamus, but not through changes in GABA and taurine.

Introduction

There is increasing evidence showing that cadmium exerts both neurotoxic (Nation *et al.,* 1989, Das *et al*. 1993, Gutierrez-Reyes *et al.* 1998; Antonio *et al.* 1999; Lafuente & Esquifino 1999) and peripheral toxic effects (Wong & Klaassen 1980; Descotes 1992; Goyer *et al*. 1995). Amino acids within the brain have been used as an indicator of the metal toxicity (Wong *et al.* 1981; Vignes *et al*. 1996; Minami *et al*. 2001; Esquifino *et al*. 2001; Lafuente *et al.* 2001a). Also, there is increasing evidence showing that amino acids are involved in the neuromodulation of the pituitary hormone secretion (Casanueva *et al*. 1984; Scott *et al*. 1987; Van den Pol *et al*. 1990; Arias *et al.* 1995; Brann 1995, Aguilar *et al.* 1996, 1997; Feleder *et al.* 1996; Dhandapani & Brann 2000), thus diverse physiological functions, regulated by these pituitary hormones may be affected.

In previous works from our laboratory, alternate subcutaneous cadmium exposure during the puberty produced a global inhibitory effect on glutamate, aspartate, glutamine, GABA and taurine (Esquifino *et al*. 2001; Lafuente *et al.* 2001a), being the effect less marked when cadmium exposure was made during adulthood (Esquifino *et al.* 2001; Lafuente *et al.* 2001a). Associated changes in pituitary hormone secretion were also observed (Zylber-Haran *et al.* 1982; Paksy *et al.* 1989; Lafuente & Esquifino 1999). The

changes induced by oral cadmium exposure on prolactin and LH in adult male rats can not be explained nor by the modifications in the NE (norepinephrine) content, nor by those in dopamine (DA) or serotonin (5-HT) metabolisms, within the brain regions involved in the regulation of pituitary hormone secretion (Lafuente & Esquifino 1999; Lafuente *et al*. 1999, 2001b).

With the above mentioned data, this work was designed (1) to evaluate the effects of oral cadmium exposure on the contents of glutamate, aspartate, glutamine, GABA and taurine within different brain regions in adult male rats and (2) to analyzed whether these amino acids are involved in cadmium toxicity at the pituitary level.

Material and methods

Materials

O-Phtalaldehyde (OPQ), 2-mercaptoethanol and the standards of each amino acid were purchased from Sigma Chemical Co., St. Louis, MO. U.S.A, Bradford Reagent from Bio-Rad Lab, Munchen, Germany. Methanol (HPLC grade) from Merck (Darmstadt, Germany). All reagents and solvents were reagent-grade purity. Doble-destilled deionized water was used for preparation of amino acid solutions and buffers.

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 $\pm$ 2°C). Food and water were available ad libitum. Two groups of 8 animals were used. One group was treated for one month with cadmium chloride ($CdCl_2$) at a dose of 272.7 μmol l of $CdCl_2$ in the drinking water. The other group received cadmium-free water to use it as control. The dose of cadmium administered to the animals in this work, is 15 times higher than the Provisional Tolerable Week Intake (PTWI) of this heavy metal (WHO 1993).

At the end of the treatment, animals were killed by decapitation at 14:00 h to avoid the diurnal secretion pattern of the pituitary hormones analyzed (Esquifino *et al.* 1997) or in the contents of the amino acids studied (Esquifino *et al*. 2000). Care was taken to avoid any major stress before sacrifice and the decapitation procedure was completed within 5–7 sec.

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).

Tissue preparation

After thawing, the median eminence, anterior, mediobasal and posterior hypothalamus, striatum and prefrontal cortex blocks were processed as previously described (Esquifino *et al*. 2000; Lafuente*et al*. 2001a).

Amino acid analysis

Amino acids (glutamine, glutamate, aspartate, GABA and taurine) were separated and analyzed using High Performance Liquid Chromatography (HPLC), with fluorescence detection after precolumn derivatizacion with OPA as previously described (Esquifino *et al.* 2000; Lafuente *et al.* 2001a).

Pituitary hormone measurements

Plasma levels of prolactin and LH were measured by specific radioimmunoassay methodology using the reagents generously provided by the National Hormone and Pituitary Program (NHPP, Rockville, MD, USA) and by to Dr A. Parlow (Harbor UCLA Medical Center, 1000 West Carson Street, Torrance CA 90509), previously described (Lafuente *et al*. 2001b).

Statistical analysis

Amino acid concentrations were expressed as pg/μg protein. The results were tested for variance homogeneity through the Snedecor test. When the results did not follow a homogeneous variance, a Mann-Whitney test was used for comparisons between groups. If the results followed a homogeneous variance, the Student's t-test was applied for comparisons between groups. The results were considered significant at $P \leq 0.05$. All values represent the mean $\pm$ S.E.M.

Results

Cadmium exposure through the drinking water for a month, diminished the content of glutamine, glutamate and aspartate in the anterior hypothalamus as

Table 1. Amino acid content in different brain areas in adult male rats treated with 272.7 μmol l of cadmium chloride ($CdCl_2$) in the drinking water during one month. The values are expressed as mean ±S.E.M. ($n = 8$ in each group). $^{*}P \leq 0.05$; $^{**}P \leq 0.01$ vs. control group.

Brain area	Experimental group	Glutamine (pg/μg protein)	Glutamate (pg/μg protein)	Aspartate (pg/μg protein)	GABA (pg/μg protein)	Taurine (pg/μg protein)
Median eminence	Control	1.83 ± 0.41	7.79 ± 2.05	1.45 ± 0.55	1.87 ± 0.42	1.50 ± 0.25
	272.7 μmol l^{-1} $CdCl_2$	1.99 ± 0.25	7.42 ± 1.67	1.90 ± 0.49	1.03 ± 0.26	1.17 ± 0.24
Anterior hypothalamus	Control	2.20 ± 0.36	8.01 ± 0.90	2.90 ± 0.38	2.84 ± 0.71	1.50 ± 0.30
	272.7 μmol l^{-1} $CdCl_2$	1.31 ± 0.20**	3.47 ± 0.64**	0.99 ± 0.15**	1.63 ± 0.17	1.40 ± 0.39
Mediobasal hypothalamus	Control	1.82 ± 0.25	6.50 ± 1.18	1.70 ± 0.20	1.67 ± 0.45	1.03 ± 0.18
	272.7 μmol l^{-1} $CdCl_2$	2.07 ± 0.41	5.76 ± 0.48	1.80 ± 0.24	2.11 ± 0.54	0.86 ± 0.08
Posterior hypothalamus	Control	2.15 ± 0.30	8.65 ± 2.18	2.10 ± 0.30	1.98 ± 0.43	1.20 ± 0.18
	272.7 μmol l^{-1} $CdCl_2$	1.94 ± 0.40	5.87 ± 1.22	1.65 ± 0.23	2.16 ± 0.68	1.03 ± 0.27
Prefrontal cortex	Control	3.15 ± 0.90	11.50 ± 1.21	3.75 ± 0.65	1.20 ± 0.18	4.42 ± 0.85
	272.7 μmol l^{-1} $CdCl_2$	2.20 ± 0.41	6.11 ± 0.38**	1.86 ± 0.32**	1.00 ± 0.17	2.84 ± 0.39*

Table 2. Plasma levels of prolactin and LH in adult male rats treated with 272.7 μmol l of cadmium chloride ($CdCl_2$) in the drinking water during one month. The values are expressed as mean ± S.E.M. ($n = 8$ in each group). $^{*}P \leq 0.05$; $^{**}P \leq 0.01$ vs. control group.

Experimental Group	rPRL-RP3 (ng/mL)	rLH-RP3 (ng/mL)
Control group	3.25 ± 0.20	0.53 ± 0.03
$CdCl_2$ (272.7 μmol l)	2.30 ± 0.07*	0.40 ± 0.02**

compared to the values found in the control group ($P \leq 0.01$; Table 1). The metal also reduced the contents of glutamate ($P \leq 0.01$), aspartate ($P \leq 0.01$) and taurine ($P \leq 0.05$) in the prefrontal cortex (Table 1). The contents of glutamine, glutamate, aspartate, GABA or taurine were not changed in the median eminence, the mediobasal or posterior hypothalamus and the striatum by cadmium exposure (Table 1).

GABA content diminished in median eminence, anterior hypothalamus and prefrontal cortex, while it increased in mediobasal hypothalamus (Table 1) although the differences did not reach statistical significance. Also taurine content diminished in median eminence and in anterior, mediobasal and posterior hypothalamus (Table 1), but as in the case of GABA the results were not statistically significant. Furthermore, glutamate content tends to decrease in median eminence and in mediobasal and posterior hypothalamus, although the results did not reach statistical significance (Table 1). In this last region aspartate content also fold down, while it increased in median eminence (Table 1), although the results were not statistically significant. Finally glutamine content also diminishes in prefrontal cortex, but the results were not statistically significant (Table 1).

Plasma levels of prolactin and LH were decreased in cadmium exposed rats as compared to the values found in the control group (Table 2; $P \leq 0.05$ for prolactin and $P \leq 0.01$ for LH).

Discussion

Ongoing results suggest that cadmium differentially affects the content of the amino acids studied within the specific brain regions analyzed. The inhibitory effect of the metal on plasma prolactin and LH levels may be partially explained by the decrease in the content of both glutamate and aspartate in the anterior hypothalamus, but not through GABA or taurine that were not changed significantly by cadmium exposure.

Glutamate decreased in the anterior hypothalamus, a region involved in pituitary hormone secretion (Morgan & King 1986). This change correlated with the effects of glutamate on plasma levels of prolactin (Brann 1995; Dhandapani & Brann 2000; Lafuente *et al.* 1999) and LH (Lafuente *et al.* 2001b). Aspartate was also reduced in the anterior hypothalamus and these changes may also explain the modifications observed in plasma prolactin and LH levels (Aguilar *et al.* 1996; Lafuente *et al.* 1999, 2001b).

The changes in prefrontal cortex of both glutamate and aspartate contents may be related to altered behaviors as this brain area is involved in the modulation of such a function (Geschwind 1980; Keenan *et al.* 2000). The reduction in taurine content in this specific area may also contribute to changes in behavior. Further studies need to be done to clarify the role of these amino acids in the modulation of several functions modulated by this central nervous system structure.

The reduction in glutamine content after cadmium exposure in the anterior hypothalamus may reflect a reduction in the metabolism of glutamate and aspartate, as their contents are also reduced by oral cadmium exposure. Also, these changes may reflect a diminished utilization as precursor of glutamate. The correlation in the changes of these three amino acids was not observed when cadmium was administered in an alternate sc. schedule (Lafuente *et al*. 2001a), thus indicating that the route of metal exposure specifically alters the content of the amino acids studied in the anterior hypothalamus. Similar effects were also observed when studying the effects of different routes of heavy metal administration on plasma LH levels (Lafuente & Esquifino 1999). The different cadmium effects on amino acid content in the brain and on plasma LH levels can be due to different degree in the metal absorption according to the administration route. Cadmium absorption from the gastrointestinal tract is as low as 2 to 8%, since in the gastrointestinal tract cells, cadmium is bound to metallothionein. Therefore, the absorption of the metal is higher when it is administered subcutaneously (sc) than when it is administered orally. However, greater effects of cadmium on amino acid content within the brain and LH were observed when the metal was given orally as compared to the effects of the metal on the same parameters when cadmium was given subcutaneously (Lafuente *et al.* 2001; Lafuente & Esquifino 1999). Thus indicating that the degree of effects of cadmium does not seem to depend on the degree of absorption of the metal.

GABA and taurine contents were not changed by cadmium exposure in any of hypothalamic areas involved in pituitary hormone secretion, in agreement with previous works from the laboratory using a different route for the metal exposure (Esquifino *et al.* 2001). So that these amino acids seemed not to be involved in metal effects on the plasma concentrations of prolactin and LH (Lafuente *et al*. 1999, 2001b).

In conclusion, the results obtained in the present study suggest that cadmium differentially affects amino acid contents within the brain, being the anterior hypothalamus and prefrontal cortex the areas most affected by the metal. The inhibitory effect of cadmium on prolactin and LH secretion may be partially explained by the decrease in the content of both glutamate and aspartate in anterior hypothalamus, but not through GABA or taurine, that were not modified at the hypothalamic level by the metal.

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