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Alternate cadmium exposure differentially affects amino acid metabolism within the hypothalamus, median eminence, striatum and prefrontal cortex of male rats

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Abstract

This work was designed to analyze the possible changes in glutamate, aspartate and glutamine content induced by cadmium exposure in the hypothalamus, striatum and prefrontal cortex of rats, using an alternate schedule of metal administration. Pubertal–adult differences were also evaluated. In adult control rats, glutamate and aspartate contents in the anterior hypothalamus decreased as compared to pubertal controls. After cadmium administration from day 30 to 60 of life, the content of anterior hypothalamic glutamate and aspartate diminished. In adult control animals, the glutamine content increased in mediobasal hypothalamus as compared to pubertal controls. After cadmium exposure from day 30 to 60 of life, the mediobasal glutamine content increased, and after cadmium treatment from day 60 to 90 of life, the mediobasal aspartate content decreased. In adult control rats the content of glutamine, glutamate and aspartate of the posterior hypothalamus decreased significantly. After cadmium administration in pubertal animals, posterior hypothalamic contents of glutamine, glutamate and aspartate diminished. Cadmium treatment of adult animals caused a decrease in glutamine content, as compared to controls. In adult control rats, only glutamate and aspartate content increased in the prefrontal cortex as compared to the values found in pubertal controls. When cadmium was administered to adult animals, only the aspartate content decreased. In the striatum, cadmium decreased the glutamine and aspartate contents when administered from day 60 to 90 of life. These data suggest that cadmium differentially affects amino acid metabolism in the hypothalamus, striatum and prefrontal cortex. Age-dependent effects of cadmium on these brain areas appeared to have occurred.

Keywords: Aspartate and glutamine; Cadmium; Glutamate

1. Introduction

There is scant evidence on the effects of cadmium accumulation on brain amino acid metabolism (Wong et al., 1981). However, these effects are suspected, considering that these amino acids affect the competition of cadmium with calcium to enter in the cell (Riveros and Orrego, 1986; Usai et al., 1999).

Besides participating in the amino acid pool involved in protein synthesis, hypothalamic glutamate and aspartate and presumably glutamine are involved in the modulation of pituitary hormone secretion (Brann and Mahesh, 1991; Carbone et al., 1992; Mahachoklertwattana et al., 1994; Brann, 1995).

Previous works from our laboratory using an alternate schedule of cadmium administration demonstrated decreased plasma levels of prolactin, FSH and testosterone, and increased plasma ACTH levels after exposure of rats to the metal during adulthood (Lafuente and Esquifino, 1999; Lafuente et al., 2000a,b). These effects were different when the rats were exposed to cadmium during puberty (Esquifino et al., 1998; Lafuente and Esquifino, 1999; Lafuente et al., 2000a,b), thus suggesting an age dependency of cadmium effects on the endocrine system. In fact, the neuroendocrine system may exhibit an age-dependent sensitivity to cadmium exposure that has been previously shown using other experimental approaches that analyzed pituitary

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hormone secretory patterns (Villanúa et al., 1990). Hormonal changes were not explained by the associated modifications in dopamine, norepinephrine and serotonin concentration at the hypothalamic areas studied or at the median eminence (Lafuente and Esquifino, 1999; Lafuente et al., 2000a,b). As these regions are involved in the regulation of pituitary hormone secretion (Esquifino et al., 1995) the changes in aminoacidergic metabolism associated with cadmium accumulation at the hypothalamus may explain the hormonal changes.

The exposure to cadmium is not constant in humans and depends on the concentration in the atmosphere or in the drinking water and food. Thus, alternate subcutaneous schedules of cadmium administration would reproduce the exposure of animals to the metal in a similar way than in humans. This schedule was used in previous work from our laboratory (Esquifino et al., 1998; Pérez, 1999; Lafuente et al., 2000a,b). In fact, the values of cadmium accumulation described in the hypothalamus by our group, in previous studies (Food), are similar to that found in liver or kidney in humans (Ellis et al., 1979). Whether cadmium in the tissues plays a major role in metal effects needs further investigation (Paksy et al., 1990; Márquez et al., 1998).

The present study was designed to determine whether the possible changes in amino acid metabolism after cadmium exposure in the hypothalamus, striatum and prefrontal cortex, using an alternate schedule of metal administration, may explain the changes in pituitary hormone secretion, described in previous works from the laboratory (Esquifino et al., 1998; Lafuente and Esquifino, 1999; Lafuente et al., 2000a,b) by comparing adult and prepubertal rats.

2. Materials and methods

2.1. Animals and treatment

Male rats of the Sprague–Dawley strain were kept under controlled conditions of light (lights on from 07.00 to 21.00 h) and temperature (22 ± 2°C) and had access to food and water 'ad libitum'. After weaning, four animals/cage were maintained in the same room for the period studied. Four groups of eight animals were used. Rats of groups 1 and 2 were 30 day old rats at the beginning of the experiment (pubertal age), and rats of groups 3 and 4 were 60 day old rats at the beginning of the experiment (young adults). 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/kg body weight) every 4th day in an alternate schedule, starting from the smaller dose as follows: 0.5 mg of $CdCl_2$/kg body weight on the first day of the treatment, and 1.0 mg of $CdCl_2$/kg body weight on the fourth. Then, the 8th day of treatment the dose of 0.5 mg of $CdCl_2$/kg body weight was repeated, and so on. The latest dose of cadmium was given 48 h in advance to killing. Groups 1 and 3 received s.c. 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 this and other laboratories (Zylber-Haran et al., 1982; Paksy et al., 1989; Laskey and Phelps, 1991; Piasek and Laskey, 1994; Lafuente et al., 2000a,b).

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 14:00 h to avoid the diurnal secretion pattern of glutamate, aspartate and glutamine. Care was taken to avoid any major stress before sacrifice, and the decapitation procedure was completed within 10–20 s. Immediately after sacrifice, the heads were placed into liquid nitrogen and kept at -80°C until analysis.

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

2.2. Tissue preparation

After thawing, the median eminence, anterior, mediobasal and posterior hypothalamus, prefrontal cortex and striatum blocks were immediately homogenized in cold 2 M acetic acid (1–4°C), heated for 5 min in 100°C water bath and centrifuged at $11000 \times g$ for 10 min, at 4°C. The supernatant was removed and kept frozen at -80°C until amino acid determination. Before heating a small aliquot of the tissue, homogenates were obtained and used to determine the protein content by the Bradford method.

2.3. Amino acid measurements

Amino acids were separated and analyzed using high-performance liquid chromatography (HPLC), with fluorescence detection after precolumn derivatization with OPA. An aliquot of the tissue supernatant containing homoserine as internal standard was neutralized with NaOH (4 M) and then was reacted at room temperature with OPA reagent (4 mM OPA, 10% methanol, 2.56 mM 2-mercaptoethanol, in 1.6 M potassium borate buffer, pH 9.5) for 1 min. At the end of this period, the reaction was stopped by adding acetic acid (0.5 v/v). Samples were immediately loaded through a Rheodyne (model 7125) injector system with a 50 μl loop sample to reach a C-18 reverse-columm (4.6 mm ID × 150 mm, Nucleosil 5, 100 A) 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, at a pressure of 14000 kPa. 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).

Amino acid 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 tested within the concentration ranges found in brain region extracts.

2.4. Statistical analysis

Amino acid concentrations were expressed as pg/mg protein and analyzed 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 for statistical significance was $P \leq 0.05$ for each analysis.

3. Results

The amino acid contents in the median eminence are shown in Table 1. In this brain area, neither age nor cadmium exposure affected amino acid metabolism.

The amino acid metabolism in anterior hypothalamus is also shown in Table 1. In adult control rats, the content of glutamate ($F = 4.84$, $P \leq 0.05$) and aspartate ($F = 9.39$, $P \leq 0.01$) decreased as compared to the values found in younger controls (Table 1). When cadmium was administered from day 30 to 60 of life, the content of glutamate and aspartate diminished as compared to age-matched controls. (Table 1; $F = 6.70$, $P \leq 0.05$ for glutamate and $F = 15.13$, $P \leq 0.001$ for aspartate). There was an interaction between the age and cadmium in glutamate and aspartate effects in animals exposed to cadmium (Table 1: $F = 5.28$, $P \leq 0.05$ and $F = 5.47$, $P \leq 0.05$, respectively). When cad-

Table 1
Glutamine, glutamate and aspartate 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 weight) every 4 days in an alternate schedule, during 1 month

	Age during the treatment	Experimental group	Glutamine (pg/μg protein)	Glutamate (pg/μg protein)	Aspartate (pg/μg protein)
Median eminence	30–60 days	Control	3.11 ± 0.86	11.87 ± 2.45	1.18 ± 0.58
		Treated	1.68 ± 0.24	11.83 ± 4.41	1.52 ± 0.32
	60–90 days	Control	1.82 ± 0.39	7.82 ± 2.10	1.57 ± 0.66
		Treated	2.29 ± 0.63	6.80 ± 2.20	1.06 ± 0.47
Anterior hypothalamus	30–60 days	Control	3.47 ± 1.18	12.38 ± 2.58	4.33 ± 1.11
		Treated	1.33 ± 0.33	4.68 ± 1.48*	0.71 ± 0.09***
	60–90 days	Control	2.17 ± 0.39	7.92 ± 1.00*	2.80 ± 0.41**
		Treated	3.74 ± 0.81	8.14 ± 1.87	1.78 ± 0.54
Mediobasal hypothalamus	30–60 days	Control	0.52 ± 0.11	3.92 ± 1.52	1.55 ± 0.43
		Treated	1.06 ± 0.19*	3.94 ± 0.41	1.58 ± 0.44
	60–90 days	Control	1.79 ± 0.27**	6.76 ± 1.24	1.67 ± 0.25
		Treated	1.05 ± 0.19	4.89 ± 2.31	0.79 ± 0.18 #
Posterior hypothalamus	30–60 days	Control	3.45 ± 0.70	15.60 ± 2.64	4.92 ± 0.12
		Treated	0.99 ± 0.14**	6.67 ± 0.93**	1.42 ± 0.28***
	60–90 days	Control	2.10 ± 0.33*	8.79 ± 2.24**	2.15 ± 0.26***
		Treated	1.10 ± 0.25 #	6.10 ± 1.70	1.74 ± 0.44
Prefrontal cortex	30–60 days	Control	2.23 ± 0.86	6.18 ± 1.19	0.92 ± 0.41
		Treated	2.57 ± 0.97	9.10 ± 1.61	1.57 ± 0.29
	60–90 days	Control	3.12 ± 0.97	11.61 ± 1.18*	3.69 ± 0.72**
		Treated	1.60 ± 0.39	6.31 ± 2.03	0.85 ± 0.11 # #
Striatum	30–60 days	Control	1.58 ± 0.42	7.38 ± 2.61	2.63 ± 1.14
		Treated	1.12 ± 0.22	6.43 ± 1.40	2.56 ± 0.46
	60–90 days	Control	1.56 ± 0.22	7.53 ± 1.28	2.41 ± 0.53
		Treated	1.10 ± 0.20	3.13 ± 1.17 #	0.83 ± 0.33 #

The values are expressed as mean ± S.E.M. ($n = 8$ in each group). *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$ vs. pubertal control group (60 days of age). #$P \leq 0.05$; # #$P \leq 0.01$ vs. adult control group (90 days of age).

mium was administered during adult age glutamate, glutamine and aspartate contents did not change significantly (Table 1).

Amino acid metabolism in mediobasal hypothalamus is shown in Table 1. In older control animals, the glutamine content increased as compared to the values found in younger animals (Table 1; $F = 7.86$, $P \leq 0.01$). Glutamate and aspartate content did not change as a function of age. Cadmium exposure from 30 to 60 days of life increased the glutamine content in this hypothalamus (Table 1; $F = 6.35$, $P \leq 0.05$) as compared to age-matched controls, whereas the glutamate and aspartate contents did not change. When cadmium was administered from day 60 to 90 of life, only the aspartate content decreased in mediobasal hypothalamus (Table 1; $F = 8.16$, $P \leq 0.05$). In the case of glutamine content, there was an interaction between age and cadmium exposure (Table 1; $F = 7.82$, $P \leq 0.01$).

Amino acid metabolism in posterior hypothalamus can be observed in Table 1. In adult control rats, the content of glutamine, glutamate and aspartate decreased as compared to the values found in young controls (Table 1; $F = 6.98$, $P \leq 0.05$ for glutamine; $F = 8.25$ $P \leq 0.05$ for glutamate and, $F = 43.58$, $P \leq 0.001$ for aspartate). Cadmium exposure throughout puberty diminished the contents of the three amino acids, as compared to the age-matched controls (Table 1; $F = 9.36$, $P \leq 0.01$ for glutamine; for $F = 18.60$, $P \leq 0.01$ glutamate and, $F = 133.29$, $P \leq 0.001$ for aspartate). After cadmium treatment in adult animals, only the glutamine content decreased (Table 1; $F = 5.94$, $P \leq 0.05$). There was an interaction between age and cadmium effects in the contents of glutamate and aspartate in the posterior hypothalamus (Table 1; $F = 6.84$, $P \leq 0.05$ and $F = 15.24$, $P \leq 0.001$, respectively).

Table 1 shows the amino acid metabolism in the prefrontal cortex. In adult control rats, glutamate and aspartate contents increased as compared to the values found in younger rats (Table 1; $F = 4.78$, $P \leq 0.05$ and $F = 7.25$, $P \leq 0.01$, respectively). Glutamine content did not change as a function of age. Cadmium exposure through puberty did not result in glutamate, aspartate or glutamine content in the hypothalamus. When cadmium was administered to adult animals, only the aspartate content decreased (Table 1; $F = 12.75$, $P \leq 0.01$). There was an interaction between age and cadmium effects in aspartate content (Table 1; $F = 12.75$, $P \leq 0.01$).

The amino acid metabolism in striatum can be observed in Table 1. Age did not modify aspartate, glutamate or glutamine content in this brain area. Only in those animals exposed to cadmium from day 60 to 90 of life did the glutamine and aspartate contents diminish (Table 1; $F = 5.94$, $P \leq 0.05$ and $F = 5.00$, $P \leq 0.05$ respectively) as compared to their age-matched controls.

4. Discussion

The present study demonstrates that cadmium exposure is capable to modify differentially glutamate, aspartate and glutamine concentration in brain as a function of age, and the variations depended on the brain area studied.

The glutamate and aspartate content of the anterior and posterior hypothalamus and prefrontal cortex were modified with age, whereas in the case of glutamine, the effect was found in mediobasal and posterior hypothalamus. Identification and measurements of these amino acids in these and other brain areas were previously undertaken by Ondo et al. (1988), Monaghan et al. (1989) and Duvilanski et al. (1998).

Pubertal cadmium exposure globally inhibited glutamate metabolism and, to a lesser extent, aspartate metabolism. The changes in amino acids of the median eminence and anterior and mediobasal hypothalamus, regions implicated in the regulation of pituitary hormones secretion (Mitchell et al., 1983; Flugge et al., 1986), can be compared to the changes in plasma levels of prolactin, LH, FSH or ACTH previously described using a similar schedule of cadmium exposure (Esquifino et al., 1998; Pérez, 1999; Lafuente and Esquifino, 1999; Lafuente et al., 2000a,b). The small part of glutamate and aspartate that acts as a neurotransmitter in aminoacidergic neurons is associated with the regulation of a great variety of physiological functions including the endocrine system (Pohl et al., 1989; Van den Pol et al., 1990; Brann and Mahesh, 1991; Carbone et al., 1992; Mahachoklertwattana et al., 1994; Brann, 1995). Both amino acid transmitters stimulated prolactin and luteinizing hormone (LH) secretion (Ondo et al., 1988; Pohl et al., 1989; Van den Pol et al., 1990; Brann and Mahesh 1991). Providing that the amino acid content is directly correlated with the amino acid transmitter pool, the observed changes in the amino acid content within the hypophysiotropic area of the hypothalamus are not directly correlated with the changes observed in plasma levels of the pituitary hormones previously studied. The changes of these amino acids in the posterior hypothalamus can be related to the activity of the autonomic nervous system. There is increasing evidence showing the modulatory role of this hypothalamic area on the peripheral nervous system activity (Fischer et al., 1995; Heberer et al., 1996). Further works are needed to clarify the role of glutamate and aspartate in the modulation of the autonomic nervous system activity.

Glutamine within the mediobasal hypothalamus was differentially affected as compared to glutamate and

aspartate. The data obtained may reflect the metabolism of these neurotransmitters or the utilization of glutamine as precursor of glutamate. Cadmium-induced changes in glutamine may reflect an anomalous amino acid metabolism within the hypothalamus after cadmium, as was demonstrated for other diseases (Heberer et al., 1996). Besides, glutamine seems to be a main growth factor for immune cells (Fischer et al., 1995) so that an immune role of this amino acid at the central nervous system cannot be ruled out.

In adult animals, the effects of the exposure to cadmium on aspartate were more pronounced than those for glutamate. Glutamate decreased only in the striatum, a region involved in pituitary hormone secretion (Cruz-Casallas, et al., 1999). These data suggest that glutamate in adults can exert indirect effects on pituitary hormone secretion through changes in the metabolism of other neuromodulators that connect the striatum and the hypothalamus (Jarry et al., 1988). Aspartate was diminished in prefrontal cortex, mediobasal hypothalamus and striatum. The changes in the striatum and mediobasal hypothalamus may be related to its involvement in pituitary secretion regulation according to data previously published (Esquifino et al., 1998; Lafuente et al., 2000a,b). The changes in prefrontal cortex may be related to behavior as this brain area is involved in the modulation of such a function (Geschwind, 1980). As it happened for pubertal animals, the changes in these amino acids within the hypophysiotropic area of the hypothalamus do not explain the observed changes in plasma levels of the pituitary hormones studied in previous works from the laboratory (Lafuente et al., 1997, 1999a,b, 2000a,b; Esquifino et al., 1998; Lafuente and Esquifino, 1998a,b, 1999; Márquez, 1999; Pérez, 1999).

The changes in glutamine after cadmium exposure in adult animals were reflected to some extent in pubertal exposed animals, as far as the metabolism of glutamate and aspartate or the utilization of glutamine as a precursor of glutamate. Cadmium-induced changes in glutamine content in the posterior hypothalamus may reflect an anomalous amino acid metabolism in this area, as was demonstrated for other pathophysiological conditions (Fischer et al., 1995).

In conclusion, cadmium exposure differentially affects glutamate content within the brain as a function of age. Considering the data obtained in this work with previous data from the laboratory using the same schedule of metal exposure (Esquifino et al., 1998; Lafuente and Esquifino, 1999; Pérez, 1999; Lafuente et al., 2000a,b), a disruption in the mechanisms that regulate the activity of the hypothalamic pituitary axis is proposed. Finally, whether the changes in amino acid concentrations may reflect cell death within the brain regions studied or gliosis processes cannot be excluded from this study.

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