J Physiol Biochem (2010) 66:213–220
DOI 10.1007/s13105-010-0027-5

ORIGINAL PAPER

Cadmium chronotoxicity at pituitary level: effects on plasma ACTH, GH, and TSH daily pattern

Ana Caride · Belén Fernández-Pérez · Teresa Cabaleiro · Marta Tarasco · Ana Isabel Esquifino · Anunciacion Lafuente

Received: 14 December 2009 / Accepted: 3 June 2010 / Published online: 22 July 2010
© University of Navarra 2010

Abstract Cadmium is an endocrine disruptor that has been shown to induce chronotoxic effects. The present study was designed to evaluate the possible cadmium effects on the daily secretory pattern of adrenocorticotropin hormone (ACTH), growth hormone (GH), and thyroid-stimulating hormone (TSH) in adult male Sprague-Dawley rats. For this purpose, animals were treated with cadmium at two different doses [25 and 50 mg/l cadmium chloride ($CdCl_2$)] in the drinking water for 30 days. Control age-matched rats received cadmium-free water. After the treatment, rats were killed at six different time intervals throughout a 24-h cycle. Cadmium exposure modified the 24-h pattern of plasma ACTH and GH levels, as the peak of ACTH content between 12:00 and 16:00 h in controls appeared at 12:00 h in the group treated with the lowest dose used, while it appeared between 16:00 and 20:00 h in rats exposed to 50 mg/l $CdCl_2$. In addition, the peak of GH content found at 04:00 h in controls moved to 16:00 h in rats exposed to 25 mg/l $CdCl_2$, and the highest dose used abolished 24-h changes of GH secretion. The metal treatment did not modify ACTH secretory pattern. Exposure to cadmium also increased ACTH and TSH medium levels around the clock with both doses used. These results suggest that cadmium modifies ACTH and TSH medium levels around the clock, as well as disrupted ACTH and GH secretory pattern, thus confirming the metal chronotoxicity at pituitary level.

Keywords Cadmium · ACTH · GH · TSH · Daily pattern

A. Caride (✉) · B. Fernández-Pérez · T. Cabaleiro · M. Tarasco · A. Lafuente
Laboratorio de Toxicología, Facultad de Ciencias, Universidad de Vigo, Campus de Orense, Las Lagunas, 32004 Orense, Spain
e-mail: anacaride@uvigo.es

A. I. Esquifino
Departamento de Bioquímica y Biología Molecular III, Facultad de Medicina, Universidad Complutense, 28040 Madrid, Spain

Introduction

Cadmium is a nonessential element that has no known beneficial biological function which exposure has been linked to toxicity in both humans and animals. The sources of exposure to this heavy metal include metal industries, production of certain batteries, intake of contaminated food or water, inhalation of tobacco smoke or polluted air [32].

Cadmium is believed to be a non-specific toxicant that reacts with a wide spectrum of cellular components and can easily enter into the cell through the L-type voltage calcium channels and receptor-mediated calcium channels [28]. It accumulates due to its binding to cytoplasm and nuclear substances [1]. Acute cadmium exposure induces alterations in lung, liver, testes, and brain, while chronic exposure to the metal leads to renal dysfunction as well as blood and

bone diseases [19, 27]. It also is reported to induce neurotoxic effects, as it may affect the integrity or permeability of the blood–brain barrier and reach the central nervous system [43]. In this sense, it has been shown that cadmium accumulates in different brain regions involved in the control of pituitary hormone release, such as the hypothalamus and the median eminence [35]. In these same regions, this xenobiotic can modify the concentration of different neurotransmitters which regulate the pituitary secretory activity such as biogenic amines and amino acids in rats [7, 35]. These neuromodulators are also modified by cadmium exposure within pituitary gland [8, 24].

On the other hand, cadmium is considered an endocrine disruptor [22] as it affects pituitary hormone secretion. Alterations of plasma prolactin, gonadotropins, adrenocorticotropin hormone (ACTH), growth hormone (GH), and thyroid-stimulating hormone (TSH) secretion have been reported after cadmium exposure in rats [3, 21, 22]. In addition, it is known that episodic ACTH release can be modified by cadmium in male rats [20]. The mechanisms by which cadmium alters the endocrine function may include inactivation of protein containing cysteine residues with –SH groups [28]; interaction with some essential elements leading to homeostasis disorders [28]; calcium displacement from its normal binding to calmodulin and protein kinase-C [28]; perturbation of signal transductions, such as protein kinase-C, mitogen-activated protein kinase, and cyclic adenosine monophosphate pathways [40]; apoptosis, evidenced in anterior pituitary cells [36]; oxidative stress [37]; and modification of the lipid content of pituitary gland [3], decreasing pituitary membrane fluidity [33].

Circadian rhythms are recurring patterns in behavioral, endocrine, and other physiological parameters that exhibit periodicities of approximately 24 h [26]. The concentration of biogenic amines and amino acid neurotransmitters that regulate pituitary function shows a daily pattern in hypothalamus [5, 7], median eminence [42], and in both anterior and posterior pituitary [8, 12]. ACTH, GH, and TSH follow a 24 h pattern of secretion, as other pituitary hormones [23, 41]. Regarding cadmium chronotoxicity, several alterations on the daily pattern of dopamine, serotonin, norepinephrine, gamma-amino butyric acid, taurine, and glutamate concentration within hypothalamus and pituitary after cadmium administration have been evidenced by our laboratory [7, 8, 23, 24].

In view that cadmium modified plasma ACTH, GH, and TSH levels [3, 22], as well as the daily pattern of neurotransmitters that are modulators of these hormones' release [7, 23, 24], the present work was undertaken to examine changes in ACTH, GH, and TSH secretory pattern induced by subchronic cadmium exposure in adult male rats.

Materials and methods

Animals, experimental designs, and tissue preparation

Experiments were carried out in adult male Sprague-Dawley rats (330–350 g), kept under controlled conditions of light (light between 07:00 and 21:00 h daily) and temperature (22±2°C). The photoperiod chosen in this study (14:10LD) is the used in the experimental model developed by our laboratory to evaluate cadmium chronotoxicity at neuroendocrine level, and it allows us to compare the results obtained in this work with previous studies. On the other hand, it is not very different to the neutral photoperiod of 12:12LD. 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 were added 25 or 50 mg/l of cadmium chloride ($CdCl_2$), respectively. The equivalence is 1.5 and 3 mg $CdCl_2$/kg body weight (bw) per day for the doses of 25 and 50 mg/l $CdCl_2$, respectively, taking account that water consumption was 20 ml/day. Cadmium doses were chosen taking into account the provisional tolerable weekly intake in humans being 7 μg/kg bw [47], the maximum permitted levels of cadmium in foodstuffs [13] and the exposition to this heavy metal of general population in Spain [11]. Moreover, we have also considered the minimum lethal dose of cadmium in rats when it is administered orally [39]. Furthermore, many works from the literature about neuroendocrine toxicity of cadmium were reported using the doses of 25 or 50 mg/l $CdCl_2$ [9, 22, 24].

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 sacrifice, and the decapitation procedure was completed within 5–7 s. Trunk blood was collected in tubes containing

EDTA (60 g/l), and plasma was obtained after centrifugation of the samples at 1,500×*g* for 15 min at 4°C. Samples were kept frozen at −20°C until hormone measurements.

The studies have been conducted according to European and Spanish legislation [10, 38].

Hormone measurement

Plasma ACTH, GH, and TSH levels were determined by specific double antibody radioimmunoassay, previously described in our laboratory [22], using material kindly supplied by the National Hormone and Pituitary Program (Rockville, MD) and by Dr. A. Parlow (Harbor UCLA Medical Center). Hormone values were expressed in terms of National Institute of Arthritis, Metabolism, and Digestive Diseases, Rat Pituitary Hormone Distribution Program (NIADD) rat-ACTH RP-3 reference, NIADD rat-GH RP-3 reference, and NIADD rat-TSH RP-3 reference preparations. The lowest level of sensitivity was 0.34 ng/ml for ACTH, and was 0.04 ng/ml for GH and 0.20 ng/ml for TSH. Samples were analyzed within the same assay to avoid inter-assay variations. The intra-assay coefficient of variation was 8.5%, 7.4%, and 8.4% for ACTH, GH, and TSH, respectively.

Statistical analysis

Hormone levels were expressed as nanograms per milliliter. 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 plasma hormone levels, in both control and treated groups, and to evaluate cadmium effects on hormone 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 plasma hormone concentration. Statistical treatment of the obtained results has been made using SPSS software, version 15.0 for windows (SPSS Inc., Chicago, IL). The level for statistical significance was $p \leq 0.05$ for each analysis. All values represent the mean±SEM.

Results

Significant 24-h changes of plasma ACTH levels occurred in both control and cadmium-treated groups, as shown by means of one-way ANOVA (Fig. 1). Adult control male rats showed a 24-h secretory pattern with ACTH maximal values between 12:00 and 16:00 h and a nocturnal peak at 04:00 h. The peak of ACTH observed between 12:00 and 16:00 h in the control group appeared at 12:00 h in animals exposed to the lowest dose used, while in the group treated with 50 mg/l $CdCl_2$ maximal values occurred at 16:00 h. Moreover, the treatment with both administered doses abolished the nocturnal peak shown in adult male rat not exposed to the metal. On the other hand, cadmium treatment increased plasma ACTH levels around the clock, obtained as a mean of the values of each time-point during the day. More concretely, with the dose of 25 mg/l $CdCl_2$ plasma ACTH levels were increased at 12:00 h and decreased at 04:00 h; with the highest dose employed, circulating plasma levels increased at 16:00, 20:00, and 00:00 h, and decreased at 12:00 h. A factorial ANOVA indicated an interaction between cadmium and time of the day on plasma ACTH levels for both administered doses.

The 24-h pattern of plasma GH levels observed in control and cadmium-treated animals is shown in

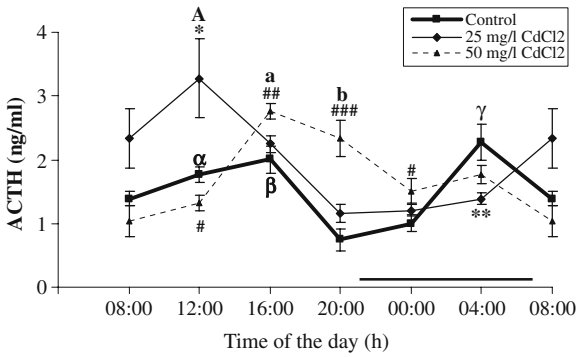

Fig. 1 Daily changes in plasma ACTH levels of adult male rats treated with cadmium-free water or cadmium chloride at the doses of 25 or 50 mg/l $CdCl_2$ in the drinking water for 30 days. Shown are means±SEM. *Letters* indicate the existence of significant differences between time points within each experimental group after a Tukey–Kramer's multiple comparisons tests. *Greek letters* refer to control group; *capital letter* and *asterisks* refer to the group treated with 25 mg/l of $CdCl_2$; and *lower case letter* and *hash sign* refer to the group treated with 50 mg/l of $CdCl_2$, respectively. α, $p \leq 0.01$ vs. 20:00 h and $p \leq 0.05$ vs. 00:00 h; β $p \leq 0.001$ vs. 20:00 h and $p \leq 0.01$ vs. 00:00 h; γ $p \leq 0.001$ vs. 20:00 and 00:00 h and $p \leq 0.01$ vs. 08:00 h; *A* $p \leq 0.001$ vs. 00:00 h and $p \leq 0.01$ vs. 04:00 and 20:00 h; *a* $p \leq 0.001$ vs. 04:00, 08:00, 12:00, and 00:00 h; *b* $p \leq 0.001$ vs. 08:00 h, $p \leq 0.01$ vs. 12:00 h and $p \leq 0.05$ vs. 00:00 h; $^{**}p \leq 0.01$ and $^{*}p \leq 0.05$; $^{###}p \leq 0.001$, $^{##}p \leq 0.01$, and $^{#}p \leq 0.05$

Fig. 2. In control rats, the secretory pattern of this hormone presents a peak at 04:00 h, but in rats treated with 25 mg/l $CdCl_2$ that peak appeared at 16:00 h, while with the dose of 50 mg/l $CdCl_2$ the metal abolished GH secretory pattern. The xenobiotic did not modify mean values of GH around the clock, but it induced a decrease of GH concentration at 12:00 h and 04:00 h, and an increase at 16:00 h with the dose of 25 mg/l $CdCl_2$. There was an interaction between cadmium treatment with 25 mg/l $CdCl_2$ and time of the day on plasma GH levels.

The effects of oral cadmium administration on the 24-h secretory pattern of TSH is shown in Fig. 3. In control animals and those exposed to 25 mg/l $CdCl_2$, TSH exhibit a 24-h secretory pattern showing two peaks at 20:00 and 04:00 h. Exposure to the highest dose used in the present study induced the disappearance of the 20:00 h peak, although the 04:00 h peak persisted in these rats. Daily medium levels of the hormone were increased after cadmium treatment. More concretely, circulating TSH levels increased at 12:00 and 16:00 h after the treatment with 25 mg/l $CdCl_2$ and at 08:00 h with the highest dose used, as compared with the values found in the animals not exposed to the metal. There was no interaction between cadmium treatment and time of the day on plasma TSH levels.

As is shown in Table 1, cadmium concentration significantly increased in metal-exposed animals as compared with control rats in blood as well as in the pituitary gland.

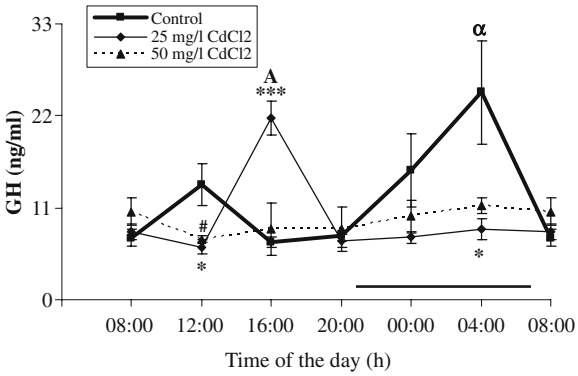

Fig. 2 Daily changes in plasma GH levels of adult male rats treated with cadmium-free water or cadmium chloride at the doses of 25 or 50 mg/l $CdCl_2$ in the drinking water for 30 days. Shown are means±SEM. *Letters* indicated the existence of significant differences between time points within each experimental group after a Tukey–Kramer's multiple comparisons tests. *Greek letters* refer to control group; *capital letter* and *asterisks* refer to the group treated with 25 mg/l of $CdCl_2$; and lower case letter and hash sign refer to the group treated with 50 mg/l of $CdCl_2$, respectively. α $p \leq 0.01$ vs. 16:00 h and $p \leq 0.05$ vs. 08:00 and 20:00 h; *A* $p \leq 0.001$ vs. all time points; ***$p \leq 0.001$ and *$p \leq 0.05$; $^{\#}p \leq 0.05$

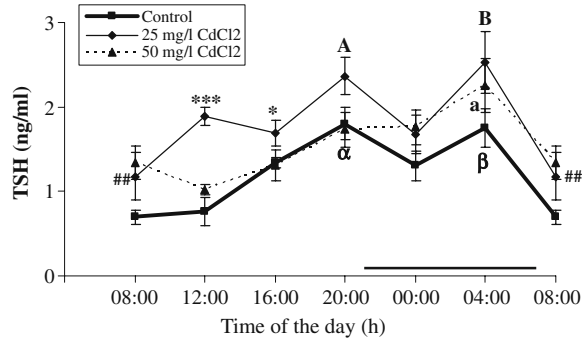

Fig. 3 Daily changes in plasma TSH levels of adult male rats treated with cadmium-free water or cadmium chloride at the doses of 25 or 50 mg/l $CdCl_2$ in the drinking water for 30 days. Shown are means±SEM. *Letters* indicated the existence of significant differences between time points within each experimental group after a Tukey–Kramer's multiple comparisons tests. *Greek letters* refer to control group; *capital letter* and *asterisks* refer to treated group with 25 mg/l of $CdCl_2$; and *lower case letter* and *hash sign* refer to the group treated with 50 mg/l of $CdCl_2$, respectively. α $p \leq 0.001$ vs. 08:00 and 12:00 h; β $p \leq 0.001$ vs. 08:00 and 12:00 h; *A* $p \leq 0.01$ vs. 08:00 h; *B* $p \leq 0.01$ vs. 08:00 h; *a* $p \leq 0.001$ vs. 12:00 h and $p \leq 0.05$ vs. 08:00 and 16:00 h; ***$p \leq 0.001$ and *$p \leq 0.05$; $^{\#\#}p \leq 0.01$

Discussion

Foregoing results are the first report showing the effects of cadmium exposure on the daily pattern of pituitary hormones ACTH, GH, and TSH in rodents. Several alterations on the secretory pattern of ACTH

Table 1 Blood and pituitary cadmium concentration of adult male rats treated with cadmium-free water or cadmium chloride at the doses of 25 or 50 mg/l in the drinking water for 30 days

	Control	25 mg/l $CdCl_2$	50 mg/l $CdCl_2$
Blood (μg/l)	1.11±0.14	5.00±0.54*	5.18±0.74*
Pituitary gland (μg/g)	3.95±0.36	17.60±0.98*	22.83±3.54**

Shown are means±SEM. **$p \leq 0.01$ and *$p \leq 0.05$

and GH have been evidenced in subchronical exposed adult male rats, while daily pattern of plasma TSH levels was not modified after this treatment.

In adult male rats not exposed to cadmium, ACTH, GH, and TSH are secreted according to specific daily patterns. These patterns were previously described in rats by other authors [41]. In the present study, 24-h changes of ACTH levels are characterized by the existence of a peak during the light phase of the photoperiod and another one during the darkness, while plasma GH and TSH concentration peaked during the dark phase of the photoperiod. These results differ from those previously reported by the authors mentioned above, possibly due to differences in the strain of rats used (Sprague-Dawley in the present work vs. Wistar in the other studies), the photoperiod employed, and the season in which the experiments were performed.

In control animals, the daily pattern of ACTH and GH release resembles the 24-h changes of median eminence norepinephrine content previously reported by our group [23]. This fact could be explained by the stimulatory activity of this catecholamine on the secretion of both ACTH, via corticotropin-releasing hormone neurons [16] and GH by increasing growth-hormone-releasing hormone (GHRH) levels via alpha (2)-adrenergic receptors [29].

Cadmium exposure is associated with a disruption of the neuroendocrine system function in experimental animals such as rats [23, 24, 44] and in human [30]. In this sense, we have previously reported several modifications on plasma ACTH, GH, and TSH concentration in rats exposed to 25 or 50 mg/l $CdCl_2$ for 30 days [21, 22] and on episodic ACTH secretion in adult male rats after acute cadmium administration [20], while the present work raises data about cadmium effects on the 24-h profile of these same hormones.

Cadmium effects on ACTH are dose-dependent [22]. More concretely, 25 and 50 mg/l $CdCl_2$ administered during 30 days increased plasma ACTH levels. This stimulatory effect has been observed by studying the daily pattern of the hormone, as the mean concentration of ACTH around the clock augmented. However, a single dose of the chemical is able to stimulate the episodic ACTH release [20], and circulating corticosterone levels were decreased in rats after oral [17] administration of the xenobiotic.

Higher mean values of ACTH in cadmium-treated animals could be due to a decrease of mean concentration of dopamine around the clock in the median eminence as well as to an increase of serotonin levels in anterior and posterior pituitary induced by the metal at a dose of 25 mg/l $CdCl_2$ [24] because ACTH release is inhibited by dopamine [45] and stimulated by serotonin in these tissues [46]. On the other hand, ACTH synthesis and secretion are regulated by a negative feedback mechanism as glucocorticoids inhibit them at pituitary level [18]. Therefore, the increased plasma ACTH levels found in the present study could be a compensatory mechanism in view of cadmium inhibits ACTH-stimulated steroidogenesis and steroid secretion from adrenocortical cells [31].

GH medium levels around the clock were not modified by cadmium exposure. However, in a previous study of our group, we evidenced decreased plasma GH concentration in adult rats at 12:00 h after exposure to 25 and 50 mg/l $CdCl_2$ [22]. These data are in agreement with those described here; decreased hormone levels were also found at the same time.

Cadmium-induced thyroid dysfunction was previously reported, showing increased thyroid weight, cadmium accumulation at this level, and elevated serum TSH concentration in rats [14, 22] and humans [15]. In cadmium-treated rats, although the daily pattern of TSH was not modified, the mean TSH levels around the clock were increased. This augment could be partially explained taking into account that this heavy metal exerted an inhibitory effect on thyroxine (T4) synthesis and/or release and a depression on 5'-monodeiodinase enzyme activity resulting in decreased serum triiodothyronine (T3) levels [14], but it does not modify TSH response to TRH [32]; so increased mean values of plasma TSH in cadmium-treated groups could be a compensatory mechanism in view of the descent of T3 and T4 concentration.

The data obtained of TSH concentration along the day agrees with that found in a previous work of our laboratory, where plasma TSH levels was measured only at 12:00 h [22]. At this time, in that work, we observed that TSH levels increased after the treatment with 25 mg/l $CdCl_2$, while the dose of 50 mg/l $CdCl_2$ did not change the hormone concentration, as it happens in the present work in the same time-point.

The daily pattern of both ACTH and GH was changed by cadmium exposure at the dose of 25 mg/l $CdCl_2$, and in animals exposed to the highest dose used, the metal altered 24-h changes of ACTH content and abolished GH secretory

pattern. The described modifications on ACTH daily pattern could be related to the altered adrenal function induced by cadmium exposure evidenced by diverse authors [25], and changes of the secretory pattern of GH could be linked to alterations on somatic growth, development, and body composition in which GH is involved [2]. The secretion of this last hormone is differentially altered by cadmium compared with ACTH and TSH, a fact that could be due to the possible damage induced by the metal in GHRH- and somatostatin-containing neurones, residing primarily in the hypothalamic arcuate nucleus and the periventricular nucleus, respectively, that regulates pulsatile GH secretion [29].

Cadmium effects reported in this study on GH and ACTH secretory pattern suggest a multiple interactive mechanism of the xenobiotic with the regulatory factors involved in ACTH and GH secretion at both pituitary [24] and hypothalamic level by modifying their neuromodulators [7] and the activity of the suprachiasmatic nucleus, endogenous clock located in the anterior hypothalamus. In fact, this toxic has been shown to change the expression of clock genes *Per 1* and *Per 2* in rat hypothalamus [6], accumulating at this level [35], and to modify the content of several neurotransmitters in this brain region [7]. Furthermore, it is important to point out that, in treated rats with 25 mg/l of $CdCl_2$, the circadian pattern of ACTH and GH release resembles the 24-h changes of median eminence norepinephrine content previously described by our group with this same cadmium dose [23], thus indicating that alterations of the secretory pattern of these hormones could be mediated by modifications on the daily pattern of norepinephrine concentration in the median eminence.

Apart from indirect actions of cadmium on pituitary gland, a direct action of the metal onto the gland could be implicated on its chronotoxicity. In this sense, apoptosis in anterior pituitary cells [36], as well as a decrease of pituitary membrane fluidity [34] and modifications of the membrane composition [3] induced by cadmium treatment have been evidenced by other authors, possibly being linked to alterations in pituitary receptor binding and in the secretory mechanisms of pituitary hormones [34]. In fact, the heavy metal has been shown to alter lactotroph activity though biochemical and morphological changes [4], thus suggesting that corticotrope, somatotroph, or thyrotrope activity may be also modified by cadmium through this same mechanisms.

In conclusion, the data obtained in the present work suggest that cadmium modifies ACTH and TSH medium levels around the clock, as well as disrupted ACTH and GH secretory pattern, thus confirming the metal chronotoxicity at pituitary level.

Acknowledgements This work was supported by grants from the Xunta de Galicia (PGIDT99PXI38301B).

References

1. Beyersmann D, Hechtenberg S (1997) Cadmium, gene regulation, and cellular signalling in mammalian cells. Toxicol Appl Pharmacol 144:247–261
2. Birzniece V, Sata A, Ho KK (2009) Growth hormone receptor modulators. Rev Endocr Metab Disord 10:145–56
3. Calderoni AM, Oliveros L, Jahn G, Anton R, Luco J, Giménez MS (2005) Alterations in the lipid content of pituitary gland and serum prolactin and growth hormone in cadmium treated rats. Biometals 18:213–220
4. Calderoni AM, Biaggio V, Acosta M, Oliveros L, Mohamed F, Giménez MS (2010) Cadmium exposure modifies lactotrophs activity associated to genomic and morphological changes in rat pituitary anterior lobe. Biometals 23:135–143
5. Cano P, Cardinali DP, Jimenez V, Chacon F, Cutrera RA, Esquifino AI (2003) Effect of aging on 24-hour changes in serotonin and dopamine turnover, and somatostatin and amino acid content, of the rat hypothalamus and pituitary gland. Biol Rhythm Res 34:279–294
6. Cano P, Poliandri AH, Jiménez V, Cardinali DP, Esquifino AI (2007) Cadmium-induced changes in *Per 1* and *Per 2* gene expression in rat hypothalamus and anterior pituitary: effect of melatonin. Toxicol Lett 172:131–136
7. Caride A, Fernández-Pérez B, Cabaleiro T, Bernárdez G, Lafuente A (2010) Cadmium chloride exposure modifies amino acid daily pattern in the mediobasal hypothalamus in adult male rat. J Appl Toxicol 30:84–90
8. Caride A, Fernández Pérez B, Cabaleiro T, Lafuente A (2010) Daily pattern of pituitary glutamine, glutamate, and aspartate content disrupted by cadmium exposure. Amino Acids 38:1165–1172
9. Cory-Slechta DA, Weiss B (1981) Aversiveness of cadmium in solution. Trends Neurosci 19:417–422
10. Council Directive (1986/609/EC) of November 24, 1986 on the approximation of laws, regulation and administrative provisions of the member states regarding the protection of animals used for experimental and other scientific purposes. The Commission of the European Communities. DOCE L 358, 18 December 1986; 1–28.
11. Cuadrado C, Kumpulainen J, Moreiras O (1995) Lead, cadmium and mercury contents in average Spanish market basket diets from Galicia, Valencia, Andalucía and Madrid. Food Addit Contam 12:107–118
12. Esquifino AI, Cano P, Jimenez V, Reyes Toso CF, Cardinali DP (2004) Changes of prolactin regulatory mechanisms in aging: 24-h rhythms of serum prolactin and median

eminence and adenohypophysial concentration of dopamine, serotonin, gamma-aminobutyric acid, taurine and somatostatin in young and aged rats. Exp Gerontol 39:45–52
13. European Community Regulation (2001/466/EC) of March 8, 2001 setting maximum levels for certain contaminants in foodstuffs. The Commission of the European Communities. DOCE L 77, 16 March 2001; 1–27
14. Hammouda F, Messaoudi I, El Hani J, Baati T, Saïd K, Kerkeni A (2008) Reversal of cadmium-induced thyroid dysfunction by selenium, zinc, or their combination in rat. Biol Trace Elem Res 126:194–203
15. Iijima K, Otake T, Yoshinaga J, Ikegami M, Suzuki E, Naruse H, Yamanaka T, Shibuya N, Yasumizu T, Kato N (2007) Cadmium, lead, and selenium in cord blood and thyroid hormone status of newborns. Biol Trace Elem Res 119:10–18
16. Itoi K, Seasholtz AF, Watson SJ (1998) Cellular and extracellular regulatory mechanisms of hypothalamic corticotropin-releasing hormone neurons. Endocr J 45:13–33
17. Jackl GA, Kollmer WE (1982) The influence of exposure to different levels of cadmium on the secretion and biological halflife of corticosterone in the rat. Sci Total Environ 25:53–59
18. Kageyama K, Suda T (2009) Regulatory mechanisms underlying corticotropin-releasing factor gene expression in the hypothalamus. Endocr J 56:335–344
19. Koçak M, Akçil E (2006) The effects of chronic cadmium toxicity on the hemostatic system. Pathophysiol Haemost Thromb 35:411–416
20. Lafuente A, Esquifino AI (1998) Modulation of episodic adrenocorticotropin hormone secretion by cadmium in male rats. Biometals 11:183–188
21. Lafuente A, González-Carracedo A, Márquez N, Pazo D, Esquifino AI (2002) Oral cadmium exposure throughout puberty does not inhibit secretion of prolactin, GH and ACTH through dopamine metabolism changes in male rat. J Trace Elem Med Biol 16:249–254
22. Lafuente A, Cano P, Esquifino A (2003) Are cadmium effects on plasma gonadotropins, prolactin, ACTH, GH and TSH levels, dose-dependent? Biometals 16:243–250
23. Lafuente A, Gonzalez-Carracedo A, Romero A, Esquifino AI (2004) Cadmium exposure differentially modifies the circadian patterns of norepinephrine at the median eminence and plasma LH, FSH and testosterone levels. Toxicol Lett 146:175–182
24. Lafuente A, González-Carracedo A, Romero A, Cabaleiro T, Esquifino AI (2005) Toxic effects of cadmium on the regulatory mechanism of dopamine and serotonin on prolactin secretion in adult male rats. Toxicol Lett 155:87–96
25. Lall SB, Dan G (1999) Role of corticosteroids in cadmium induced immunotoxicity. Drug Chem Toxicol 22:401–409
26. Levi F, Schibler U (2007) Circadian rhythms: mechanisms and therapeutic implications. Annu Rev Pharmacol Toxicol 47:593–628
27. Manca D, Ricard AC, Trottier B, Chevalier G (1991) Studies on lipid peroxidation in rat tissues following administration of low and moderate doses of cadmium chloride. Toxicology 67:303–323
28. Martelli A, Rousselet E, Dycke C, Bouron A, Moulis JM (2006) Cadmium toxicity in animal cells by interference with essential metals. Biochimie 88:1807–1814
29. McMahon CD, Radcliff RP, Lookingland KJ, Tucker HA (2001) Neuroregulation of growth hormone secretion in domestic animals. Domest Anim Endocrinol 20:65–87
30. Meeker JD, Rossano MG, Protas B, Diamond MP, Puscheck E, Daly D, Paneth N, Wirth JJ (2009) Multiple metals predict prolactin and thyrotropin (TSH) levels in men. Environ Res 109:869–873
31. Mgbonyebi OP, Smothers CT, Mrotek JJ (1998) Modulation of adrenal cell functions by cadmium salts. 5. Cadmium acetate and sulfate effects on basal and ACTH-stimulated steroidogenesis. Cell Biol Toxicol 14:301–311
32. Nawrot T, Plusquin M, Hogervorst J, Roels HA, Celis H, Thijs L, Vangronsveld J, Van Hecke E, Staessen JA (2006) Environmental exposure to cadmium and risk of cancer: a prospective population-based study. Lancet Oncol 7:119–126
33. Pavia Júnior MA, Paier B, Noli MI, Hagmüller K, Zaninovich AA (1997) Evidence suggesting that cadmium induces a non-thyroidal illness syndrome in the rat. J Endocrinol 154:113–117
34. Pillai A, Laxmi Priya PN, Gupta S (2002) Effects of combined exposure to lead and cadmium on pituitary membrane of female rats. Arch Toxicol 76:671–675
35. Pillai A, Priya L, Gupta S (2003) Effects of combined exposure to lead and cadmium on the hypothalamic–pituitary axis function in proestrous rats. Food Chem Toxicol 41:379–384
36. Poliandri AH, Cabilla JP, Velardez MO, Bodo CC, Duvilanski BH (2003) Cadmium induces apoptosis in anterior pituitary cells that can be reversed by treatment with antioxidants. Toxicol Appl Pharmacol 190:17–24
37. Poliandri AH, Machiavelli LI, Quinteros AF, Cabilla JP, Duvilanski BH (2006) Nitric oxide protects the mitochondria of anterior pituitary cells and prevents cadmium-induced cell death by reducing oxidative stress. Free Radic Biol Med 40:679–688
38. Real Ordinance 1201/2005 of October 10, 2005 sobre protección de los animales utilizados para experimentación y otros fines científicos. BOE 252, 21 October 2005; 34367–34391
39. Repetto M, López-Artíguez M (1995) Estado actual de la Toxicología del cadmio. In: Toxicología Avanzada. Díaz de Santos, Madrid, pp 393–418
40. Satoh M, Kaji T, Tohyama C (2003) Low dose exposure to cadmium and its health effects. Toxicity in laboratory animals and cultured cells. Nippon Eiseigaku Zasshi 57:615–623
41. Selgas L, Arce A, Esquifino AI, Cardinali DP (1997) Twenty-four-hour rhythms of serum ACTH, prolactin, growth hormone, and thyroid-stimulating hormone, and of median-eminence norepinephrine, dopamine, and serotonin, in rats injected with Freund's adjuvant. Chronobiol Int 14:253–265
42. Sellix MT, Egli M, Poletini MO, McKee DT, Bosworth MD, Fitch CA, Freeman ME (2006) Anatomical and functional characterization of clock gene expression in neuroendocrine dopaminergic neurons. Am J Physiol Regul Integr Comp Physiol 290:R1309–1323
43. Shukla A, Shukla GS, Srimal RC (1996) Cadmium-induced alterations in blood-brain barrier permeability and its possible correlation with decreased microvessel antioxidant potential in rat. Hum Exp Toxicol 15:400–405
44. Takiguchi M, Yoshihara S (2006) New aspects of cadmium as endocrine disruptor. Environ Sci 13:107–116

45. Tong W, Li FY, Chen SQ, Chen JL, Ding T, Kuang AK, Xu MY (1992) A study on the regulation of ACTH secretion in rat pituitary cells. Sheng Li Xue Bao 44:414–419
46. Van de Kar LD (1997) 5-HT receptors involved in the regulation of hormone secretion. In: Baumgarten HG, Göthert M (eds) Handbook of experimental pharmacology. Serotoninergic neurons and 5-HT receptors. Springer, Berlin, pp 537–562
47. World Health Organization (WHO) (2000) Evaluation of certain food additives and contaminants. In: 55th Report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva.