

# Modulatory Effects of Melatonin on Cadmium-Induced Changes in Biogenic Amines in Rat Hypothalamus

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**Abstract** This study was undertaken to examine whether cadmium oral exposure modifies biogenic amine concentration at hypothalamic level in adult male rats, and to investigate the possible modulatory effects of melatonin against cadmium-induced changes on these neurotransmitters. For this purpose, rats were exposed to cadmium (25 mg/l of CdCl<sub>2</sub> in the drinking water) with or without melatonin (30 µg/rat/day intraperitoneally) for 30 days. Norepinephrine (NE), dopamine (DA), serotonin (5-HT), 3,4-dihydroxyphenyl acetic acid (DOPAC), and 5-hydroxyindoleacetic acid (5-HIAA) were quantified by high performance liquid chromatography (HPLC). Oral cadmium administration led to decrease of NE, DA, and 5-HT content and DA turnover within the three hypothalamic regions examined, and therefore an inhibition of 5-HT turnover at posterior hypothalamus. Sensitivity to melatonin was specific to the hypothalamic region evaluated. Thus, the anterior hypothalamus was not nearly sensitive to exogenously administered melatonin, whereas the neurohormone decreased the content of these amines in the mediobasal hypothalamus, and melatonin increased it in the posterior hypothalamic region. Melatonin effectively prevented some cadmium-induced alterations on hypothalamic amine concentration. This is the case of DA in the

anterior and posterior hypothalamus, and 5-HT metabolism in the posterior hypothalamic region. In conclusion, the obtained results indicate that melatonin treatment may be effective modulating some neurotoxic effects induced by cadmium exposure, and, more to the point, a possible role of this indolamine as a preventive agent for environmental or occupational cadmium contamination.

**Keywords** Melatonin · Cadmium · Biogenic amines · Hypothalamus

## Introduction

Cadmium is one of the most important toxic chemicals due to its accumulation in the environment as a result of industrial and agricultural practices (Acuna-Castroviejo et al. 1995; Goering et al. 1995; Satarug et al. 2003). Cadmium in soil and water is taken up by plants and it is concentrated and transferred to upper links of the food chain, including humans (World Health Organization 1995; Satarug et al. 2003). Due to its long biological half-life (10–30 years), cadmium accumulation in the organism can increase the risk of toxicity (Sugita and Tsuchiya 1995).

The hypothalamus seems to be a target brain region for this heavy metal (Lafuente et al. 2001), it can penetrate blood–brain barrier accumulating at this level (Pillai et al. 2003), inducing alterations on aminoacidergic (Esquifino et al. 2001; Minami et al. 2001; Lafuente et al. 2005a, b; Caride et al. 2010), and aminergic (Pillai et al. 2003; Lafuente et al. 2005a) transmitter systems. Modifications of hypothalamic neurotransmitter concentration could have adverse consequences; the hypothalamus is involved in the control and regulation of body temperature, hunger and satiety, thirst, sexual activity, the affective conduct, the

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activity of the autonomic nervous system (Swaab 2003), as well as on endocrine regulation (Ugrumov 2009).

Among the mechanisms of cadmium-induced neurotoxicity is included oxidative stress, reported by diverse authors in different tissues, such as the kidney (Bagchi et al. 1997), liver (Liu et al. 2002), and brain (Kumar et al. 1996). Cadmium is not a redox-active metal; it does not produce free radicals directly by Fenton-like reactions, but it may increase reactive oxygen species levels through indirect ways, as depleting glutathione or affecting mitochondrial electron transfer chain (Poliandri et al. 2006a). Indeed, DNA repair mechanisms inhibition and antioxidant defenses have been reported (Kasprzak et al. 2001; Waalkes 2003). Cadmium has also been observed to induce lipid peroxidation at brain level (Mendez-Armenta et al. 2003), which depends on oxygen free radicals (Kumar et al. 1996).

Consequently, we have paid attention to antioxidant compounds, which should have protective effects against cadmium-induced toxic effects (Casalino et al. 2002; Eybl et al. 2006). Melatonin (*N*-acetyl-5-methoxytryptamine), the pineal gland's main secretory product has been shown to have remarkable antioxidant properties (Reiter et al. 2000), acting as scavenger for direct free radical and indirect antioxidant. In terms of its scavenging activity, melatonin has been shown to quench the hydroxyl radical, superoxide anion radical, singlet oxygen, peroxy radical, and the peroxynitrite anion. Additionally, melatonin's antioxidant actions probably derive from its stimulatory effect on superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase, and glucose-6-phosphate dehydrogenase and its inhibitory action on nitric oxide synthase (NOS). Finally, melatonin acts stabilizing cell membranes, thereby making them more resistant to oxidative attack, and it is able to form stable complexes with heavy metals (Reiter et al. 2000). Melatonin protective effects against metal-induced oxidative damage is well established and it was evidenced in different tissues (Ortega-Gutierrez et al. 2002; Parmar et al. 2002; Eybl et al. 2006), including brain (Millan-Plano et al. 2003). It is important to point out that melatonin has been shown to protect against ROS-mediated neuronal degeneration, evidenced on serotonergic and dopaminergic neurons (Hirata et al. 1998; Antolin et al. 2002; Lin et al. 2008). At hypothalamic level, melatonin effect on redox pathway is mainly exerted via down-regulation of gene expression of the pro-oxidant enzymes nitric oxide synthase 1 and 2 (NOS-1 and NOS-2), and heme-oxygenase 1 and 2 (HO-1 and HO-2) (Jimenez-Ortega et al. 2009).

This study's scope was to investigate the possible modulatory role of melatonin against cadmium-induced neurotoxic effects on biogenic amines concentration and metabolism in different hypothalamic areas.

## Materials and Methods

### Animals

Adult male Sprague-Dawley rats (Animal Production of Santiago University, Spain), weighing 300–320 g, were used at the beginning of the experiment. They were maintained under a controlled photo-period (14:10LD; light between 07:00 and 21:00 h daily) and temperature ( $22 \pm 2^\circ\text{C}$ ), and they were fed with rat's chow (Panlab, Barcelona, Spain) and water ad libitum.

The studies have been conducted according to the effective European and Spanish legislation (Council Directive of the European Communities 1986; Real Ordinance 2005).

### Experimental Procedure

Animals were treated during 30 days with cadmium and/or melatonin. The metal was added to tap water from the public supply at a dose of 25 mg/l of cadmium chloride ( $\text{CdCl}_2$ ), which is equivalent to 1.5 mg  $\text{CdCl}_2/\text{kg}(\text{bw})/\text{day}$ , and 0.77 mg  $\text{Cd}^{2+}/\text{kg}(\text{bw})/\text{day}$ , recording that rat water consumption is 20 ml/day. This dose of the metal is approximately 835 times higher than provisional tolerable weekly intake (PTWI) for this metal (World Health Organization 2000), it was employed in previous works of our investigation group (Lafuente et al. 2003, 2004, 2005a), and it is similar to the doses employed by other authors (Cory-Slechta and Weiss 1981; Sugawara et al. 1981). Melatonin was dissolved in a small volume of vehicle solution (10% absolute ethanol and diluted in 0.9% NaCl to a dose of 150 mg per 100 g body weight). Melatonin injections were administered intraperitoneally (i.p.) at a dose of 30  $\mu\text{g}/\text{rat}/\text{day}$  at 19:00 h (after 12 h light on). The administered melatonin dose was within the pharmacological range, and it was administered at this time to mimic the peak occurring at endogenous level in the first phase of the photoperiod's darkness. This type of administration was previously used by other authors (Esquifino et al. 1989; Villanua et al. 1989).

This study is a  $2 \times 2$  design, in which the following groups of 10 animals each were used: control group (0 mg cadmium plus 0  $\mu\text{g}$  melatonin); cadmium group (25 mg/l of  $\text{CdCl}_2$ ); melatonin group (30  $\mu\text{g}$  melatonin/day); and melatonin plus cadmium group (25 mg/l of  $\text{CdCl}_2$  plus 30  $\mu\text{g}$  melatonin/day).

At the end of the experimental period, the animals were killed by decapitation. Care was taken to avoid any major stress to the animals before killing them, and the decapitation procedure was completed within 5–7 s. Brains were immediately removed and the hypothalamus was quickly

dissected out, and sectioned in the frontal plain, the anterior, mediobasal, and posterior regions comprising one-third of the each block. Tissues were homogenized in cold (0–1°C) 2 M acetic acid. After centrifugation (at 15,000×g for 30 min, at 4°C), the supernatants were kept frozen at –80°C until biogenic amines measurement.

#### Amine Measurement

The hypothalamus from 10 animals of each group were used to measure norepinephrine (NE), dopamine (DA), serotonin (5-HT), 3,4-dihydroxyphenylacetic acid (DOPAC), and 5-hydroxyindol acetic acid (5-HIAA) contents. Intranural dopaminergic metabolism, expressed as the ratio DOPAC/DA, as well as 5-HT metabolism, measured as the quotient between 5-HIAA and 5-HT concentration, were studied at the anterior, mediobasal, and posterior hypothalamus. NE, DA, 5-HT, DOPAC, and 5-HIAA were determined by HPLC, using electrochemical detection (Coulochem, II, ESA; USA). A C-18 reverse phase column, eluted with a mobile phase (pH 4; 0.1 M sodium acetate, 0.1 M citric acid, 0.7 mM sodium octyl-sulphate, and 0.57 mM EDTA containing 10% methanol v/v) was employed. Flow rate was 1 ml/min, at a pressure of 2200 psi. Fixed potentials against H<sub>2</sub>/H<sup>+</sup> reference electrode were: conditioning electrode –0.4 V; preoxidation electrode +0.10 V; working electrode +0.35 V. Amine concentrations were calculated from the chromatographic peak areas by using external standards. The linearity of the detector response for NE, DA, 5-HT, DOPAC, and 5-HIAA was tested within the concentration ranges found in supernatants of mediobasal hypothalamus (Lafuente et al. 2003). Results were expressed as pg/μg protein. DA and 5-HT turnover, evaluated from DOPAC to

DA and 5-HIAA to 5-HT ratios were studied at the anterior, mediobasal, and posterior hypothalamus.

#### Protein Determination

The protein concentration in homogenates of the three hypothalamic region was measured by using Bio-Rad protein assay kit (Bio-Rad laboratories Inc., Hercules, CA, USA), according to *Bradford* method (Bradford 1976).

#### Statistical Analysis

The results of the parameters measured in this study were tested for variance homogeneity through the Snedecor test. When the results did not have a homogeneous variance, they were compared through a Mann–Whitney test. If the variance was homogeneous, an analysis of variance (ANOVA) and post-hoc Tukey–Kramer's multiple comparisons test was applied. To study if there is an interaction between cadmium and melatonin, two-way ANOVA was applied. Statistical analysis of results was performed by SPSS software, version 15.0 for windows (SPSS Inc., Chicago, IL). The results were considered significant at  $P \leq 0.05$ . All values represent the mean  $\pm$  S.E.M.

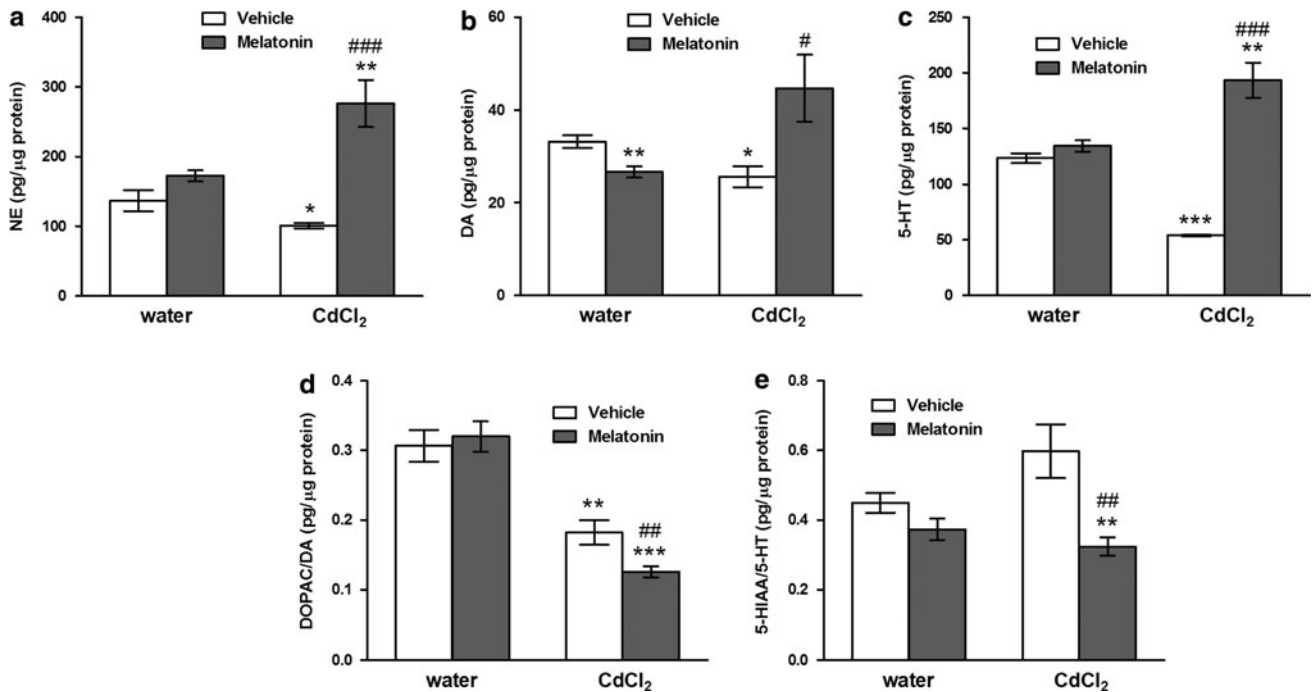
#### Results

Globally, oral cadmium administration decreased biogenic amine concentration and both DA and 5-HT turnover in each analyzed hypothalamic area (anterior, mediobasal, and posterior hypothalamus), while melatonin effects are different depending on the amine and the studied hypothalamic region, and these changes are modified by cadmium treatment (Table 1; Figs. 1, 2, 3).

**Table 1** Overview of the effects of melatonin and/or cadmium on the three studied hypothalamic regions

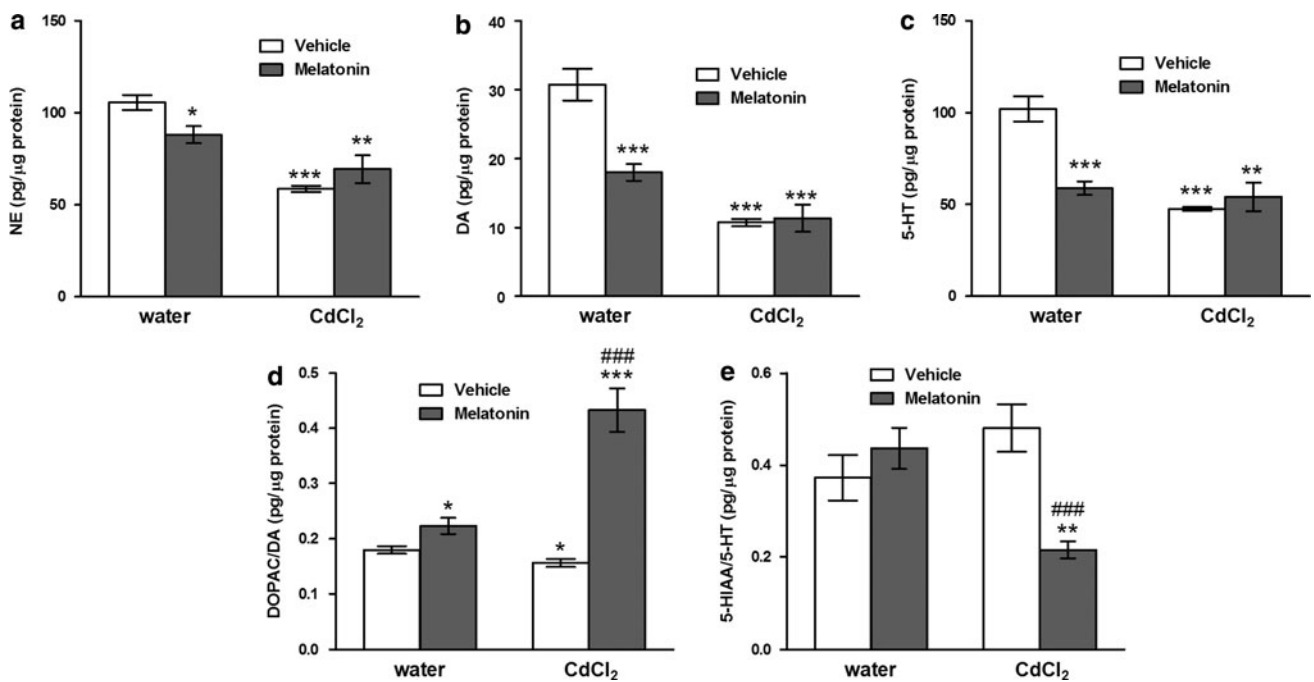
Hypothalamic region	Treatment	Norepinephrine concentration	Dopamine concentration	Dopamine metabolism	Serotonin concentration	Serotonin metabolism
Anterior	Cadmium	↓	↓	↓	↓	–
	Melatonin	–	↓	–	–	–
	Cadmium + melatonin	↑	–	↓	↑	↓
Mediobasal	Cadmium	↓	↓	↓	↓	–
	Melatonin	↓	↓	↑	↓	–
	Cadmium + melatonin	↓	↓	↑	↓	↓
Posterior	Cadmium	↓	↓	↓	↓	↓
	Melatonin	↑	↑	↓	↑	–
	Cadmium + melatonin	↑	–	↓	↑	–

The global results obtained in this study: biogenic amine concentration or metabolism increased (↑), decreased (↓) or not modified (–) in the each hypothalamic region analyzed



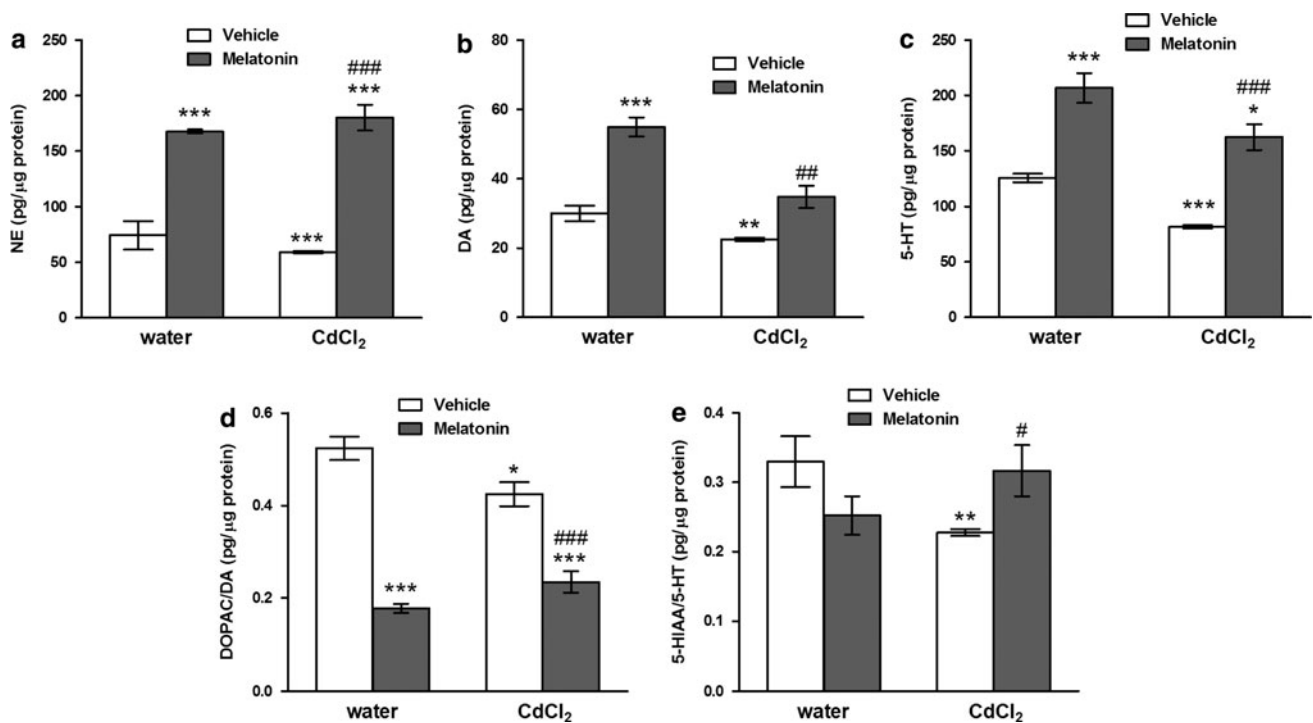
**Fig. 1** Effects of cadmium and/or melatonin on NE, DA, and 5-HT concentration, as well as on DOPAC/DA and 5-HIAA/5-HT ratios in the anterior hypothalamus. Groups of 10 rats received CdCl<sub>2</sub> (25 mg/l in drinking water) and/or melatonin (30 μg/rat/day i.p.); controls were

given vehicle solution. Results are expressed as pg/μg protein. The values are expressed as mean ± S.E.M. (*n* = 10 animals per group). \**P* ≤ 0.05, \*\**P* ≤ 0.01, and \*\*\**P* ≤ 0.001 vs. control group; #*P* ≤ 0.05, ##*P* ≤ 0.01, and ###*P* ≤ 0.001 vs. cadmium-treated rats



**Fig. 2** Effects of cadmium and/or melatonin on NE, DA, and 5-HT concentration, as well as on DOPAC/DA and 5-HIAA/5-HT ratios in the mediobasal hypothalamus. Groups of 10 rats received CdCl<sub>2</sub> (25 mg/l in drinking water) and/or melatonin (30 μg/rat/day i.p.);

controls were given vehicle solution. Results are expressed as pg/μg protein. The values are expressed as mean ± S.E.M. (*n* = 10 animals per group). \**P* ≤ 0.05, \*\**P* ≤ 0.01, and \*\*\**P* ≤ 0.001 vs. control group; ###*P* ≤ 0.001 vs. cadmium-treated rats



**Fig. 3** Effects of cadmium and/or melatonin on NE, DA, and 5-HT concentration, as well as on DOPAC/DA and 5-HIAA/5-HT ratios in the posterior hypothalamus. Groups of 10 rats received CdCl<sub>2</sub> (25 mg/l in drinking water) and/or melatonin (30 μg/rat/day i.p.); controls

were given vehicle solution. Results are expressed as pg/μg protein. The values are expressed as mean ± S.E.M. ( $n = 10$  animals per group). \* $P \leq 0.05$ , \*\* $P \leq 0.01$ , and \*\*\* $P \leq 0.001$  vs. control group; # $P \leq 0.05$ , ## $P \leq 0.01$ , and ### $P \leq 0.001$  vs. cadmium-treated rats

#### Effects of Melatonin on Cadmium-Induced Changes in Biogenic Amines Concentration in Anterior Hypothalamus

The concentration of NE was decreased by cadmium exposure in the anterior hypothalamus (Fig. 1a;  $P \leq 0.05$  vs. control group), whereas melatonin administration had no effect on the concentration of this neurotransmitter. A significant interaction between cadmium and melatonin occurred ( $F = 10.75$ ;  $P \leq 0.01$ ), i.e., treatment with cadmium plus melatonin increased NE concentration in anterior hypothalamus with respect to the values observed in the control group ( $P \leq 0.01$ ), as well as in cadmium-treated rats ( $P \leq 0.001$ ).

The effects of cadmium and/or melatonin on DA content within the anterior hypothalamus are shown in Fig. 1b. Animals exposed to the metal or melatonin showed a significant decrease of DA content with respect to the values found in the control group ( $P \leq 0.05$  and  $P \leq 0.01$ , respectively). On the other hand, a factorial ANOVA indicated an interaction between cadmium and melatonin ( $F = 5.22$ ,  $P \leq 0.05$ ). As is shown in Fig. 1b, DA content in animals treated with melatonin and cadmium together was not modified with respect to control group, but was increased with respect to the values observed in cadmium-exposed animals ( $P \leq 0.05$ ).

Figure 1c shows the effect of cadmium and/or melatonin on 5-HT content. Cadmium treatment decreased the concentration of this indolamine (Fig. 1c;  $P \leq 0.001$  vs. control group), whereas melatonin administration had no effect on the content of 5-HT. A significant interaction between cadmium and melatonin occurred in anterior hypothalamus ( $F = 39.41$ ,  $P \leq 0.001$ ). In this sense, we have observed that animals treated with the heavy metal and melatonin showed an increase of 5-HT content as compared to the values found in rats just exposed to cadmium ( $P \leq 0.001$ ) and in control rats ( $P \leq 0.01$ ).

The effects of cadmium and/or melatonin on DA turnover are summarized in Fig. 1d. Exposure to cadmium-induced a decrease of intraneural DA metabolism ( $P \leq 0.01$  vs. control group), and melatonin administration had no effect on this parameter. In addition, administration of both substances also decreased DA metabolism with respect to control animals ( $P \leq 0.001$ ) and cadmium-exposed rats ( $P \leq 0.01$ ).

The effects of cadmium and/or melatonin on 5-HT turnover were presented in Fig. 1e. Treatment with the metal or melatonin alone did not modified the indolamine metabolism, while exposure to cadmium and melatonin together decreased 5-HT metabolism as compared to the values found in control group ( $P \leq 0.01$ ) and in cadmium-treated animals ( $P \leq 0.01$ ).

### Effects of Melatonin on Cadmium-Induced Changes in Biogenic Amines Concentration in Mediobasal Hypothalamus

Figure 2a depicts cadmium and/or melatonin effects on NE content in mediobasal hypothalamus. The concentration of this biogenic amine was decreased by cadmium exposure ( $P \leq 0.001$ ), as well as by melatonin treatment ( $P \leq 0.05$ ). A significant interaction between cadmium and melatonin occurred ( $F = 6.14$ ,  $P \leq 0.05$ ), as animals treated with cadmium and melatonin together showed lower NE concentration than control rats ( $P \leq 0.01$ ).

The effects of cadmium and/or melatonin on DA content within the mediobasal hypothalamic region were shown in Fig. 2b. Animals exposed to the metal alone and melatonin alone showed lower DA content in comparison to the values found in the control group ( $P \leq 0.001$  for both cases). An interaction between cadmium and melatonin occurred ( $F = 11.01$ ,  $P \leq 0.01$ ), as administration of the metal and the neurohormone induced a decrease of DA concentration ( $P \leq 0.001$ ).

In Fig. 2c we showed the effect of cadmium and/or melatonin on 5-HT content in the mediobasal hypothalamus. Administration of cadmium or melatonin alone decreased the concentration of this indolamine ( $P \leq 0.001$  vs. control group for both cases). A significant interaction between cadmium and melatonin has been observed ( $F = 13.40$ ,  $P \leq 0.01$ ). In this sense, we have found that animals treated with the heavy metal and melatonin together showed lower 5-HT content in this hypothalamic region as compared to the values found in control rats ( $P \leq 0.01$ ).

The effects of cadmium and/or melatonin on DA turnover in mediobasal hypothalamus were summarized in Fig. 2e. The metabolism of this biogenic amine was inhibited by cadmium exposure ( $P \leq 0.05$  vs. control rats), but stimulated by melatonin treatment ( $P \leq 0.05$  vs. control rats). An interaction between cadmium and melatonin treatment appeared for DA turnover ( $F = 4.68$ ,  $P \leq 0.05$ ). When these two substances were administered together, DA turnover was increased with respect to control and cadmium-treated rats ( $P \leq 0.001$  for both cases).

Figure 2e shows the effects of cadmium and/or melatonin in 5-HT turnover in mediobasal hypothalamus. When the animals were treated with cadmium or melatonin alone, the 5-HT turnover remained unaltered. However, a significant interaction between cadmium and melatonin occurred in mediobasal hypothalamus ( $F = 7.09$ ,  $P \leq 0.01$ ). In this sense, animals treated with the metal and melatonin showed a decrease of 5-HT metabolism compared to the values found in cadmium-treated animals ( $P \leq 0.001$ ) and in control group ( $P \leq 0.01$ ).

### Effects of Melatonin on Cadmium-Induced Changes in Biogenic Amines Concentration in Posterior Hypothalamus

Figure 3a illustrates cadmium and/or melatonin effects on NE content in posterior hypothalamus. The concentration of this biogenic amine was decreased by cadmium exposure ( $P \leq 0.001$  vs. control group) but increased by melatonin treatment ( $P \leq 0.001$  vs. control group). “Cadmium plus melatonin” administration induced increased the neurotransmitter concentration in comparison with the values found in the control group ( $P \leq 0.001$ ), and in cadmium-exposed rats ( $P \leq 0.001$ ).

The effects of cadmium and/or melatonin on DA content in posterior hypothalamus were analyzed are shown in Fig. 3b. Animals exposed to the metal showed a significant decrease of DA content ( $P \leq 0.01$  vs. control group). However, melatonin increased this biogenic amine concentration ( $P \leq 0.001$  vs. control group). A factorial ANOVA indicated an interaction between cadmium and melatonin ( $F = 4.20$ ,  $P \leq 0.05$ ) as the inhibitory effect of cadmium on DA content observed in posterior hypothalamus was counteracted by the neurohormone. The concentration of this catecholamine was increased in animals treated with cadmium and melatonin as compared to the values found in animals just exposed to the metal ( $P \leq 0.01$ ).

The concentration of 5-HT was decreased by cadmium exposure (Fig. 3c;  $P \leq 0.001$  vs. control group), but increased by melatonin administration ( $P \leq 0.001$  vs. control group). A significant interaction between cadmium and melatonin has been observed ( $F = 0.33$ ,  $P \leq 0.05$ ). In this sense, we have found that animals treated with the heavy metal and melatonin showed an increase of 5-HT content in comparison to the values found in animals just exposed to cadmium ( $P \leq 0.001$ ), and in control rats ( $P \leq 0.05$ ).

DA turnover was inhibited by cadmium or melatonin administered alone (Fig. 3d;  $P \leq 0.05$  and  $P \leq 0.001$  vs. control group, respectively). An interaction between cadmium and melatonin treatment appeared for DA turnover ( $F = 19.95$ ,  $P \leq 0.01$ ). Treatment with cadmium and melatonin decreased the biogenic amine metabolism ( $P \leq 0.001$  vs. control and cadmium-exposed animals).

Figure 3e shows the effects of cadmium and/or melatonin in 5-HT turnover. The metabolism of this indolamine is inhibited in posterior hypothalamus by cadmium exposure ( $P \leq 0.01$  vs. control group). We found a significant interaction between cadmium and melatonin ( $F = 6.52$ ,  $P \leq 0.05$ ), as cadmium effects on 5-HT turnover were prevented by melatonin within posterior hypothalamus. In addition, the metal and melatonin administered together



increased 5-HT turnover with respect to cadmium-exposed animals ( $P \leq 0.05$ ).

Table 1 summarizes the effects of cadmium and melatonin in the whole hypothalamus. Globally, in anterior, mediobasal, and posterior hypothalamus cadmium inhibits dopamine and serotonin release, whereas melatonin inhibits both neurotransmitter systems in anterior and mediobasal hypothalamus and stimulated them in the posterior hypothalamic region. In addition, melatonin may abolish the cadmium-induced neurotoxic injury on dopaminergic neurons in the anterior and posterior hypothalamus and serotonergic system in the posterior hypothalamus.

## Discussion

The obtained results, showed in Table 1 and Figs. 1, 2, 3, suggest that some of cadmium effects on biogenic amine concentration and metabolism observed in different hypothalamic areas, might be reverted by melatonin administration in rats.

A cadmium inhibitory effect on the studied biogenic amines was observed in anterior, mediobasal, and posterior hypothalamus; the same effect was previously described by our laboratory in adult rats exposed to the same dose of the metal (Lafuente et al. 2003, 2004, 2005a; González-Carracedo 2005), and by other authors (Das et al. 1993; Pillai et al. 2003). Decreased DA concentration could be explained, at least partially, by an inhibition of the biogenic amine synthesis, or may be due to the metal-stimulated catecholamine oxidation, that results in quinone formation, closely linked to mitochondrial dysfunction, inflammation, and oxidative stress (Dawson et al. 2000). The observed DA depletion could be related to the inhibitory cadmium effect on NE, as DA is a metabolic precursor of NE (Von Bohlen und Halbach and Dermietzel 2002). Regarding to 5-HT, the metal seems to inhibit its synthesis in all the hypothalamic regions studied, and also its metabolism in the posterior hypothalamus, as is reflected by the reduction of the ratio 5-HIAA/5-HT.

According to the data obtained, the sensitivity to melatonin is specific to the analyzed hypothalamic region, it was pointed out by Miguez et al. (1996). In this sense, melatonin binding has been shown to be maximal in the mediobasal hypothalamus (Cardinali et al. 1979); at anterior hypothalamic level the neurohormone affects 5-HT concentration through specific receptors, while in extra-suprachiasmatic areas a non-receptor mediated action was suggested (Miguez et al. 1996). In general terms, we have found an inhibitory effect of melatonin on the biogenic amines synthesis and/or secretion within mediobasal hypothalamus, and a stimulatory activity in the posterior hypothalamus, while the anterior hypothalamus is not

nearly sensitive to exogenously administered neurohormone. The inhibitory effect of melatonin could be explained by the reduction of calcium entry into the pre-synaptic nerve endings induced by the neurohormone (Zisapel and Laudon 1983). Other authors found that melatonin had no effect on NE or 5-HT concentration in the whole hypothalamus (Yoshioka et al. 2000; Yu et al. 2001), but it inhibited the electrically stimulated DA release from this brain region (Zisapel et al. 1982).

One of the mechanisms involved in cadmium-induced neurotoxicity is oxidative stress (Kumar et al. 1996), and the brain is highly sensitive to reactive oxygen species (ROS)-induced damage because of its high rate of oxygen utilization and deficient antioxidant defense mechanisms (Calabrese et al. 2000). Numerous studies showed that cadmium inhibits the activity of antioxidant defense systems (Gupta and Shukla 1995; El-Missiry and Shalaby 2000); it might be due to binding of the metal to sulphhydryl groups of enzymes and oxidative modifications of amino acid chains (Pari and Murugavel 2007). Also, a disruption of the 24-h rhythmicity and overall expression of redox enzyme at mediobasal hypothalamus level was evidenced by Jimenez-Ortega et al. (2010). In addition, cadmium produces a breakdown of the mitochondrial membrane potential, which could mediate the induction of ROS (Lopez et al. 2006). In this sense, it is important to note that treatment with melatonin has been shown to reduce the brain mitochondrial impairment (Carretero et al. 2009).

A melatonin slight modulatory activity against cadmium neurotoxicity is evidenced in the present study, thus in the melatonin treated animals cadmium was not able to modify DA concentration in both anterior and posterior hypothalamus, neither 5-HT metabolism in the posterior hypothalamic region, alterations observed in rats not treated with the neurohormone. In addition, melatonin treatment partially reversed cadmium-induced alterations on NE concentration in mediobasal hypothalamus, as well as on 5-HT content in mediobasal and posterior hypothalamus. The mechanism by which melatonin prevent cadmium toxicity at hypothalamic level remain to be defined. On one hand, this metal forms metal complexes with melatonin, thus decreasing the fraction of free cadmium and contributing to metal detoxification (Limson et al. 1998). In fact, Chweliuk et al. (2006) evidenced that co-treatment of melatonin with cadmium decreases renal, hepatic, and intestinal cadmium concentrations. On the other hand, cadmium is able to activate responses mediated by estrogenic receptors (ER) alpha (Johnson et al. 2003), and melatonin presents antiestrogenic properties (Sanchez-Barcelo et al. 2005), thus the hormone could compensate the effects of the metalloestrogen at hypothalamic level, where estrogenic receptors are present (Micevych et al. 2010). In addition, it is well established that melatonin provides protection

against cadmium-mediated free radical damage due to its antioxidant properties (Eybl et al. 2006). In fact, in rat hypothalamic–pituitary axis, melatonin down-regulated the pro-oxidant NOS1, NOS2, HO-1, and HO-2 (Jimenez-Ortega et al. 2009). At hypothalamic level, the hormone prevented the metal-induced lipid peroxidation and the increased expression of HO-1, and NOS1 and 2 (Poliandri et al. 2006a), as well as reverted its effects on expression of Per 1 and Per 2 clock genes (Cano et al. 2007). In other tissues, melatonin also was evidenced to be effective in combating cadmium-induced oxidative stress. This fact was observed in rat liver (Eybl et al. 2006; El-Sokkary et al. 2010), testes (Kara et al. 2007), heart and lung (Karbownik et al. 2001). However, according to the results obtained in this study, melatonin does not seem to prevent in a great extent the cadmium-induced neurotoxic effects. This fact could be due to the too high metal dose. Melatonin protective effects against cadmium toxicity is evidenced by other authors, they were used doses lower than administered in this study, i.e., most authors used 5 mg CdCl<sub>2</sub>/l (Poliandri et al. 2006b; Cano et al. 2007), and other doses, such as 6.5 mg CdCl<sub>2</sub>/l were used (Kara et al. 2007). On the other hand, some of the melatonin and cadmium-induced changes on biogenic amine concentration may be due to the pharmacological properties of the neurohormone, instead of its protective effect against cadmium-induced toxicity. In addition, physiological interaction between the metal and melatonin is very complex, because the toxic effects of cadmium are still debated and little is known about the modulatory role of melatonin in terms of variations of specific neurotransmitters or the interactions between neurotransmitters in specific hypothalamic areas. It could be possible that the neurohormone and the metal change some of the effects induced by each other on neurotransmitter systems through changes at nervous, endocrine, and/or immune level, as well as on other neurotransmitters concentration, as the nervous, endocrine, and immune systems are connected by shared neurotransmitters, hormones, and cytokines (Mazzoccoli et al. 2010).

As was mentioned above, cadmium-induced oxidative stress may not be prevented completely by melatonin that could be related to other toxic action mechanisms of the metal at hypothalamic level different to the oxidative stress, so hypothalamus, compared with other brain regions, is poor in nonheme iron, which is catalytically involved in the production of free radicals (Hill and Switzer 1984).

Serotonin is thought to play a primary role in the suprachiasmatic nuclei function (Tominaga et al. 1992), that is a major component of the mammalian biological clock, and cadmium effects on serotonergic concentration in anterior hypothalamus may be implicated on its chronotoxicity, previously reported by our group (Lafuente

et al. 2005a). In fact, circadian rhythms are regulated by melatonin (Reiter 1991), so the neurohormone may be effective on preventing the metal chronotoxicity although it was not able to absolutely prevent cadmium alterations on the concentration of the biogenic amine at hypothalamic level. In this line, the neurohormone treatment is effectively on revert cadmium effects on expression of Per 1 and Per 2 clock genes as it was evidenced by Cano et al. (2007).

In summary, the findings of this study illustrate that the endogenous mechanisms defenses of the body are insufficient to counteract the toxic effects metal-induced hypothalamic damage, and hence exogenous antioxidant supplementation can afford therapeutic benefit against cadmium-induced hypothalamus neurotoxicity.

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**Conflict of interest** None.

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