Cadmium Effects on Hypothalamic-Pituitary-Testicular Axis in Male Rats

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This study analyzes cadmium effects at the hypothalamic-pituitary-testicular axis. Male rats were given cadmium during puberty or adulthood. Cadmium exposure through puberty increased norepinephrine content in all hypothalamic areas studied, but not in the median eminence. Metal exposure increased serotonin turnover in median eminence and the anterior hypothalamus, while decreased it in mediobasal hypothalamus. Also, decreased plasma levels of testosterone were found. Cadmium exposure during adulthood increased norepinephrine content in posterior hypothalamus and decreased the neurotransmitter content in anterior and mediobasal hypothalamus. Decreased circulating levels of luteinizing hormone (LH) and testosterone and increased plasma follicle stimulating hormone (FSH) levels were also observed. Cadmium accumulated in all analyzed tissues. Various parameters showed age-dependent changes. These data suggest that cadmium globally effects hypothalamic-pituitary-testicular axis function by acting at the three levels analyzed and that an interaction between cadmium exposure and age emerge.

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Key words: cadmium; LH; FSH; testosterone; norepinephrine; serotonin turnover

The gonad is considered the main target for environmental toxins (1). Cadmium, among them, is very dangerous to testicular function (2–9). Different studies have shown that cadmium affects plasma gonadotropin levels (10–15) and the content of several neurotransmitters in discrete areas of the brain that are not involved in the regulatory mechanisms of these hormones (15–23). Changes in dopamine, serotonin, and norepinephrine contents in half brains after metal exposure were described (17, 23). Also, modifications in serotonin, 5-hydroxyindolacetic acid, dopamine, and norepinephrine contents in the cortex, striatum, and hippocampus from rats exposed to the metal alone or in combination with lead were reported by Nation *et al.* (18). Changes in dopamine, DOPAC (3,4-dihydoxyphenil acetic acid), serotonin, 5-hydroxyindol acetic acid contents, and their metabolic rates in the frontal cortex, nucleus accumbens, olfactory bulb, and the striatum of rats exposed to this heavy metal were also shown (21). Only Shukla and Shinghal (23) and Das *et al.* (20) measured the contents of serotonin in the whole hypothalamus, because this area is involved in pituitary hormone release. These results do not explain the specific changes at the hypothalamic areas involved in the regulation of pituitary hormone secretion (24–26). There is no work that poses changes at the regulatory areas of the hypothalamus, with plasma levels of gonadotropins, prolactin, and testosterone.

The study of the hypothalamic-pituitary-gonadal axis in animals exposed to the metal is of great interest since the levels of cadmium in air, water, soil, and foods have increased several-fold in many parts of the world as a result of emissions from industrial activities. It is also of interest since the natural biogeochemical cycle of cadmium has been overwhelmed (27). The safety limit of cadmium intake for adult humans is of 51 to 71 μg/day in industrialized countries (28).

This work was undertaken to answer the following questions: (i) if the exposure to cadmium modifies the hypothalamic-pituitary-testicular axis function; (ii) if cadmium effects on the male reproductive axis are dependent on sexual development (prepubertal or postpubertal) during the exposure to the metal; and (iii) if these possible changes in the reproductive axis are related to cadmium accumulation within the axis. Serotonin turnover (as the index 5-HIAA, 5-hidroxyindol acetic acid/5-HT serotonin) and norepinephrine content in various hypothalamic areas, as

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well as plasma levels of gonadotropins and testosterone, were measured. Cadmium accumulation at the hypothalamus, the pituitary, and the testis was also evaluated.

Materials and Methods

Animals and Treatment. Male rats of the Sprague-Dawley strain kept under controlled conditions of light (lights on from 07:00 to 21:00 hr) and temperature (22° ± 2°C) and having access to food and water *ad libitum* were used. Four groups of 16 animals were used. Groups 1 and 2 were 30-day-old rats (prepubertal) and groups 3 and 4 were 60-day-old male rats (young adults) at the beginning of the experiment. Groups 2 and 4 were exposed to the metal (as cadmium chloride [$CdCl_2$] at a dose of 50 ng/ml in the drinking water) from Day 30 to 60 or from Day 60 to 90, respectively. Groups 1 and 3 received water from University supply as controls. The daily dose of cadmium was 2 mg/kg body wt, considering that water consumption is about 20 ml per day/rat and that it was not modified by cadmium addition. The dose of the metal was not significantly modified by the content in the water supply (20 ng/20 ml). This dose of cadmium is one-half of the lethal dose for rats divided by a factor of security of 500. However, this dose is 40 times higher that the range of the dietary intake by the human in several countries (29–31). Considering that the mean-half life of cadmium is over 30 years, the metal will accumulate in the tissues. Moreover, other factors may increase the intake of cadmium. For example, the smoking of 20 cigarettes may increase cadmium intake by 0.5 and 2 μg (31).

At the 60th day of life for groups 1 and 2, and at 90th day of life for groups 3 and 4, animals were sacrificed by decapitation at 14:00 hr (25). The decapitation procedure was completed within 5 to 10 sec to avoid stressors. Trunk blood was collected in tubes containing EDTA (60 g/l) and plasmas were collected after centrifugation at 1500*g* for 15 min at 4°C, and the samples were kept frozen at –20°C until LH, FSH, and testosterone were measured. The hypothalamus from eight animals of each group were used to measured cadmium accumulation and the other eight were used to measured norepinephrine, 5-HT, and 5-HIAA contents in the median eminence, and anterior, mediobasal, and posterior hypothalamus.

The studies were conducted in accordance with the principles and procedures outlined in the National Institutes of Health Guide for the Care and Use of the Laboratory Animals (32).

Amine Measurements. The hypothalamus was quickly removed as described in previous works of the group (24). Tissue was weighed and homogenized in chilled (0°–1°C) 2 M acetic acid. After centrifugation (at 15,000*g* for 30 min at 4°C), the supernatants were analyzed by high performance liquid chromatography (HPLC) using electrochemical detection (Coulochem, II, ESA). A C-18 reverse phase column was eluted with a mobile phase consisting of: 0.1 M sodium acetate, 0.1 M citric acid, 0.7 mM sodium octylsulphate, and 0.57 mM EDTA containing 10% methanol (v/v), pH 4. The flow rate was 1 ml/min at a pressure of 2200 psi. Fixed potentials against H_2/H^+ reference electrodes were conditioning electrode –0.4 V, pre-oxidation electrode +0.10 V, and working electrode +0.35V. Amine concentrations were calculated from the chromatographic peak areas by using external standards. The linearity of the detector response for norepinephrine, 5-HT, and 5-HIAA was tested within the concentration ranges found in supernatants of anterior, mediobasal, and posterior hypothalamus and median eminence (33).

Hormone Measurement. Plasma LH and FSH levels were measured by homologous specific double antibody radioimmunoassay using material kindly supplied by the National Hormone and Pituitary Program (NHPP, Rockville, MD) and by Dr. A. Parlow (Harbor UCLA Medical Center). The intraassay coefficients of variation were 7.3% and 9.5% for LH and FSH, respectively. Sensitivities of the assays were 0.025 and 0.04 ng/mL, respectively, using r-LH-RP-3 and r-FSH-RP-2 reference preparations, respectively. All samples were measured in the same assay to avoid interassay variation. Plasma testosterone levels were measured using a commercial kit form Diagnostic Systems Laboratories Inc. (Webster, TX), validated in our laboratory. The intraassay coefficient of variation was 9% and the sensitivity of the assay was 0.1 ng/mL.

Cadmium Determination. Cadmium concentration was determined in the water supply and in the hypothalamus, pituitary, and testis of eight animals. Tissue cadmium concentrations were determined by graphite furnace atomic absorption spectrophotometry after microwave digestion (GFAAS). The samples were mineralized in a Parr 4780 microwave, acid digestion bomb, and a Samsung M-745 microwave oven. The mineralization step was performed by treating 25 mg of dried homogenized samples with 3.0 ml of ultra pure nitric acid and 1 ml of distilled water. In the case of pituitary and hypothalamus, the whole tissue was treated. Then, the samples were digested in the microwave acid digestion bomb. The mineralization was completed after two digestions at 450 W for 2 min and 20 s. For cadmium determination, an atomic absorption spectrophotometer (Perkin-Elmer, Varian Spectra 250 plus) with Zeeman background correction was used. Accuracy was obtained by calibration against aqueous standards (RSD 5%). Sensitivity of the method was 0.02mg/l. Samples of the whole experiment were analyzed within the same assay to avoid interassay variations, and the intraassay coefficient of variation was 4%.

Statistical Analysis. Stadistical analysis of results was performed by using a factorial ANOVA followed by a Scheffé test (StatView for Macintosh). The results were considered significant at $P < 0.05$. All values represent the means ± SEM.

Results

In older control rats, the norepinephrine content increased in the anterior, mediobasal, and posterior hypothala-

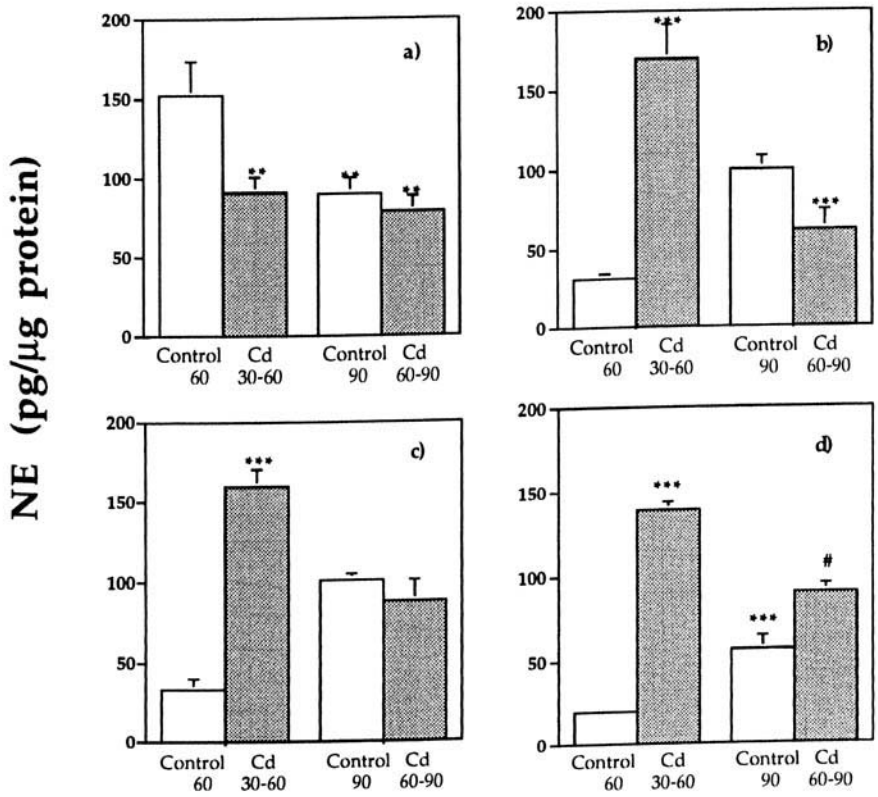

Figure 1. Norepinephrine content in median eminence (a), and in anterior (b), mediobasal (c), and posterior hypothalamus (d) in 60- or 90-day-old male rats treated with cadmium-free water or with cadmium chloride at a dose of 50 ppm in the drinking water for 1 month beginning from Day 30 or 60 of life, respectively. The values are expressed as means ± SEM (n = 8 in each group). $^{**}P < 0.01$; $^{***}P < 0.001$ versus controls of 60 days of life. $\#P < 0.05$, $\#\#\#P < 0.001$ versus controls of 90 days of age.

mus as compared with the values found in younger controls ($P < 0.001$; Fig. 1, b, c, and d). This increase was not observed in the median eminence (Fig. 1a). Serotonin turnover decreased in the anterior mediobasal and posterior hypothalamus in 90-day-old rats as compared with the values found in 60-day-old control animals ($P < 0.01$; Fig. 2, b, c, and d).

In rats treated with $CdCl_2$ from 30 to 60 days of life, the norepinephrine content increased in the anterior, mediobasal, and posterior hypothalamus (Fig. 1, b, c, and d) as

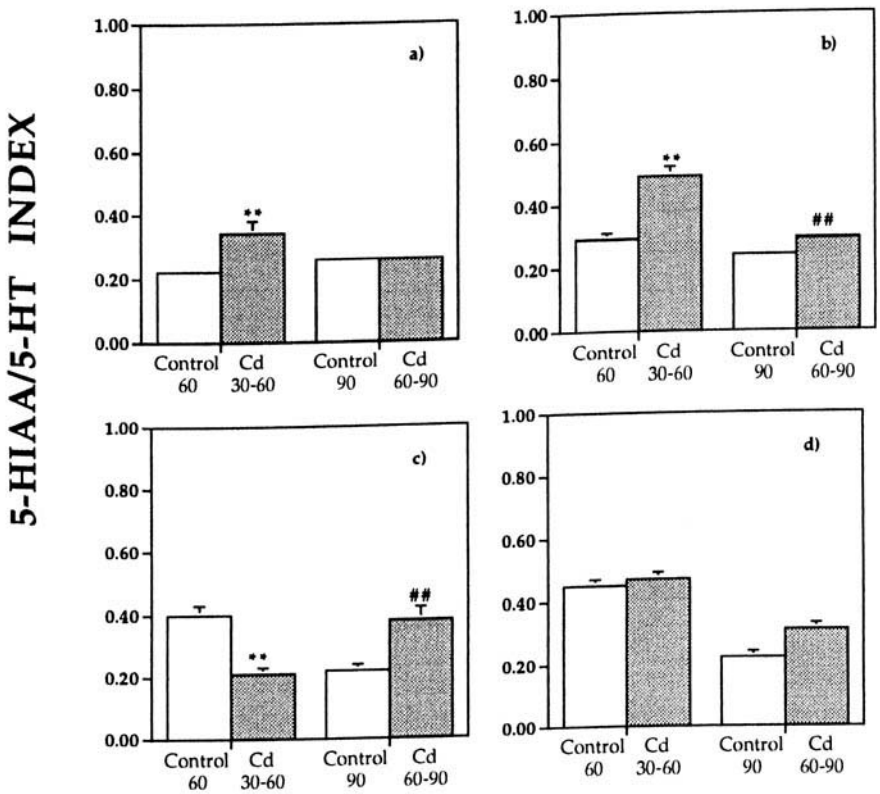

Figure 2. Serotonin turnover measured as 5-hydroxyindole acetic acid/serotonin ratio in median eminence (a), and in anterior (b), mediobasal (c), and posterior hypothalamus (d) in 60- or 90-day-old male rats treated with cadmium-free water or with cadmium chloride at a dose of 50 ppm in the drinking water for 1 month beginning from Day 30 or 60 of life, respectively. The values are expressed as means ± SEM (n = 8 in each group). $^{**}P < 0.01$ versus controls of 60 days of life. $\#\#P < 0.01$ versus controls of 90 days of age.

compared with the values found in their age-matched controls ($P < 0.001$). In these animals, serotonin turnover increased in the median eminence and anterior hypothalamus (Fig. 2a) as compared with the values found in age-matched controls ($P < 0.01$). However, in the mediobasal hypothalamus, serotonin turnover decreased ($P < 0.001$; Fig. 2 c).

When cadmium was administered from Day 60 to 90 of life, the norepinephrine content increased in the posterior hypothalamus ($P < 0.05$; Fig. 1d) and decreased in the anterior hypothalamus ($P < 0.05$; Fig. 1 b) as compared with the values found in their age-matched control rats. The serotonin turnover increased in mediobasal and posterior hypothalamus (Fig. 2, c and d; $P < 0.001$ and < 0.05, respectively) as compared with controls.

The analysis of norepinephrine content within the hypothalamus indicates the existence of a global cadmium effect in mediobasal ($F = 43.10$; $P < 0.0001$), anterior ($F = 21{,}668$; $P < 0.0002$), and posterior ($F = 205.33$; $P < 0.0001$) hypothalamus. An interaction between age and cadmium appeared in the same areas of the hypothalamus ($P < 0.0001$ for each comparison). However, no effects of the metal or interaction between the metal and the age were determined in the median eminence.

Serotonin turnover analysis indicates the existence of a global cadmium effect at the anterior ($F = 6.62$; $P < 0.01$) and mediobasal ($F = 23.94$; $P < 0.0001$) hypothalamus and the median eminence ($F = 22.72$; $P < 0.0001$). An interaction between age and cadmium appeared in mediobasal hypothalamus ($P < 0.0001$) and median eminence ($P < 0.01$), but not in the anterior hypothalamus.

In older animals, plasma levels of LH and testosterone increased as compared with the values found in younger rats ($P < 0.001$; Fig. 3), while plasma FSH levels decreased ($P < 0.001$; Fig. 3). After cadmium treatment from 30 to 60 days of life, only plasma levels of testosterone decreased ($P < 0.01$; Fig. 3) as compared with the values found in the control group (Fig. 3). Metal exposure during adulthood (60 to 90 days of life) decreased plasma levels of LH and testosterone ($P < 0.01$ and < 0.001 versus age-matched controls; Fig. 3) and increased plasma FSH levels ($P < 0.01$; Fig. 3).

Hormone analysis shows a global effect of cadmium on LH ($F = 5.14$; $P < 0.02$), FSH ($F = 18.82$; $P < 0.00019$), and testosterone ($F = 6.2$; $P < 0.01$). An interaction between cadmium and age appeared for LH and FSH ($P < 0.04$ and < 0.006, respectively). However, the interaction was not detectable for testosterone.

Prepubertal exposure to cadmium (from 30 to 60 days of life) increased metal content in the hypothalamus ($P < 0.05$; Fig. 4) and the testis ($P < 0.05$ versus control; Fig. 4), but not in the pituitary (Fig. 4) as compared with the values found in age-matched control rats. Cadmium treatment in adult rats (from 60 to 90 days of age) increased metal content in the hypothalamus, the pituitary, and the testis ($P < 0.001$, < 0.01, and < 0.001, respectively, versus control; Fig. 4) as compared with their age-matched controls.

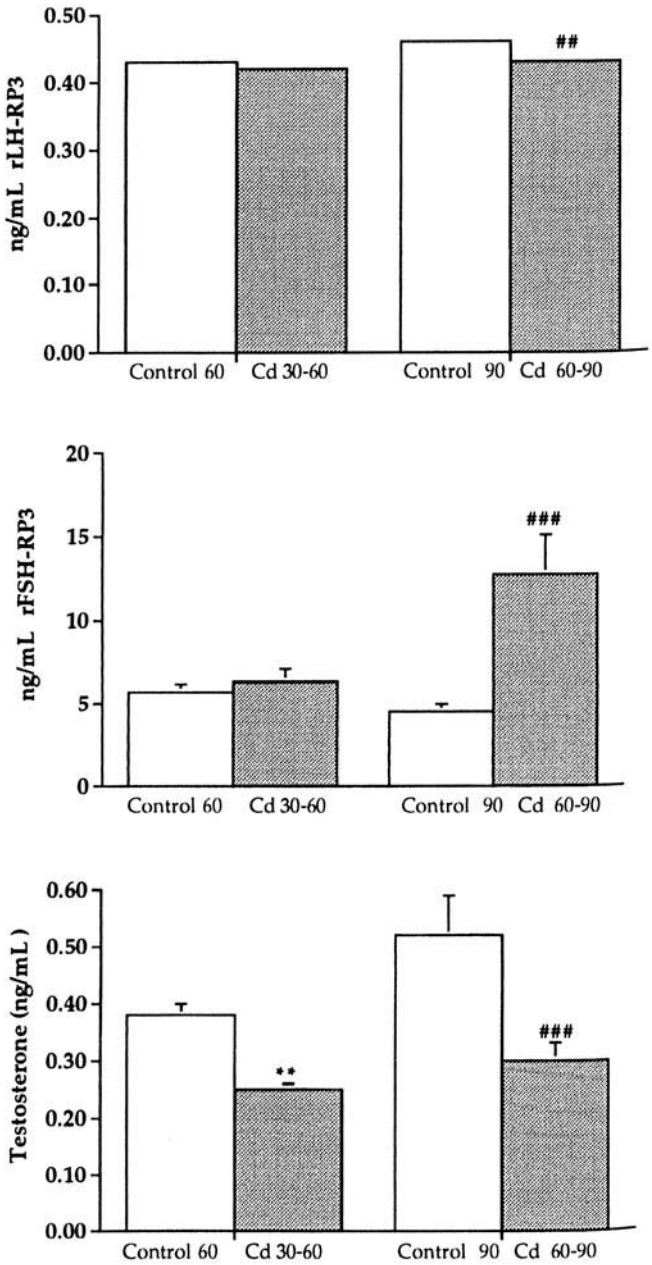

Figure 3. Plasma LH, FSH, and testosterone levels in 60- or 90-day-old male rats treated with cadmium-free water or with cadmium chloride at a dose of 50 ppm in the drinking water for 1 month beginning from Day 30 or 60 of life, respectively. The values are expressed as means ± SEM ($n = 16$ in each group). $^{**}P < 0.01$ versus controls of 60 days of age. $\#\#P < 0.01$; $\#\#\#P < 0.001$ versus controls of 90 days of life.

In Figure 5, absolute body weight gain can be observed. In animals exposed to the metal during puberty, this parameter was not changed. However, when rats were exposed to the xenobiotic during adulthood, body weight gains significantly decreased ($P < 0.01$).

Discussion

To our knowledge, this is the first report showing the effects of oral exposure to cadmium on several hypothalamic areas involved in the regulation of pituitary hormone secretion (anterior and mediobasal hypothalamus) and in

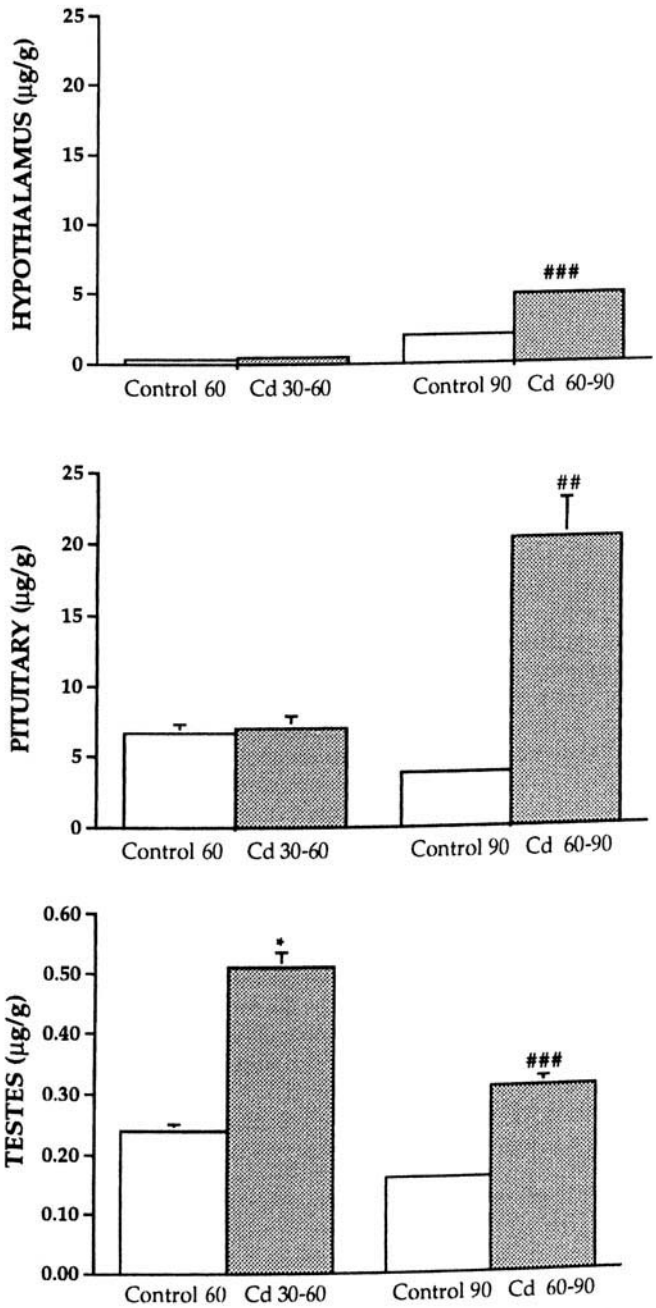

Figure 4. Cadmium accumulation in the hypothalamus, pituitary, and testis in 60- or 90-day-old male rats treated with cadmium-free water or with cadmium chloride at a dose of 50 ppm in the drinking water for 1 month beginning from Day 30 or 60 of life, respectively. The values are expressed as means ± SM ($n = 8$ in each group). $^*P < 0.001$ versus controls of 60 days of age. $\#\#P < 0.01$; $\#\#\#P < 0.001$ versus controls of 90 days of life.

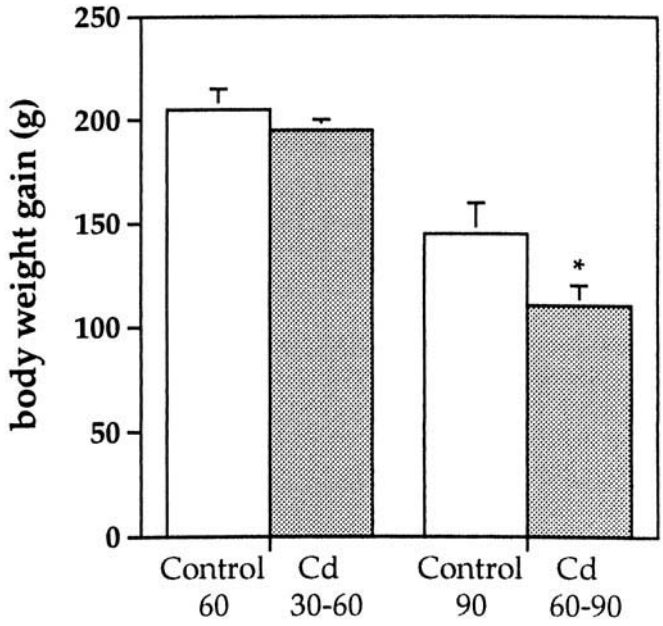

Figure 5. Body weight gain in male rats treated with cadmium chloride at a dose of 50 ppm in the drinking water for 1 month beginning from Day 30 or 60 of life, respectively. The values are expressed as means ± SEM ($n = 8$ in each group). $^*P < 0.05$ versus controls of 90 days of age.

median eminence and in the posterior hypothalamus, an area not directly related to this function. The changes found at the hypothalamus, together with those observed in plasma levels of gonadotropins and testosterone, suggest a global effect of the metal on the reproductive function and that an interaction exists between the metal and the age during cadmium exposure.

The changes in norepinephrine content within the hypothalamus observed in this study agree with those described for the whole brain (34). However, norepinephrine content did not change after postpubertal treatment, which agrees with the data reported by Andersson *et al.* (21). These data indicated age-dependent effects of the metal on norepinephrine, which may be explained by the sensitivity of the hypothalamic-pituitary axis to exogenous stimulation with age (35). Serotonin metabolism changes were only evident in prepubertal animals and the data agree with those reported earlier using other brain areas (15, 18–20). Metal accumulation in the hypothalamus and pituitary was higher after postpubertal than after prepubertal metal exposure, although the changes observed in norepinephrine content and serotonin metabolism were more marked in younger than in older rats, thus confirming previous data in growing rats (18).

In cadmium-exposed animals from 60 to 90 days of life, plasma levels of LH and FSH were differentially modified. These changes did not correlate with norepinephrine content and 5-HT metabolism at the hypothalamic level, as these neurotransmitters were unchanged after metal exposure. This may be the basis to explain the age-dependent effect of cadmium on reproduction, as both neurotransmitters (norepinephrine and serotonin) are involved in the regulation of gonadotropin (36–38). Other neuromodulators not studied in this work, implicated in gonadotropin secretion regulation, may be responsible of the observed changes.

Globally, the data indicate multiple levels of action for the metal on the hypothalamic-pituitary axis, as specific levels of this axis were affected. This may indicate that cadmium differentially affects both hypothalamic and pituitary levels in the reproductive axis, depending upon the age of the animals during the metal exposure, as indicated by the

interaction between the metal and the age in most of the parameters studied.

We found increased plasma levels of FSH after cadmium administration from 60 to 90 days of life. This finding could be due to the accumulation of cadmium in the testis. In this context, it was shown that cadmium affects Sertoli cell activity by decreasing inhibin synthesis and release. Thus, the increased plasma levels of FSH could be explained, as this peptide is the main inhibitory signal for FSH secretion (39). These data confirm that the severity of cadmium-induced damage at the testicular level increased with age (40, 41).

The decrease in plasma testosterone levels observed after both prepubertal and postpubertal cadmium exposure may reflect direct effects of the metal at the testis as this metal accumulates in this tissue (this study; 42–44). This effect may be explained considering the results described by Laskey and Phelps (9) from *in vitro* studies that showed a reduction in both human chorionic gonadotrophin and dibutyl cyclic adenosine monophosphate-stimulated testosterone production in rats treated with cadmium.

The changes in testosterone did not correlate with the modifications in gonadotropin secretion. This might suggest that selective accumulation of the metal on the reproductive axis disrupts the bidirectional regulatory mechanism that operates in this specific axis.

The decrease in body weight gain in the postpubertal cadmium-treated animals may be correlated to the greater accumulation of the metal in both hypothalamus and pituitary in these animals. This decrease does not seem not to be caused by alterations in growth hormone secretion, as serum levels of this hormone are not changed by postpubertal cadmium administration (45, 46). The changes that we have found could be explained by differential target sensitivity with the age, or different functionality of the hypothalamic-pituitary-testicular axis after sexual development. However, further studies are needed to fully understand the mechanism through which cadmium decreases body weight gain.

In conclusion, these data suggest that cadmium exerts age-dependent effects on the hypothalamic-pituitary-testicular axis function that may be related to the selective cadmium accumulation within this axis.

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1. Sokol RZ. The hypothalamic-pituitary-gonadal axis as a target for toxicants. In: Sipes IC, McQueen CA, Gandolfi AJ, Eds. Comprehensive Toxicology. Oxford: Elsevier Science, Vol **10**:pp87–98, 1997.
2. Garside DA, Harvey PW. Endocrine toxicology of the male reproductive system. In: Atterwill CK, Flack JD, Eds. Endocrine Toxicology. Cambridge: Cambridge University Press, pp285–312, 1992.
3. Li LH, Heindel JJ. Sertoli cell toxicants. In: Korach KS, Ed. Reproductive and Developmental Toxicology. New York: Marcel Dekker, pp655–691, 1998.
4. Parízek J, Záhor Z. Effect of cadmium salts on testicular tissue. Nature **177**:1036–1037, 1956.
5. Cameron E, Foster CL. Observations on the histological effects of sub-lethal doses of cadmium chloride in the rabbit. J Anat **97**:269–274, 1963.
6. Kotsonis FN, Klaaseen CD. Toxicity and distribution of cadmium administered to rats at sublethal doses. Toxicol Appl Pharmacol **41**:667–680, 1977.
7. Nath R, Prasad R, Palinal VK, Chopra RK. Molecular basis of cadmium toxicity. Prog Food Nutr Sci **8**:109–164, 1984.
8. Singhal RL, Vijayvargiya R, Shukla GS. Toxic effects of cadmium and lead on reproductive functions. In: Thomas JA, Korach KS, McLachlan JA, Eds. Endocrine Toxicology. New York: Raven Press, pp149–180, 1985.
9. Laskey JW, Phelps PV. Effect of cadmium and other metal cations on *in vitro* Leydig cell testosterone production. Toxicol Appl Pharmacol **108**:296–306, 1991.
10. Lafuente A, Márquez N, Piquero S, Esquifino AI. Cadmium affects the episodic luteinizing hormone secretion in male rats: Possible age-dependent effects. Toxicol Lett **104**:27–33, 1999.
11. Zylber-Haran EA, Gershman H, Rosenmann E, Spitz MI. Gonadotrophin, testosterone and prolactin interrelationships in cadmium-treated rats. J Endocrinol **92**:123–130, 1982.
12. Paksy K, Varga B, Horvath E, Tatrai E, Ungvary G. Acute effects of cadmium on preovulatory serum FSH, LH and prolactin levels and on ovulation and ovarian hormone secretion in oestrus rats. Reprod Toxicol **3**:241–247, 1989.
13. Varga B, Paksy K. Toxic effects of cadmium on LHRH-induced LH release and ovulation in rats. Reprod Toxicol **5**:199–203, 1991.
14. Lafuente A, Esquifino AI. Acute and chronic cadmium administration on pituitary hormone secretion in adult male rats. In: Collery P, Corbella J, Domingo JL, Etienne JC, Llobet JM, Eds. Metal Ions in Biology and Medicine. Paris: John Libbey Eurotext, Vol **4**:pp459–461, 1996.
15. Lafuente A, Blanco A, Márquez N, Alvarez-Demanuel E, Esquifino AI. Effects of acute and subchronic cadmium administration on pituitary hormone secretion in rat. Rev Esp Fisiol **53**:265–270, 1997.
16. Hrdina PD, Peters DA, Singhal RL. Effects of chronic exposure to cadmium, lead and mercury of brain biogenic amines in the rat. Res Commun Chem Pathol Pharmacol **15**:483–493, 1976.
17. Chandra SV, Kalia K, Hussain T. Biogenic amines and some metals in brain of cadmium-exposed diabetic rats. J Appl Toxicol **5**:378–381, 1985.
18. Nation JR, Frye, GD, Von Stultz J, Bratton GR. Effects of combined lead and cadmium exposure: Changes in schedule-controlled responding and in dopamine, serotonin, and their metabolites. Behav Neuroendocr **103**:1108–1114, 1989.
19. Gupta A, Gupta A, Murthy RC, Thakur SR, Dubey MP, Chandra SV. Comparative neurotoxicity of cadmium in growing and adult rats after repeated administration. Biochem Int **21**:97–105, 1990.
20. Das KP, Das PC, Dasgupta S, Dey CC. Serotoninergic-cholinergic neurotransmitters' function in brain during cadmium exposure in protein restricted rat. Biol Trace Element Res **36**:119–127, 1993.
21. Andersson H, Petersson-Grave K, Lindqvist E, Luthman J, Oskarsson A, Olson L. Low-level cadmium exposure of lactating rats causes alterations in brain serotonin levels in the offspring. Neurotoxicol Teratol **19**:105–115, 1997.
22. Antonio MT, Corpas I, Leret ML. Neurochemical changes in newborn rat's brain after gestational cadmium and lead exposure. Toxicol Lett **104**:1–9, 1999.
23. Shukla GS, Singhal RL. The present status of biological effects of toxic metals in the environment: Lead, cadmium and manganese. Can J Physiol Pharmacol **62**:1015–1031, 1984.
24. Esquifino AI, Moreno ML, Arce A, Agrasal C, Pérez-Diaz J, Villanua MA. Effects of cyclosporine at the hypothalamic-pituitary axis in pituitary-grafted young female rats. J Endocrinol **144**:159–164, 1995.
25. Selgas L, Arce A, Esquifino AI, Cardinali DP. Twenty-four hour

rhythms of serum ACTH, prolacitn, 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, 1997.
26. Duvilanski BH, Selgas L, Garcia-Bonacho M, Esquifino AI. Daily variations of amino acid concentration in mediobasal hypothalamus in rats injected with Freund's adjuvant. Effect of cyclosporine. J Neuroimmunol **87**:189–196, 1988.
27. Nriagu JO. Food contamination with cadmium in the environment. In: Nriagu JO, Simmons MS, Eds. Food Contamination from Environmental Sources. New York: John Wiley & Sons, pp59–84, 1990.
28. Word Health Organization. Evaluation of Certain Food Additives and Contaminants. 41st Report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Tech Rep Ser 837 (Geneva, Switzerland), pp53, 1993.
29. Piscator M. Dietary exposure to cadmium and health effects: Impact of environmental changes. Environ Health Perspect **63**:127–132, 1985.
30. Coni E, Baldini M, Stacchini P, Zanasi F. Cadmium intake with diet in Italy: A pilot study. J Trace Elem Electrolytes Health Dis **6**:175–181, 1992.
31. López-Artíguez M, Soria ML, Cameán A, Repetto M. Cadmium in the diet of the local population of Seville (Spain). Bull Environ Contam Toxicol **50**:417–424, 1993.
32. National Research Council. Guide for the Care and Use of Laboratory Animals. Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, National Academy of Sciences (USA), National Institutes of Health, 1996.
33. Esquifino AI, Arce A, Muñoz RM, Villanúa MA, Cardinali DP. Changes in mediobasal hypothalamic dopamine and indolamine metabolism after superior cervical ganglionectomy of rats. J Neural Transmission **103**:287–298, 1996.
34. Arito H, Sudo A, Suzuki Y. Aggressive behavior of the rat induced by repeated administration of cadmium. Toxicol Lett **7**:457–461, 1981.
35. Esquifino AI, Ramos JA, Tresguerres JAF. Possible role of dopamine in changes in LH and prolactin concentrations after experimentally induced hyperprolactinaemia in rats. J Endocrinol **100**:141–148, 1984.
36. Douva S, Gallo R. Catecholamine involvement in episodic luteinizing hormone release in adult ovariectomized rats. Endocrinology **99**:651–658, 1976.
37. Wuttke W, Hancke JL, Hoöhn KG, Baumgarten HG. Effect of intraventricular injection of 5,7-dihydroxytryptamine on serum gonadotropins and prolactin. Ann NY Acad Sci **305**:423–436, 1978.
38. Ellis GB, Desjardins C, Fraser HM. Control of pulsatile LH release in male rats. Neuroendocrinology **37**:177–183, 1983.
39. Ultee-van Gessel AM, Leemborg FG, de Jong FH, van der Molen HJ. Influence of neonatal hemicastration on *in vitro* secretion of inhibin, gonadotrophins and testicular steroids in male rats. J Endocrinol **106**:259–265, 1985.
40. Wong KL, Klaassen CD. Age difference in the susceptibility to cadmium-induced testicular damage in rats. Toxicol Appl Pharmacol **55**:456–466, 1980.
41. Phelps PV, Laskey JW. Comparison of age-related changes in *in vivo* and *in vitro* measures of testicular steroidogenesis after acute cadmium exposure in the Sprague-Dawley rat. J Toxicol Environ Health **27**:95–105, 1989.
42. Márquez N, Alvarez-Demanuel E, Piquero S, Esquifino AI, Lafuente A. Chronic alternate or daily cadmium exposure differentially affects its accumulation within the tissues. Effects of age. Toxicol Lett **95**(Suppl 1):125, 1998.
43. Clark DE, Nation JC, Bourgeois AJ, Hare MF, Baker DM, Hinderberger EJ. The regional distribution of cadmium in the brains of orally exposed adult rats. Neurotoxicology **6**:109–114, 1985.
44. Paksy K, Naray M, Varga B, Kiss I, Folly G, Ungvary G. Uptake and distribution of Cd in the ovaries, adrenals, and pituitary in pseudopregnant rats: Effect of cadmium on progesterone serum levels. Environ Res **51**:83–90, 1990.
45. Pérez D. Alteraciones inducidas por cadmio sobre el sistema neuroendocrinológico en rata macho. PhD thesis, University of Vigo, 1999.
46. Lafuente A, Esquifino AI. Cadmium effects on hypothalamic activity and pituitary hormone secretion in the male. Toxicol Lett **110**:209–218, 2000.