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Cadmium does not inhibit pulsatile prolactin secretion through TRH

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This work was undertaken to analyse the effects of acute cadmium administration on the pulsatile pattern of prolactin release, in adult male rats. For this purpose, animals were cannulated 40 h before the experiment to allow a continuous blood withdrawal. Two hours after the administration of one dose of cadmium chloride (4.5 mg kg^{1}), the pulsatile pattern of prolactin, during three hours, was studied. The effects of two pulses of thyrotropin-releasing hormone (TRH) (1 μg per rat), given 60 and 120 min after starting the period of blood sampling, were studied. The mean values of prolactin during the bleeding period and the absolute pulse amplitude were decreased by acute cadmium chloride administration. However, no changes in any other parameters of prolactin pulsatility were observed. TRH administration to control rats increased mean prolactin levels, and absolute and relative pulse amplitudes, but decreased the mean half-life of the hormone. In animals pretreated with cadmium, TRH increased the mean levels of prolatin, and absolute and relative amplitudes of the hormone pulses. No other parameter studied was changed by TRH in cadmium pretreated rats. These data suggest that acute administration of cadmium did not inhibit the pulsatile prolactin release through TRH.

Keywords: cadmium, prolactin, pulsatile secretion, TRH

Introduction

Cadmium is a contaminant of food and drinks, and exhibits a high toxicity. The latter may be due to its wide distribution and numerous industrial uses in modern technology. It is likely that cadmium uptake through the food web creates a health risk for both humans and animals. Indeed, field and laboratory studies indicate that bioaccumulation of metals, including cadmium, occurs in primary and secondary consumers of the food web (Levine *et al*. 1989, Brueske & Barret 1991).

It has been shown that cadmium exposure produces a wide spectrum of disorders in diverse physiological processes (Friberg *et al*. 1986, WHO 1992). Reproductive organs and developing organisms are more likely than others to be damaged by cadmium (Clarkson *et al*. 1985). The effects of cadmium on gonadal function are well documented (Laskey & Phelps 1991, Paksy *et al*. 1992, Piasek & Laskey 1994).

However, little is known about possible direct effects of cadmium on pituitary hormone release. Cadmium exposure has been shown to cause changes in prolactin secretion, although controversial reports showing decreases (Zylber-Haran *et al*. 1982, Lorenson *et al*. 1983, Winstel & Callahan 1992) or increases in release have been reported (Cooper *et al*. 1987, Paksy *et al*. 1989). Cadmium may influence prolactin secretion at the hypophyseal level through a competition with calcium effects within the lactotrophs, as has been shown in other tissues (Waalkes & Poirier 1984, Milos *et al*. 1989). Cadmium may also interfere with the secretory

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granules of the hormone within the lactotrophs (Lorenson *et al*. 1983).

On the other hand, regulation of prolactin release is a complex process, and involves a great variety of hypothalamic (Negro-Vilar *et al*. 1984, Tuomisto & Manisto 1985) and non-hypothalamic (Tresguerres & Esquifino 1983, Esquifino *et al*. 1989) factors. It has been shown that cadmium administration (0.3 mg 100 g^{-1} body weight for two weeks) to adult male rats was able to reduce serotonin and acetylcholine contents in various discrete regions of the brain (Das *et al*. 1993). These neurotransmitters are involved in the modulation of prolactin secretion; thus, cadmium may influence pituitary hormone secretion through hypothalamic effects.

Another component of the regulatory mechanism of prolactin is thyrotropin-releasing hormone (TRH). This neuropeptide stimulates prolactin secretion *in vivo* (Deis & Alonso 1975, Martínez de la Escalera & Weiner 1988, Agrasal *et al*. 1989, Lafuente *et al*. 1994) and *in vitro* (Mitsuma *et al*. 1990, Arita *et al*. 1992). The mechanisms through which TRH stimulates prolactin release from pituitary cells have been widely studied, showing a calcium-dependent mechanism (Martínez de la Escalera & Weiner 1988, 1990, Bjoro *et al*. 1990, Login *et al*. 1991). All these data have been obtained using single sampling in the experimental designs. However, prolactin, like other pituitary hormones, is released in an episodic fashion (Shin & Chi 1979, López *et al*. 1989, Lafuente *et al*. 1992, 1993, 1996a, 1996b, Esquifino *et al*. 1996). Previous work from our laboratory demonstrated that TRH is involved in the modulation of the episodic release of prolactin in female rats (Lafuente *et al*. 1994). The results based on cadmium and prolactin interaction suggested that this metal may exert effects on several physiological functions through changes in the pulsatile secretion pattern of the hormone.

In a previous study we analysed the effects of a single dose of cadmium on adrenocorticotropin hormone (ACTH) pulsatile release (Lafuente & Esquifino 1998). The results indicate that the metal greatly influenced ACTH secretion (by lowering the mean levels 10 times). However, the circulating levels of prolactin were less affected by cadmium (Lafuente *et al*. 1996b). These differential effects accounted for different mechanisms of the metal in changing pituitary hormone secretion. Thus, we considered it of great interest to analyse the effect of cadmium on the pulsatile pattern of prolactin.

Considering the above mentioned data the aim of this work was to answer two questions: (1) if a single administration of cadmium was able to modify the pulsatile pattern of prolactin; and (2) if cadmium effects on the pulsatile pattern of the hormone are mediated by TRH.

Materials and methods

Animals

Adult male Sprague-Dawley rats weighing 250–260 g were used in all experiments. They were maintained in a room with controlled photo-period (14 h light/10 h darkness; lights on from 07.00 to 21.00 h) and temperature (22 + 2°C), and 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 100 g^{-1} body weight) and atrial cannulas were implanted through the external jugular vein according to procedures used in previous studies (Lafuente *et al*. 1992, 1993, 1994), which allow the animals to move freely in their cages during the period of bleeding.

Experimental design and blood sampling

Four experimental groups of six rats per group were used in this study. Groups 1 and 2 were 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 or with 0.9% NaCl solution. Group 3 was injected with 0.9% NaCl solution and stimulated with two intravenous pulses of 1 μg of TRH (Sigma Chemical Co., St Louis, MO, USA), 60 and 120 min after starting the period of blood sampling. Group 4 was treated with $CdCl_2$ (4.5 mg kg^{-1}) and with two pulses of 1 μg of TRH, 60 and 120 min after starting the period of blood sampling.

The dose of cadmium selected was based on doses used in the literature (Paksy *et al*. 1989, Katsuta *et al*. 1993, Piasek & Laskey 1994). This amount of the metal is within the low to medium range of the doses utilized previously, when acute administration designs were used to study toxic effects of the metal.

The blood was sampled by a method described before (Lafuente & Esquifino 1998).

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.

Prolactin measurements

Prolactin levels, in all series from each rat, were determined by a specific double-antibody radioimmunoassay. The reagents were kindly supplied by the NIDDK'S National Hormone and Pituitary Program and Professor A.F. Parlow (Habor, UCLA Medical Center, Torrance, USA). Prolactin values are expressed in terms of NIADD rat PRL RP-3 reference preparation. The sensitivity of the assay was 5 pg per tube. To analyse the variability of the

assay, a series of plasma consisting of 10 replicates at four different concentrations of the prolactin standard curve were run. At the level of 1.5 ng ml^{-1} the coefficient of variation was 9.1%, at the level of 6.25 ng ml^{-1} in the standard curve it was 6.4%, at the level of 12.5 ng ml^{-1} it was 6.0%, and at the level of 25 ng ml^{-1} in the standard curve it was lower (4.9%). Samples were analysed within the same assay to avoid interassay variations.

Data analysis

To identify and characterize prolactin 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, as in previous studies (Lafuente *et al.* 1993, 1996a, 1996b, Lafuente & Esquifino 1998).

Statistics

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

Results

Prolactin secretion in animals from the four experimental groups studied was pulsatile. A representative profile from one animal in each experimental group is shown in Figure 1.

Acute cadmium chloride administration significantly decreased mean serum prolactin levels and absolute amplitude of the prolactin pulses (Figures 2 and 3) as compared with control animals. No changes in any other parameters of prolactin pulsatility were observed by acute cadmium administration (Figures 4 to 7).

As expected, TRH administration increased the mean prolactin levels, and absolute and relative amplitudes of the prolactin pulses (Figures 2, 3 and 5)

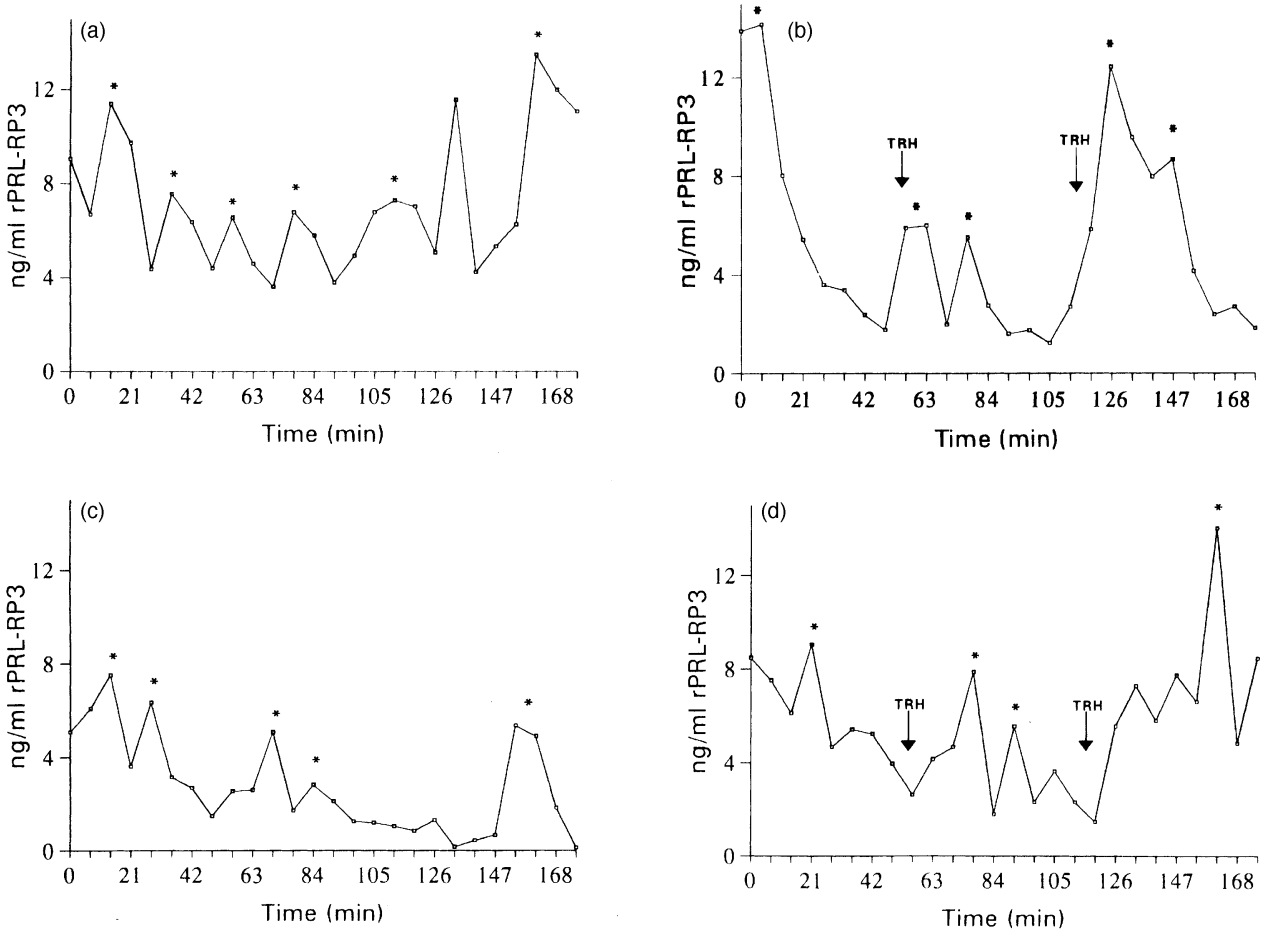

Figure 1. Basal individual pulsatile prolactin patterns in adult male rats treated with: (a) saline; (b) saline and TRH-stimulated secretion; (c) cadmium chloride at a dose of 4.5 mg kg^{-1} body weight; and (d) cadmium chloride and TRH-stimulated secretion. The asterisks indicate the prolactin peaks during the period studied.

as compared with control animals, while decreasing the mean half-life of the hormone (Figure 4). Administration of TRH did not change the duration and frequency of prolactin pulses (Figures 6 and 7) compared with control rats.

TRH administration in rats treated with cadmium chloride increased mean prolactin levels, and absolute and relative amplitudes of prolactin pulses (Figures 2, 3 and 5, respectively), while the mean half-life of the hormone decreased and the other parameters of prolactin pulsatility studied did not change (Figures 4, 6 and 7).

Discussion

Foregoing results suggest that acute cadmium administration (4.5 mg kg^{-1}) changes the pulsatile secretion pattern of prolactin previously described in adult male rats, and reduces the mean levels of the hormone. TRH stimulation tests in control rats confirmed the role of this neuropeptide in the regulation of the episodic secretion of prolactin (Lafuente *et al.* 1994). Surprisingly, cadmium administration did not change TRH effects on prolactin release, thus suggesting that cadmium effects on prolactin secretion were not mediated through this neuropeptide.

Control rats showed an irregular pulsatile secretion pattern of prolactin, which is characteristic of this hormone, and agrees with previous works from our laboratory (Lafuente *et al.* 1993, 1996a, 1996b) and from the literature (López *et al.* 1989).

TRH administration was followed by an increase in the absolute and relative amplitudes of the prolactin pulses. These changes may explain the increased mean values of the hormone during the period studied. These stimulatory effects of TRH on prolactin release in adult male rats are similar to those described in adult female rats (Lafuente *et al.* 1994) except for the duration of prolactin peaks, which are

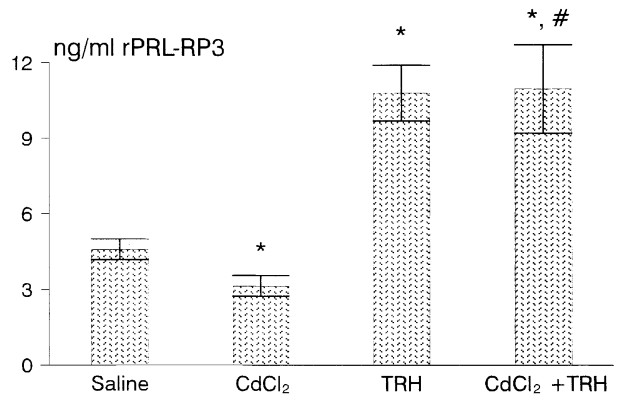

Figure 3. Absolute pulse amplitude of prolactin in adult male rats treated with saline, or with cadmium chloride at a dose of 4.5 mg kg^{-1} body weight; and TRH-stimulated secretion in male rats treated with saline, or with cadmium chloride. $*P \leq 0.05$ versus saline; $\#P \leq 0.05$ versus cadmium chloride.

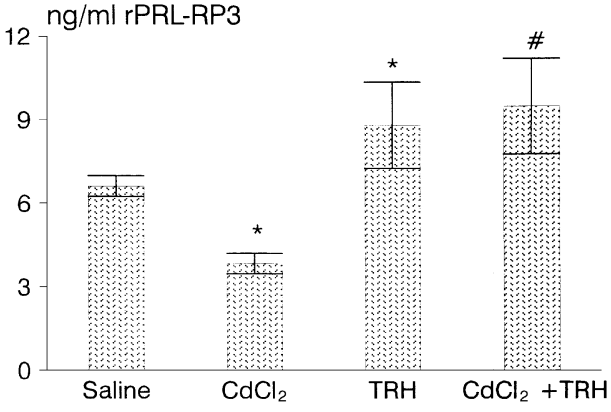

Figure 2. Mean serum prolactin levels in adult male rats treated with saline, or with cadmium chloride at a dose of 4.5 mg kg^{-1} body weight; and TRH-stimulated secretion in male rats treated with saline, or with cadmium chloride. $*P \leq 0.05$ versus saline; $\#P \leq 0.05$ versus cadmium chloride.

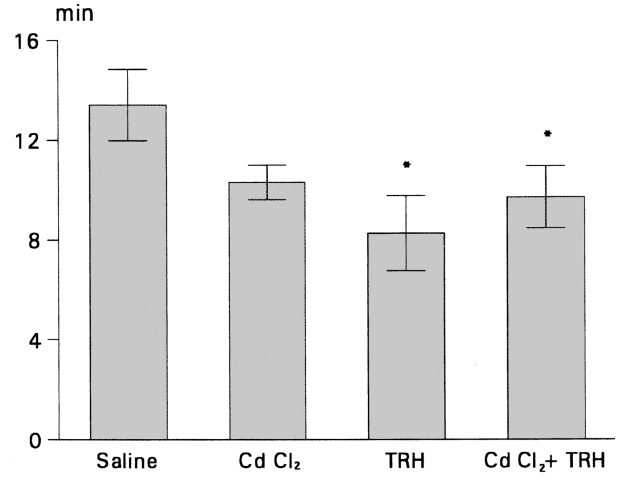

Figure 4. Mean half-life of prolactin in adult male rats treated with saline, or with cadmium chloride at a dose of 4.5 mg kg^{-1} body weight; and TRH-stimulated secretion in male rats treated with saline, or with cadmium chloride. $*P \leq 0.05$ versus saline.

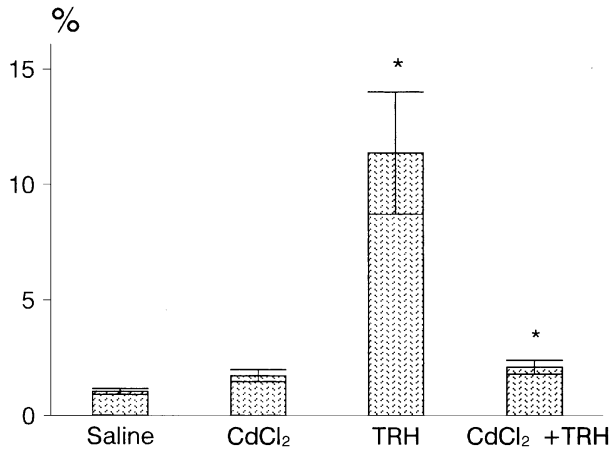

Figure 5. Relative pulse amplitude of prolactin in adult male rats treated with saline, or with cadmium chloride at a dose of 4.5 mg kg^{-1} body weight; and TRH-stimulated secretion in male rats treated with saline, or with cadmium chloride. $*P \leq 0.05$ versus saline.

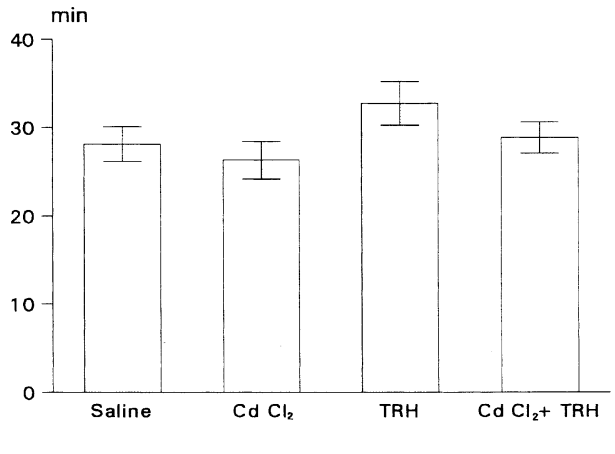

Figure 7. Pulse duration of prolactin in adult male rats treated with saline, or with cadmium chloride at a dose of 4.5 mg kg^{-1} body weight; and TRH-stimulated secretion in male rats treated with saline, or with cadmium chloride.

longer in female than in male rats, and for the degree of response, which was lower in male than in female rats (Lafuente *et al.* 1994). As in female rats, TRH did not change pulse frequency (Lafuente *et al.* 1994). The differences between male and female rats may be due to the estrogenic component of the prolactin regulatory mechanism, which is stronger in female than in male rats (Haisenleder *et al.* 1991). Other mechanisms mediated through dopamine (Martínez de la Escalera & Weiner 1990, Login *et al.* 1991) or through other hypothalamic factors, which were not studied in the work, cannot be excluded (Tuomisto & Manisto 1985), although TRH seemed not to act on prolactin secretion through changes in opiates or serotonin (Hylka *et al.* 1986).

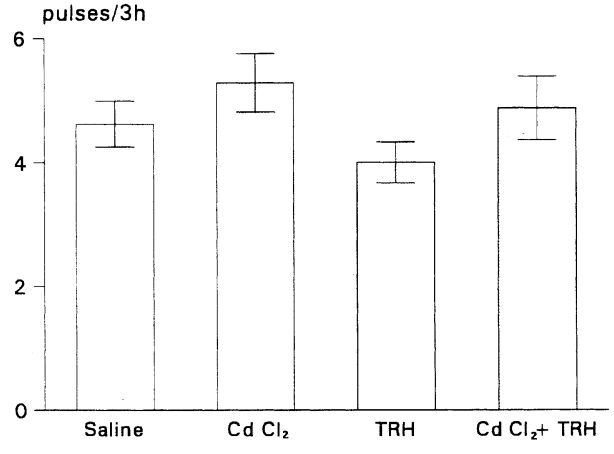

Figure 6. Frequency of the prolactin pulses in adult male rats treated with saline, or with cadmium chloride at a dose of 4.5 mg kg^{-1} body weight; and TRH-stimulated secretion in male rats treated with saline, or with cadmium chloride.

Cadmium-treated rats showed an irregular prolactin pulsatile pattern, with no changes in the number of peaks during the study period as compared with controls. However, cadmium decreased the absolute amplitude of prolactin pulses. These changes may explain the decrease in the mean prolactin levels observed during the bleeding period, which agrees with previous work from the literature (Zylber-Haran *et al.* 1982, Winstel & Callahan 1992). These changes have been obtained with a cadmium dose of 4.5 mg kg^{-1} body weight, which was similar to that used in previous studies (Zylber-Haran *et al.* 1982, Das *et al.* 1993). However, higher doses of cadmium increased serum prolactin levels (Paksy *et al.* 1989), thus suggesting a dose-dependent effect of cadmium on prolactin secretion.

The inhibitory effect of cadmium on prolactin secretion shown in this study may be explained by changes in the dopaminerg ic tone. In fact, a reduction in the prolactin response to metoclopramide in cadmium-treated rats has been reported (Zylber-Haran *et al.* 1982). A reduction in hypothalamic serotonin content has also been observed after cadmium treatment (Das *et al.* 1993), suggesting that this metal may affect prolactin secretion through serotonin metabolism changes.

On the other hand, cadmium may act directly on the lactotrophs, through an interaction with a disulphide group of the amino terminal of the

prolactin molecule, thus inhibiting the release of the hormone from the storage granules, as this form of storage is sensitive to divalent metals (Lorenson *et al*. 1983). However, the normal response after TRH administration may suggest that cadmium does not compete with calcium at the hypophyseal level, as was previously suggested (Waalkes & Poirier 1984, Milos *et al*. 1989). This affect disagrees with the *in vitro* results, showing that cadmium treatment significantly decreased TRH-stimulated prolactin release from the anterior pituitary lactotrophs (Winstel & Callahan 1992). This discrepancy may be due to differences in the experimental models used (*in vitro* versus *in vivo* in this study). In *in vivo* experimental designs, the pituitary gland is under the regulatory influence of the hypothalamus, which is not present in *in vitro* studies. All these data and those obtained in this study suggest that cadmium effects on prolactin secretion may be exerted mainly at the hypothalamic level. The data may also indicate that changes in prolactin secretion exerted by cadmium administration may reflect alterations in other functions that are regulated by the hormone, like the immune system (Esquifino *et al*. 1991, Reber 1993, Arce *et al*. 1997).

In conclusion, our results confirm that prolactin is secreted following an irregular episodic pattern (Lafuente *et al*. 1993, 1996a, 1996b) and that TRH stimulates prolactin secretion through changes in the pulsatile parameters. Acute cadmium administration inhibits the episodic release of prolactin through specific changes in the pulsatile parameters measured in this study. Cadmium did not inhibit prolactin response to TRH, thus suggesting that TRH and cadmium modify the pulsatile pattern of prolactin release through different mechanisms.

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