Amino Acids (2010) 38:1165–1172
DOI 10.1007/s00726-009-0327-7

ORIGINAL ARTICLE

Daily pattern of pituitary glutamine, glutamate, and aspartate content disrupted by cadmium exposure

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Received: 7 May 2009 / Accepted: 13 July 2009 / Published online: 28 July 2009

Abstract Cadmium is a neurotoxic heavy metal and is considered endocrine disruptor. In this work, we investigate the effects of cadmium on the 24 h changes of aspartate, glutamate, and glutamine content in the pituitary. Adult male Sprague–Dawley rats were treated with 25 or 50 mg/l of cadmium chloride ($CdCl_2$) in the drinking water for 30 days. Metal exposure with the lowest dose induced the disappearance of the nocturnal peak of anterior pituitary amino acid content, and the appearance of a peak of glutamine concentration during the resting phase of the photoperiod. After exposure to 50 mg/l of $CdCl_2$, the peaks of anterior pituitary amino acid content at 12:00 and 00:00 h disappeared, and two minimal values at these same hours and a peak at 08:00 h appeared. In the posterior pituitary, cadmium treatment with the lowest dose induced the appearance of a peak of aspartate and glutamate concentration at 12:00 h, and the disappearance of the peak of glutamine content at 16:00 h. After exposure to 50 mg/l of $CdCl_2$ aspartate and glutamate daily pattern presented two maximal values between 00:00 and 04:00 h, and the metal abolished glutamine daily pattern. These results suggest that cadmium disrupted aspartate, glutamate, and glutamine daily pattern in the pituitary.

Keywords Cadmium · Pituitary · Daily pattern · Aspartate · Glutamate · Glutamine

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Introduction

Cadmium is a toxic heavy metal obtained as a by-product of zinc refining and used industrially in plating of steel, pigments, plastics, alloys, in nickel–cadmium batteries, and in nuclear and electronic engineering. Primary exposure sources of this toxicant for the general population include food and tobacco smoking. Cadmium has a long biologic half-life (Fox 1983), and a low excretion rate (Jones and Cherian 1990).

After chronic low level exposure, cadmium may affect the integrity or permeability of the blood brain barrier and reaches the central nervous system (CNS) (Murphy 1997), causing neurobehavioral disturbances (Leret et al. 2003) and modifying synaptic neurotransmission (Minami et al. 2001), as well as biochemical changes in the CNS. Cadmium could exert these effects on transmitter synthesis and/or release by several mechanisms: blocking the influx of calcium through membrane channels into the nerve terminal following the action potential (Nelson 1986); altering AMPA receptor-mediated synaptic transmission (Wang et al. 2008); inducing oxidative stress (Nemmiche et al. 2007) and cytotoxicity (Gotti et al. 1987); modifying zinc metabolism (Palmiter 1994); and even inducing apoptosis and necrosis evidenced in rat cortical neurons (López et al. 2003).

Cadmium neurotoxicity is reported to be associated with alterations on aminergic and aminoacidergic neurotransmitters content. More specifically, at brain level, this heavy metal has been shown to modify serotonin content and turnover (Gupta et al. 1990; Lafuente et al. 2001a) and norepinephrine concentration (Flora and Tandon 1987; Lafuente et al. 2001a). Moreover, dopamine concentration and metabolism are also altered by cadmium exposure (Antonio et al. 1998). In adult rats treated with cadmium,

changes in amino acid neurotransmitter content has also been reported in different brain areas such as hypothalamus, median eminence, striatum and prefrontal cortex (Minami et al. 2001; Lafuente et al. 2001b, 2005a).

Pituitary secretion activity has been shown to be affected by metals (Ronis et al. 1998). In fact, this endocrine gland is particularly a sensitive target to cadmium toxicity (Lafuente et al. 2001a; Poliandri et al. 2006; Cano et al. 2007). These endocrine effects can be mediated by several alterations on neurotransmitters involved in the regulation of pituitary activity (Schantz and Widholm 2001). It is important to note that pituitary gland regulates several important physiological functions such as metabolism and reproduction (Sam and Frohman 2008), so changes on pituitary gland could cause disturbances at these levels.

On the other hand, organisms exhibit daily rhythms at physiological and behavioral level (Herzog 2007). This is the case of neuroendocrine system (Kalsbeek et al. 2008). Glutamate, aspartate, and glutamine (Esquifino et al. 2002) content presents circadian rhythms, which may be modified by cadmium exposure, as this metal exerts a chronotoxicity in mammals (Lafuente et al. 2004; Cano et al. 2007; Caride et al. 2009). The disruption of neurotransmitter daily pattern by this xenobiotic has been evidenced by our laboratory in previous studies. Concretely, cadmium modifies the 24 h changes of biogenic amine content in several hypothalamic regions (Lafuente et al. 2003a), the daily pattern of hypothalamic–pituitary–testicular axis activity (Lafuente et al. 2004), and of prolactin release and its neuromodulators dopamine and serotonin in median eminence and pituitary gland (Lafuente et al. 2004, 2005b). Moreover, 24 h variations of gamma-amino butyric acid (GABA) and taurine content in the median eminence and anterior pituitary have been modified by cadmium administration in adult male rats (Caride et al. 2009).

Considering that (1) cadmium alters glutamine, glutamate and aspartate content in several brain regions and in median eminence; (2) these amino acids are involved in the regulation of pituitary hormone secretion; (3) these amino acid content and hormone secretion show a specific daily pattern; the present work has been designed to evaluate the possible effects of subchronic cadmium exposure on the 24 h variations of anterior and posterior pituitary aspartate, glutamate and glutamine content.

Materials and methods

Animals, experimental designs, and tissue preparation

Experiments were carried out in adult male Sprague–Dawley rats (320–350 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. Three groups of 60 animals were used. Group 1 received tap water from the public supply, containing 36 μg Cd^{2+}/l, and group 2 and 3 received for 30 days tap water from the public supply, in which 25 or 50 mg/l of cadmium chloride ($CdCl_2$) were added, respectively. The equivalence is 1.49 and 2.98 mg $CdCl_2$/kg bw per day, for the doses of 25 and 50 mg/l of $CdCl_2$, respectively, as water consumption was 20 ml/day. The doses of cadmium administered to the animals in this work were chosen taken into account the provisional tolerable weekly intake (PTWI) in humans (World Health Organization 2000), the maximum permitted levels of cadmium in foodstuffs (European Community Regulation 2001/466/EC of 8/3/2001) and the exposition to this heavy metal of general population in Spain (Cuadrado et al. 1995). Moreover, we have also considered the minimum lethal dose of cadmium in rats when it is administered orally (Repetto and López-Artíguez 1995). Furthermore, many works from the literature about neuroendocrine toxicity of cadmium were reported using the doses of 25 or 50 mg/l of $CdCl_2$ (Cory-Slechta and Weiss 1981; Lafuente et al. 2003a, 2005a).

At the end of the treatment, groups of ten animals were killed by decapitation at six different time intervals around the clock, beginning at 08:00 h. Samples were taken every 4 h. Care was taken to avoid any major stress to the animals before killing, and the decapitation procedure was completed within 5–7 s. The anterior and posterior pituitary were immediately removed and homogenized in cold 2 M acetic acid (1–4°C), processed as previously described (Lafuente et al. 2007) and kept frozen at −80°C until aspartate, glutamate, and glutamine determinations. In each sample, protein content was determined by the Bradford method (Bradford 1976).

The studies have been conducted according to European and Spanish legislation (Guideline of the Council of the European Communities 86/609/EEC of 24/11/1986 and Real Ordinance 1201/2005 of 10/10/2005), and they have been approved by the Ethics Committee of the University.

Amino acids measurement

Aspartate, glutamate, and glutamine were separated and analyzed using High Performance Liquid Chromatography (HPLC), with fluorescence detection after precolumn derivatization with *O*-phtalaldehyde (OPA), as previously described (Lafuente et al. 2005a, 2007). An aliquot of the tissue supernatant containing homoserine as internal standard was neutralized 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. After 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 column (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 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 consistent of a solvent delivery system coupled to a filter fluorometer (excitation 340 nm, emission 455 nm). Amino acid contents in each tissue were calculated from the chromatographic peak areas by using standard curves and the internal standard.

Statistical analysis

Aspartate, glutamate, and glutamine concentration was expressed as ng/µg protein. For statistical analysis of results, the following tests were applied: (a) one-way analysis of variance (ANOVA) followed by post hoc Tukey–Kramer′s multiple comparisons test, to study 24 h changes of aspartate, glutamate, and glutamine content in the anterior and posterior pituitary, in both control and treated groups, and to evaluate cadmium effects on amino acid mean levels around the clock; (b) two-way ANOVA in order to analyze the possible interaction between cadmium treatment and time of the day on aspartate, glutamate, and glutamine content. Statistical treatment of the obtained results has been made using SPSS software, version 15.0 for windows (SPSS Inc., Chicago, ILL.). The level for statistical significance was $P \leq 0.05$ 0.05 for each analysis. All values represent the mean ± SEM.

Results

Cadmium effect on the daily pattern of aspartate content in the anterior pituitary is shown in Fig. 1. There were specific 24 h variations in aspartate content in both control ($F = 91.03$, $P \leq 0.001$) and treated animals ($F = 8.91$, $P \leq 0.01$; $F = 39.96$, $P \leq 0.001$, for the doses of 25 and 50 mg/l of $CdCl_2$, respectively). Cadmium, at the lowest dose used, increased the amplitude of the 12:00 h peak and induced the disappearance of the nocturnal peak (at 00:00 h), which is found in untreated animals. After exposure to 50 mg/l of $CdCl_2$, the daily pattern of aspartate content was characterized by the existence of a peak of content at 08:00 h and minimal values at 12:00 and 00:00 h. Cadmium treatment with 25 mg/l of $CdCl_2$ increased aspartate concentration at 12:00 ($P \leq 0.01$) and 16:00 h ($P \leq 0.001$) and decreased it at 00:00 h ($P \leq 0.01$), as compared to control group. Exposure to 50 mg/l of $CdCl_2$ induced an increase of aspartate content at 08:00 ($P \leq 0.01$) and 16:00 h ($P \leq 0.01$), and decreased it at 12:00 ($P \leq 0.001$) and 00:00 h ($P \leq 0.001$) as compared to values found in control group. However, the mean levels of this amino acid, obtained as a mean of the values of each time-point during the day, were not modified by cadmium treatment. A factorial ANOVA indicated an interaction between cadmium treatment and time of the day in which aspartate concentration was measured in this pituitary lobe ($F = 27.76$, $P \leq 0.001$; $F = 63.87$, $P \leq 0.001$, for the lowest and the highest dose, respectively).

Changes in the daily pattern of glutamate content in the anterior pituitary induced by oral cadmium administration are shown in Fig. 2. There were specific time of the day variations in the amino acid content in both control ($F = 58.95$, $P \leq 0.001$) and metal-exposed rats ($F = 4.66$, $P \leq 0.05$; $F = 47.21$, $P \leq 0.001$, for the doses of 25 and 50 mg/l of $CdCl_2$, respectively). Cadmium treatment with 25 mg/l of $CdCl_2$ increased the amplitude of the 12:00 h peak and induced the disappearance of the peak occurred at 00:00 h in control animals. With the highest administered dose, the metal disrupted the two peaks observed in the control group, showing a peak at 08:00 h and two minimal values at 12:00 and 00:00 h. The mean values of glutamate around the clock were not altered by cadmium exposure at any administered dose, although animals treated with 25 mg/l of $CdCl_2$, showed an increase of glutamate levels at 12:00 ($P \leq 0.01$) and 16:00 h ($P \leq 0.05$) and a decrease at 00:00 h ($P \leq 0.05$) compared to the values found in the control group. Besides, the dose of 50 mg/l of $CdCl_2$

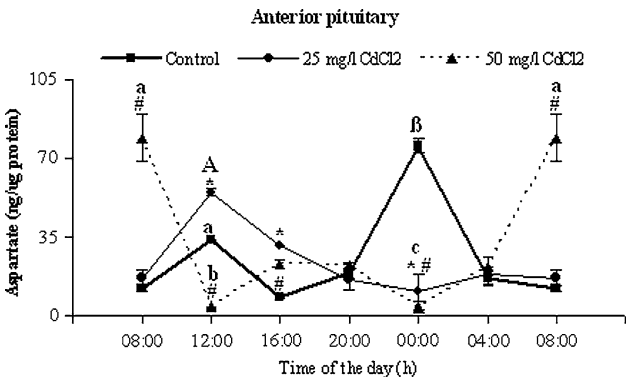

Fig. 1 Daily changes of aspartate content in the anterior pituitary of adult male rats treated with cadmium free water or cadmium chloride at the doses of 25 or 50 mg/l of $CdCl_2$ in the drinking water for 30 days. Shown are mean ± SEM. *Letters* indicated the existence of significant differences between time points within each experimental group after a Tukey–Kramer's multiple comparisons tests, as follows: $^{\alpha}P \leq 0.01$ versus 08:00 and 16:00 h, $P \leq 0.05$ versus 04:00 and 20:00 h; $^{\beta}P \leq 0.001$ versus all time points; $^{A}P \leq 0.01$ versus 04:00, 08:00, 20:00 and 00:00 h; $^{a}P \leq 0.001$ versus all time points; $^{b}P \leq 0.05$ versus 04:00, 16:00 and 20:00 h; $^{c}P \leq 0.05$ versus 04:00, 16:00 and 20:00 h; $^{*}P \leq 0.05$ and $^{\#}P \leq 0.05$. For further statistical analysis, see text

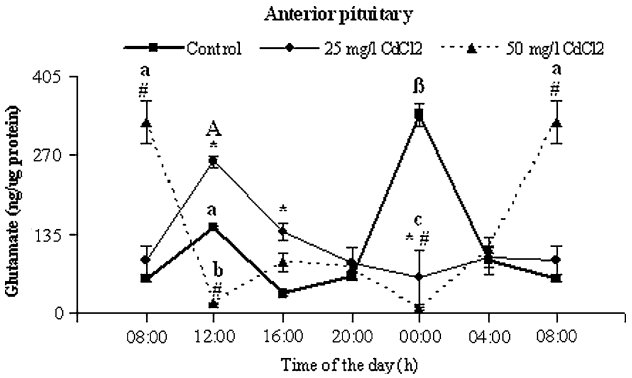

Fig. 2 Daily changes of glutamate content in the anterior pituitary of adult male rats treated with cadmium free water or cadmium chloride at the doses of 25 or 50 mg/l of $CdCl_2$ in the drinking water for 30 days. Shown are mean ± SEM. *Letters* indicated the existence of significant differences between time points within each experimental group after a Tukey–Kramer's multiple comparisons tests, as follows: $^{\alpha}P \leq 0.01$ versus 16:00 h, $P \leq 0.05$ versus 08:00 and 20:00 h; $^{\beta}P \leq 0.001$ versus all time points; $^{A}P \leq 0.05$ versus 04:00, 08:00, 20:00 and 00:00 h; $^{a}P \leq 0.001$ versus all time points; $^{b}P \leq 0.01$ versus 04:00 h, $P \leq 0.05$ versus 16:00 h; $^{c}P \leq 0.01$ versus 04:00 and 16:00 h, $P \leq 0.05$ versus 20:00 h; $^{*}P \leq 0.05$ and $^{\#}P \leq 0.05$. For further statistical analysis, see text

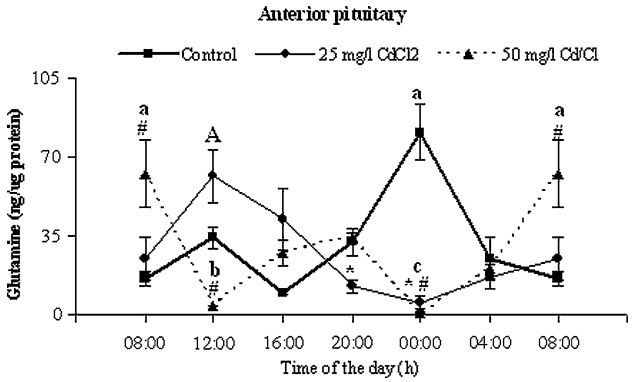

Fig. 3 Daily changes of glutamine content in the anterior pituitary of adult male rats treated with cadmium free water or cadmium chloride at the doses of 25 or 50 mg/l of $CdCl_2$ in the drinking water for 30 days. Shown are mean ± SEM. *Letters* indicated the existence of significant differences between time points within each experimental group after a Tukey–Kramer's multiple comparisons tests, as follows: $^{\alpha}P \leq 0.001$ versus 08:00 and 16:00 h, $P \leq 0.01$ versus 04:00 and 20:00 h, $P \leq 0.05$ versus 12:00 h; $^{A}P \leq 0.01$ versus 00:00 h, $P \leq 0.05$ versus 04:00 and 20:00 h; $^{a}P \leq 0.001$ versus 04:00, 12:00 and 00:00 h, $P \leq 0.01$ versus 16:00 h, $P \leq 0.05$ versus 20:00 h; $^{b}P \leq 0.05$ versus 20:00 h; $^{c}P \leq 0.01$ versus 20:00 h, $P \leq 0.05$ versus 16:00 h; $^{*}P \leq 0.05$ and $^{\#}P \leq 0.05$. For further statistical analysis, see text

increased glutamate content al 08:00 h ($P \leq 0.01$) and decreased it at 12:00 ($P \leq 0.001$) and 00:00 h ($P \leq 0.001$). There was an interaction between cadmium treatment and time of the day on glutamate concentration in this gland region ($F = 14.59$, $P \leq 0.001$; $F = 68.87$, $P \leq 0.001$, for the doses of 25 and 50 mg/l of $CdCl_2$, respectively).

In control and cadmium exposed animals, 24 h changes of glutamine content in the anterior pituitary were detected by one-way analysis of variance (Fig. 3; $F = 9.69$, $P \leq 0.001$; $F = 6.42$, $P \leq 0.01$; $F = 13.57$, $P \leq 0.001$, for control and treated with 25 and 50 mg/l of $CdCl_2$, respectively). In control animals, the pattern of glutamine concentration was characterized by the existence of a peak at 00:00 h. Treatment with 25 mg/l of $CdCl_2$ advanced that peak to 12:00 h, while after administration of the highest dose, we found a peak of glutamine content at 08:00 h and minimal values at 12:00 and 00:00 h. Moreover, cadmium treatment decreased glutamine content at 00:00 h ($P \leq 0.01$ and $P \leq 0.001$, for the dose of 25 and 50 mg/l of $CdCl_2$, respectively), at 20:00 h ($P \leq 0.05$ for the dose of 25 mg/l of $CdCl_2$) and at 12:00 h ($P \leq 0.001$ for the dose of 50 mg/l of $CdCl_2$). The highest dose used also increased glutamine concentration at 08:00 h ($P \leq 0.05$) as compared to the values found in control animals. However, the mean levels of this neurotransmitter did not change by cadmium exposure with any cadmium dose administered. On the other hand, we have found an interaction between cadmium treatment and time of the day on glutamine concentration in the anterior pituitary ($F = 7.69$, $P \leq 0.010$; $F = 18.51$, $P \leq 0.001$, for exposed to 25 or 50 mg/l of $CdCl_2$ animals, respectively).

Significant 24 h changes of aspartate content in the posterior pituitary occurred in both control and cadmium-treated groups (Fig. 4; $F = 6,85$, $P \leq 0.01$; $F = 22.92$, $P \leq 0.001$; $F = 11.75$, $P \leq 0.001$, for control and exposed to 25 or 50 mg/l of $CdCl_2$ animals, respectively). The pattern of aspartate concentration in control animals was characterized by the existence of a single peak at 20:00 h. After administration of 25 mg/l of $CdCl_2$, the amplitude of this small peak was increased and it was advanced to 12:00 h, while the treatment with 50 mg/l of $CdCl_2$ delayed this peak, and maximal values between 00:00 and 04:00 h were observed. Cadmium did not modify mean content of aspartate around the clock. However, increased levels were found at 00:00 and 04:00 h with both doses of cadmium ($P \leq 0.001$ for 25 mg/l of $CdCl_2$ and $P \leq 0.05$ for 50 mg/l of $CdCl_2$), and at 12:00 h ($P \leq 0.001$) for the lowest dose. In addition, a decrease of aspartate concentration was observed at 12:00 ($P \leq 0.05$) and 20:00 h ($P \leq 0.01$) after exposure to the highest dose. On the other hand, there was an interaction between cadmium treatment and time of the day on aspartate concentration in this gland region ($F = 20.06$, $P \leq 0.001$; $F = 9.44$, $P \leq 0.001$, for the lowest and the highest dose, respectively).

In the posterior pituitary, glutamate levels changed as a function of time in control and cadmium-treated groups (Fig. 5; $F = 51.51$, $P \leq 0.001$; $F = 129.22$, $P \leq 0.001$; $F = 11.91$, $P \leq 0.001$, for control and treated with 25 or 50 mg/l of $CdCl_2$ animals, respectively). In control

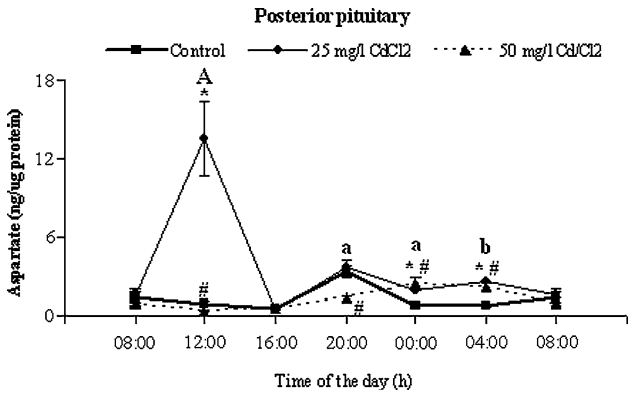

Fig. 4 Daily changes of aspartate content in the posterior pituitary of adult male rats treated with cadmium free water or cadmium chloride at the doses of 25 or 50 mg/l of $CdCl_2$ in the drinking water for 30 days. Shown are mean ± SEM. *Letters* indicated the existence of significant differences between time points within each experimental group after a Tukey–Kramer's multiple comparisons tests, as follows: $^{\alpha}P \leq 0.01$ versus 00:00 h, $P \leq 0.05$ versus 04:00, 08:00, 12:00 and 16:00 h; $^{\beta}P \leq 0.01$ versus 16:00 h, $P \leq 0.05$ versus 00:00 h; $^{A}P \leq 0.001$ versus all time points; $^{a}P \leq 0.001$ versus 08:00, 12:00 and 16:00 h; $^{b}P \leq 0.001$ versus 08:00, 12:00 and 16:00 h; $^{*}P \leq 0.05$ and $^{\#}P \leq 0.05$. For further statistical analysis, see text

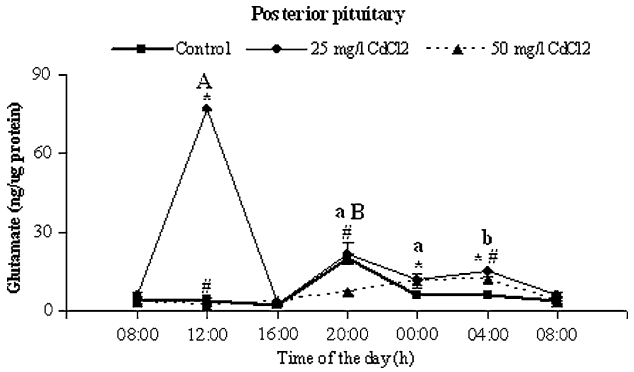

Fig. 5 Daily changes of glutamate content in the posterior pituitary of adult male rats treated with cadmium free water or cadmium chloride at the doses of 25 or 50 mg/l of $CdCl_2$ in the drinking water for 30 days. Shown are mean ± SEM. *Letters* indicated the existence of significant differences between time points within each experimental group after a Tukey–Kramer's multiple comparisons tests, as follows: $^{\alpha}P \leq 0.001$ versus all time points; $^{A}P \leq 0.001$ versus all time points; $^{B}P \leq 0.01$ versus 08:00 and 16:00 h; $^{a}P \leq 0.001$ versus 12:00 and 16:00 h, $P \leq 0.01$ versus 08:00 h; $^{b}P \leq 0.001$ versus 12:00 and 16:00 h, $P \leq 0.01$ versus 08:00 h; $^{*}P \leq 0.05$ and $^{\#}P \leq 0.05$. For further statistical analysis, see text

animals, the pattern of glutamate content was characterized by the existence of a peak of content at 20:00 h in this tissue. Cadmium, at the dose of 25 mg/l of $CdCl_2$, induced the appearance of another peak at 12:00 h. Besides, with the dose of 50 mg/l of $CdCl_2$ this peak was delayed and its amplitude was decreased. Glutamate medium levels around the clock were higher in the

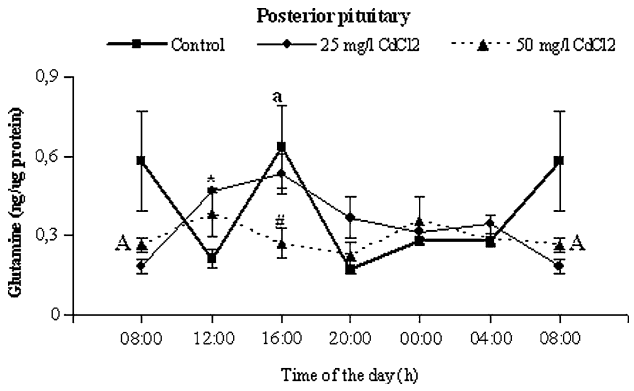

Fig. 6 Daily changes of glutamine content in the posterior pituitary of adult male rats treated with cadmium free water or cadmium chloride at the doses of 25 or 50 mg/l of $CdCl_2$ in the drinking water for 30 days. Shown are mean ± SEM. *Letters* indicated the existence of significant differences between time points within each experimental group after a Tukey–Kramer's multiple comparisons tests, as follows: $^{\alpha}P \leq 0.01$ versus 20:00 h, $P \leq 0.05$ versus 12:00 and 00:00 h; $^{A}P \leq 0.01$ versus 16:00 h, $P \leq 0.05$ versus 12:00 h; $^{*}P \leq 0.05$ and $^{\#}P \leq 0.05$. For further statistical analysis, see text

25 mg/l of $CdCl_2$-treated group ($F = 5.03$, $P \leq 0.05$), as increased glutamate levels at 12:00, 00:00 and 04:00 h ($P \leq 0.001$) were evidenced, while the dose of 50 mg/l of $CdCl_2$ decreased this amino acid concentration at 12:00 ($P \leq 0.05$) and 20:00 h ($P \leq 0.001$), and increased it at 04:00 h ($P \leq 0.05$) as compared to the values found in control animals. There was an interaction between cadmium treatment and time of the day on glutamate content in the posterior pituitary ($F = 109.75$, $P \leq 0.001$; $F = 15.96$, $P \leq 0.001$, for the lowest and the highest dose, respectively).

The effects of oral cadmium exposure on the 24 h variations of glutamine concentration in the posterior pituitary are shown in Fig. 6. Significant 24 h changes of the amino acid content occurred in both control and treated animals with 25 mg/l of $CdCl_2$ ($F = 6.45$, $P \leq 0.01$; $F = 5.52$, $P \leq 0.05$, for control and treated with 25 mg/l of $CdCl_2$, respectively). Cadmium, with the lowest dose administered, disrupted glutamine daily pattern, as induced the disappearance of the 16:00 h peak and the appearance of a minimal value at 08:00 h compared to control group. With the highest dose used in this work, cadmium abolished the 24 h variations of glutamine content. The metal did not modify glutamine medium levels around the clock, but it increased the amino acid content at 12:00 h ($P \leq 0.05$) with the dose of 25 mg/l of $CdCl_2$ and decreased it at 16:00 h ($P \leq 0.05$) after exposure to the highest dose employed. There was an interaction between cadmium treatment and time of the day on glutamine levels in the posterior pituitary ($F = 5.66$, $P \leq 0.01$; $F = 6.81$, $P \leq 0.001$, for exposed to 25 or 50 mg/l of $CdCl_2$ animals, respectively).

Discussion

The obtained results show a disruption of the daily pattern of pituitary aspartate, glutamate and glutamine content in cadmium treated rats. Besides, in our knowledge, this is the first study showing the daily pattern of aspartate, glutamate, and glutamine concentration in both anterior and posterior pituitary.

The existence of 24 h changes of aminergic and aminoacidergic neurotransmitter systems in rodent brain is well known (Duvilanski et al. 1998; Castañeda et al. 2004). In anterior pituitary, 24 h variations of dopamine, norepinephrine, serotonin, GABA and taurine were evidenced in young and older Wistar rats (Cano et al. 2001; Esquifino et al. 2004). Our group has showed GABA and taurine daily pattern in the anterior lobe of the gland of Sprague–Dawley rats (Caride et al. 2009), and those of dopamine and serotonin in both anterior and posterior pituitary in rats of the same strain (Lafuente et al. 2005b).

Cadmium chronotoxicity was previously reported by our laboratory analyzing dopamine, norepinephrine and serotonin content in different tissues, such as hypothalamus and median eminence (Lafuente et al. 2004, 2005b), and GABA and taurine concentration pattern in the median eminence (Caride et al. 2009). In pituitary gland, dopamine, serotonin, GABA and taurine daily pattern is disrupted by cadmium exposure (Lafuente et al. 2005b; Caride et al. 2009), and in the present work, these effects have been observed in aspartate, glutamate, and glutamine daily changes.

Cadmium effects on neurotransmitter daily pattern in the pituitary could be due to a direct effect of the metal in this gland, where it accumulates (Lafuente et al. 2003b). In the mechanism by which cadmium affects this gland may be implicated oxidative stress, evidenced by Poliandri et al. (2006) in the anterior pituitary, as well as apoptosis, demonstrated in the anterior lobe of the gland through both in vivo and in vitro studies (Yang et al. 2005). In addition, it is well known that cadmium modifies calcium homeostasis and even it interacts with proteins that are normally regulated by calcium (McNulty and Taylor 1999). This fact could be related to the alterations on the 24 h changes of aspartate, glutamate, and glutamine content, as intracellular calcium in the suprachiasmatic nucleus regulates mammalian circadian clock (Ikeda 2004). In addition, cadmium increased phospholipid content in the pituitary gland of adult male rats (Calderoni et al. 2005), that could be related to changes in membrane fluidity and altered physiological responses including metabolic regulation, cell proliferation, mitogenesis, secretion, etc. (Liscovitch et al. 2000). We also may take account that cadmium exposure alters the expression of adenohypophysial clock genes *Per 1* and *Per 2* (Cano et al. 2007).

The altered glutamate secretion here described could be associated to the disrupted hypothalamic glutamate daily pattern evidenced by our laboratory (Fernández-Pérez 2008) as neurosecretory glutamatergic neurons located within the paraventricular, anterior periventricular, supraoptic nuclei and the preoptic area of the hypothalamus secrete glutamate into the fenestrated vessels of the median eminence and the pituitary (Hrabovszky et al. 2007). In the mechanisms of cadmium toxicity could be also involved this amino acid excitotoxicity, as the metal treatment with the lowest dose administered increased glutamate medium levels. This neurotransmitter has been shown to induce neuronal cell death in cultured anterior pituitary cells by activating metabotropic glutamate receptors (mGluRs) (Caruso et al. 2004).

The modifications on amino acid neurotransmitters daily pattern showed in the present work could be related to the altered pituitary hormone secretion induced by this heavy metal. In this sense, cadmium disruption on prolactin and gonoadotropins secretion was evidenced by our group (Lafuente et al. 2005b) and could be linked to the altered pituitary 24 h changes of amino acid content induced by this toxicant exposure evidenced in the present work, as pituitary aspartate, glutamate, and glutamine regulate pituitary hormone secretion (Furuchi and Homma 2005).

In conclusion, these findings suggest that cadmium exposure affects the 24 h changes of aspartate, glutamate, and glutamine content in both anterior and posterior pituitary. The effects of the metal are different in each lobe of this endocrine gland and dependent on the administered dose of the xenobiotic. These alterations confirm cadmium chronotoxicity and could be related to several modifications on pituitary hormone release.

Acknowledgments This work was supported by a grant from the Xunta de Galicia (PGIDT99PXI38301B).

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