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Modulation of episodic adrenocorticotropin hormone secretion by cadmium in male rats

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The effects of cadmium on adrenocorticotropin hormone (ACTH) secretion are controversial and seem to depend on the dose and duration of the exposure to the metal. This work was undertaken to analyze the effects of acute cadmium administration on the episodic pattern of ACTH release in adult male rats. For this purpose, animals were cannulated 40 h before the experiment to allow a continuous blood withdrawal. Two and a half hours after the administration of a single dose of cadmium chloride (4.5 mg kg^{-1} bodyweight), the episodic pattern of ACTH was analyzed during three hours (from 10:30 to 13:30, samples being collected every seven minutes) in conscious and freely moving adult male rats. The mean values of ACTH during the bleeding period and the absolute pulse amplitude were decreased by acute cadmium chloride administration ($P \leqslant 0.001$, $P \leqslant 0.01$, respectively). By contrast, the frequency of ACTH pulses increased ($P \leqslant 0.01$). However, no changes in any other parameters of episodic ACTH secretion were observed compared with control animals. These data suggest that cadmium interferes with the regulatory mechanism of ACTH.

Keywords: ACTH, cadmium, episodic secretion

Introduction

Cadmium is present in the environment as a result of the application of modern technology in diverse industries. It is taken by mammals via the food web and creates a health risk for both humans and animals as primary and secondary consumers of the food web (Levine *et al.* 1989, Brueske & Barrett 1991).

It has been shown that cadmium exposure is associated with changes in the gonadal (Laskey & Phelps 1991, Piasek & Laskey 1994), adrenal (Anca *et al.* 1982, Hidalgo & Armario 1987, Mgbonyebi *et al.* 1993) and immune functions (Descotes 1992, Teocharis *et al.* 1994). Most of these effects may be explained by taking into consideration the fact that cadmium differentially modifies prolactin, gonadotropins and GH secretions, when measured as single point times (Zylber-Haran *et al.* 1982, Lorenson *et al.* 1983, Lafuente *et al.* 1996, 1997); these are the pituitary hormones involved in the modulation of the above functions. This hypothesis has been confirmed, as cadmium has been shown to change the metabolism of the hypothalamic neuromodulators which regulate pituitary hormone secretion (Hrdina *et al.* 1976, Chandra *et al.* 1985, Nation *et al.* 1989, Das *et al.* 1993).

Cadmium exposure also changes adrenocorticotropin hormone (ACTH) secretion, although controversial data showing decreases (Alvarez *et al.* 1996), increases (Hidalgo & Armario 1987) or no

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changes (Nishiyama & Nakamura 1984) have been reported. All these data have been obtained using single sampling in the experimental designs studied, and a wide variety of doses and means of administration. Furthermore, ACTH is secreted in an episodic fashion (ultradian) that follows a circadian rhythm (Gambacciani *et al.* 1987, López & Negro-Vilar 1988, Carnes *et al.* 1989, Mershon *et al.* 1992, Gudmundsson & Carnes 1997). This pattern is similar to that described for prolactin (Lafuente *et al.* 1993, 1994, Selgas *et al.* 1997).

To better understand the effects of cadmium on ACTH, a single exposure to the metal was followed by a serial sampling during three hours.

Materials and methods

Animals and treatment

Adult male Sprague-Dawley rats weighing 275–300 g were used in all experiments. They were maintained in a room with controlled photoperiod (14 h light/10 h darkness; lights on from 0700 to 21.00 h) and temperature (22 + 2°C), and were supplied with rat chow and water available *ad libitum*.

Cannula implantation

Forty hours before the day of the experiment, animals were anaesthetized with 2.5% tribromoethanol in saline (1 ml per 100 g body weight) and atrial cannulas were implanted through the external jugular vein, according to procedures used in previous studies (Lafuente *et al.* 1993, 1994, 1996). This procedure allows the animals to move freely in their cages during the period of bleeding.

Experimental design and blood sampling

Two experimental groups of six rats per group were used in this study. One group was treated at 08.00 h with a single intraperitoneal (i.p.) injection of $CdCl_2$ at a dose of 4.5 mg kg^{-1} body weight. We administered 0.9% NaCl solution to the other group, which was used as control.

The dose of cadmium used in this study is in the range of dietary intake by the adult human in several countries (Piscator 1985, Coni *et al.* 1992, López-Artíguez *et al.* 1993) and the data obtained can be compared with those described in previous works using the same dose of the metal (Zylber-Haran *et al.* 1982, Katsuta *et al.* 1993, Piasek & Laskey 1994). It must be considered that the mean life of cadmium is long enough (over 30 years) to allow continuous increases of the metal in the tissues. Moreover, other factors may increase the intake of cadmium and its presence in several tissues in humans. For example, 20 cigarettes smoked may add 0.5–2 μg of cadmium to the dietary intake; this can be absorbed and deposited in the various tissues (López-Artíguez *et al.* 1993).

On the day of experiment, conscious and freely moving rats from each group were continuously infused with 0.9% saline (0.5 ml h^{-1}) for four hours, beginning at 9.30 h. One hour after the beginning of the intravenous infusion of saline, and 15 min after the administration of 300 IU of heparin, rats were bled continuously through a peristaltic pump at a flow rate of 50 μl, every seven minutes. Blood samples were collected in Hamilton microliter syringes every seven minutes for three hours, from 10.30 h to 13.30 h. The samples were collected into assay tubes containing phosphate buffer (0.01 mol l^{-1}) with 0.1% gelatin and kept on ice. Hematocrits remained stable with this bleeding protocol (41–36%). To blood samples was added aprotinin (1 U ml^{-1}) to avoid the effects of the seric proteases. All samples were centrifuged for 15 min at 1500 *g* at 4°C and the serum was kept frozen until analyzed.

The studies were conducted in accordance with the principles and procedures outlined in the NIH guide for the Care and Use of the Laboratory Animals.

ACTH measurements

ACTH levels, in all series from each rat, were determined by a specific double-antibody radioimmunoassay system. The reagents were kindly supplied by the National Hormone and Pituitary Program (NHPP, Rockville, MD, USA). ACTH values are expressed in terms of NIADD rat ACTH-RP3 reference preparation. The sensitivity of this assay was 1 pg per tube. Samples of the whole experiment were analyzed within the same assay to avoid interassay variations. To ascertain the variability of the assay, a series of ten replicates corresponding to five different concentrations of ACTH in the standard curve was used. The mean intraassay coefficient of variation (CV) was 4.2%.

Data analysis

To identify and characterize ACTH pulses appearing in the hormonal profile of each rat, a computer program (Ultra-analysis) described by Van Cauter (1990) and reviewed by Richard *et al.* (1990), was used. In this program, a pulse was defined as an increase exceeding a multiple of the dose-adjusted coefficient of variance (CV), followed by a significant decrease. The intraassay CV was calculated from values of five different concentrations of ACTH in its standard curve. Thus, the CV and the mean hormone level were determined for all hormone values that comprised the ascending and descending phases of each potential pulse. The pulse was defined when this CV was triple that of the intraassay CV determined at comparable mean ACTH level. To test the specificity of a pulse detection, a series of 26 samples from a pool of serum was analyzed using a threshold of 3CV for ACTH pulses. Extensive simulation studies using computer-generated series have indicated that, for series that have large and frequent pulses, a threshold of three CV values minimizes both false positive and negative errors (Van Cauter 1988).

The episodic ACTH secretion pattern was characterized by the mean hormone levels, absolute and relative amplitudes of ACTH peaks, and their frequency and pulse duration. The program also calculates the mean half-life of the hormone. The absolute pulse amplitude was defined as the difference between the hormone level at the maximum of the peak and the hormone level at the preceding nadir. The relative pulse amplitude was calculated as the quotient between absolute pulse amplitude and preceding nadir value. Pulse frequency was the number of pulses observed during the bleeding period. Pulse duration was the time between the beginning of the ascending phase of the peak and the end of the descending phase of the peak. The mean hormone level was calculated by the mean of all samples collected from each rat during the three hour period, and the average for the experimental group from the individual means. The half-life of the hormone is calculated from the tangent of the descending phase of the peak.

Statistics

Comparison of values was done by analysis of variance (ANOVA) followed by Duncan's multiple range test. The results were considered significant at $P \leqslant 0.05$. All values represent the mean ± SEM.

Results

Adrenocorticotropin hormone secretion in animals from the two experimental groups studied was episodic, and a representative profile from one animal of each experimental group is shown in Figure 1.

Acute cadmium administration ($CdCl_2$ at a dose of 4.5 mg kg^{-1} body weight) significantly decreased mean serum ACTH levels and the absolute amplitude of the ACTH pulses during the bleeding period (Table 1, $P \leqslant 0.001$, $P \leqslant 0.01$, respectively). Surprisingly, cadmium chloride treatment markedly increased the frequency of ACTH peaks (Table 1, $P \leqslant 0.01$). However, relative pulse amplitude, duration of ACTH pulses and mean half-life of the hormone did not change after cadmium administration (Table 1).

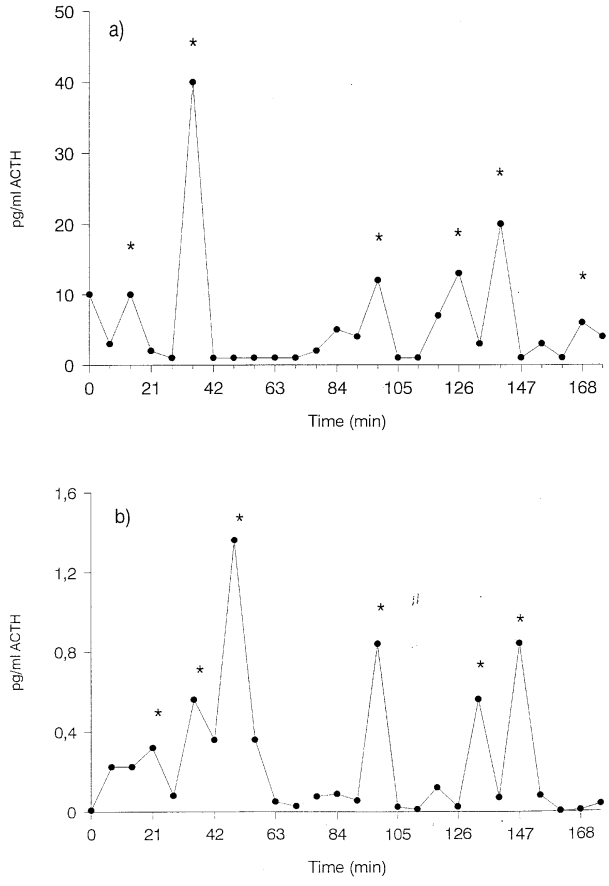

Figure 1. Individual episodic ACTH patterns in adult male rats treated with saline (a) or a single i.p. injection of cadmium chloride at a dose of 4.5 mg kg^{-1} body weight (b). The asterisks indicate the ACTH peaks during the study period.

Table 1. Mean serum ACTH levels, absolute and relative pulse amplitude, frequency and duration of the pulses, and mean half-life of ACTH, in control and treated adult male rats with $CdCl_2$ at a dose of 4.5 mg kg^{-1} body weight

Group	ACTH (pg ml^{-1})	Absolute amplitude (pg ml^{-1})	Relative amplitude (%)	Frequency (pulses per 3 h)	Duration (min)	Half-life (min)
Control	6.68 ± 0.44	0.16 ± 0.06	24.20 ± 5.15	5.66 ± 0.49	19.83 ± 1.37	3.08 ± 0.37
$CdCl_2$	0.60 ± 0.19***	0.003 ± 0.001**	18.84 ± 3.40	7.14 ± 0.26**	22.28 ± 0.52	4.23 ± 0.64

The relative pulse amplitude was calculated as the quotient between absolute pulse amplitude and preceding nadir value. Values are expressed as mean ± SEM. The number of animals per group is six.
**$P \leqslant 0.01$, ***$P \leqslant 0.001$ versus control group.

Discussion

Foregoing results suggest that a single i.p. injection of cadmium chloride (4.5 mg kg^{-1} body weight) modifies the episodic secretion pattern of ACTH in adult male rats, indicating that cadmium interferes with the regulatory mechanism of the hormone.

In this study, because of the time interval in which we collected the samples (seven minutes), we can only discuss all matters concerning macropulsatility, when considering the micropulses described by Carnes *et al.* (1988) where samples were collected every two minutes. However, control rats showed an irregular episodic secretion pattern of ACTH, which is characteristic of this hormone, and which agrees with previous works from the literature. (Gambacciani *et al.* 1987, López & Negro-Vilar 1988, Carnes *et al.* 1989, Mershon *et al.* 1992, Gudmundsson & Carnes 1997).

In spite of the marked reduction in the mean values of ACTH during the bleeding period, it is of interest to note that the irregular episodic pattern of the hormone was maintained in cadmium treated rats. This may reflect an inherent pulsatility of the anterior pituitary cells (Gambacciani *et al.* 1987, Shin & Reifel 1981). The increase in the number of peaks during the studied period may suggest that the decrease in the circulating hormone is of such a magnitude (over 10 times), that stimulatory signals from the periphery try to overcome the inhibitory effects of the metal. In fact, considering the effect of cadmium on other pituitary hormones, the greatest changes may be ascribed to ACTH.

Furthermore, cadmium administration decreased the absolute amplitude of ACTH peaks, though neither relative pulse amplitude nor duration of the ACTH pulses nor mean half-life of the hormone decreased. However, this last parameter is not very important when studying the effects of cadmium on episodic ACTH release, because the half-life of this hormone may vary from one episode to another, and the different types of secretory episodes may reflect the multifactorial control of ACTH secretion by the hypothalamus (López & Negro Vilar 1988).

Nevertheless, the changes in absolute amplitude of ACTH peaks are by themselves enough to explain the decrease in the mean ACTH levels observed during the bleeding period; this agrees with previous works from our laboratory, with a dose of cadmium chloride of 3 mg kg^{-1} day^{-1} for eight days, and using a single sample protocol (Alvarez *et al.* 1996).

The effects described in this study suggest a multiple interactive mechanism of cadmium with the regulatory factors involved in ACTH secretion at both hypothalamic (Hrdina *et al.* 1976, Chandra *et al.* 1985, Nation *et al.* 1989, Das *et al.* 1993, Hagan & Brooks 1996) and hypophyseal levels (Lorenson *et al.* 1983), or through cytokines released by the immune cells (Teocharis *et al.* 1994). The changes in the episodic ACTH secretion exerted by cadmium administration may reflect alterations in other functions that are hormonally regulated, like the adrenal function (Mgbonyebi *et al.* 1993).

Other data from the literature suggest that corticotropin-releasing hormone (CRH) is responsible for amplitude modulation of ACTH pulses (Carnes *et al.* 1990); on the other hand, dopamine inhibits CRH-induced ACTH release (Murakami *et al.* 1984), while episodic ACTH release is tonically inhibited by dopaminergic pathways (Hagan & Brooks 1996). In addition, chronic cadmium treatment significantly increases the dopamine levels in various areas of the central nervous system (Nation *et al.* 1989). Therefore, the reduction in the absolute amplitude of ACTH pulses that we observed in treated animals could be through dopamine.

In conclusion, our results confirm that ACTH is secreted following an irregular episodic pattern, and suggest that acute cadmium administration might be able to inhibit the pulsatile release of ACTH through specific changes in the episodic parameters measured in this study. Further studies are needed to clarify through which hypothalamic neuromodulator(s) cadmium modifies the ACTH release.

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