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# Changes in prolactin secretion in the short- and long-term after adrenalectomy

*Efeito da evolução temporal da adrenalectomia na secreção de prolactina*

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## ABSTRACT

**Objective:** To evaluate the modulation of the hypothalamus-pituitary-adrenal axis (HPA) on prolactin secretion in rats after adrenalectomy (ADX). **Materials and methods:** Plasma corticosterone, ACTH, and prolactin concentrations were measured by radioimmunoassay in rats after bilateral ADX in the short- (3 hours and 1 day) and long-term (3, 7, and 14 days). **Results:** Animals that underwent ADX showed undetectable corticosterone levels and a triphasic ACTH response with a transient increase (3h), a decrease (1d), and further increase in the long-term after ADX. Sham animals showed a marked increase in corticosterone and ACTH levels three hours after surgery, with a decrease to basal levels thereafter. Plasma prolactin levels were not changed after ADX. **Conclusion:** There are different points of equilibrium in the HPA axis after the glucocorticoid negative feedback is removed. Prolactin plasma secretion is not altered in the short or long-term after ADX, suggesting that the peptidergic neurons essential for prolactin release are not activated after ADX. *Arq Bras Endocrinol Metab.* 2012;56(4):244-9

## Keywords

Adrenalectomy; prolactin; corticosterone; adrenocorticotrophic hormone

## RESUMO

**Objetivo:** Avaliar a modulação do eixo hipotálamo-hipófise-adrenal (HHA) sobre a secreção de prolactina após adrenalectomia (ADX). **Materiais e métodos:** Quantificamos por RIE corticosterona, ACTH e prolactina plasmáticos em ratos após curtos (3 horas e 1 dia) e longos (3, 7 e 14 dias) períodos de ADX bilateral. **Resultados:** Animais ADX mostraram níveis indetectáveis de corticosterona. As concentrações plasmáticas de ACTH apresentaram resposta trifásica: aumento transitório (3h), diminuição (1d) e novo aumento após longos períodos de ADX. Animais Sham mostraram aumento de corticosterona/ACTH após três horas de cirurgia, diminuindo posteriormente aos níveis basais. As concentrações plasmáticas de prolactina não se alteraram após ADX. **Conclusão:** Existem diferentes pontos de equilíbrio do eixo HHA após a remoção da retroalimentação negativa exercida pelos glicocorticoides. A secreção de prolactina não se alterou após curtos/longos períodos de ADX, sugerindo que os neurônios peptidérgicos essenciais para a liberação de prolactina não estão ativados durante os diferentes períodos de ADX. *Arq Bras Endocrinol Metab.* 2012;56(4):244-9

## Descritores

Adrenalectomia; prolactina; corticosterona; hormônio adrenocorticotrófico

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## INTRODUCTION

Prolactin is a polypeptide hormone synthesized and secreted from specialized cells of the anterior pituitary gland, called lactotrophs. In humans, an alternative

promoter can drive the expression of this hormone also in extra-pituitary sites. Besides its biological actions in the uterus and mammary gland, an important role of prolactin has been well established in the modulation

of the immune system activity, and in the maintenance of the internal environment by regulation of osmotic balance in some species (1,2). More recently, circulating and locally-produced PRL has also been implicated as risk factors for cancer development (3,4).

Although hypothalamic dopamine provides inhibitory control over prolactin synthesis and secretion, other factors produced in the brain, pituitary gland, and peripheral organs have also been shown to inhibit or stimulate prolactin secretion. In addition, prolactin itself can act on the hypothalamus to regulate its own secretion by means of a feedback mechanism (1,2). Prolactin-releasing stimuli not only include nursing, but also light, noise, smells, and stress (2,5). Prolactin secretory response differs depending on the stress stimulus, such as ether (6,7), restraint (6,8-12), thermal stress (13), hemorrhage (9), social conflict (14), and even academic stress in humans (15).

Prolactin immunoreactivity is found in numerous hypothalamic areas in a variety of mammals. In the rat hypothalamus, prolactin immunoreactivity is detectable in the dorsomedial, ventromedial, supraoptic, and paraventricular (16,17) nuclei. Vasopressin and oxytocin are synthesized in the magnocellular neurons located mainly in the posterior division of the paraventricular and supraoptic nuclei, and may be involved in the regulation of prolactin secretion (18,19). Previous studies have clearly indicated that disturbance in water and electrolyte regulation alters adeno-hypophysial prolactin secretion (20). We have previously studied the effect of acute extracellular volume expansion by intravenous injection of isotonic or hypertonic saline on the secretion of prolactin, corticosterone, vasopressin, oxytocin, and atrial natriuretic peptide. We demonstrated that the increment in prolactin and oxytocin was blocked by the inhibition of the hypothalamus-pituitary-adrenal (HPA) axis by dexamethasone (21). Therefore, prolactin and oxytocin responses induced by isotonic or hypertonic extracellular volume expansion are likely to be modulated by the HPA axis.

HPA axis is regulated by circadian rhythm, stressful stimuli, and glucocorticoid negative feedback. Hypothalamic-pituitary activity is dramatically enhanced after adrenalectomy (ADX), which causes changes in pituitary corticotrophs, and might play a role in prolactin response after stress (22-24). Therefore, in the present study, we evaluated the modulation of HPA axis on plasma prolactin secretion in rats in the short- and long-term after ADX.

## MATERIALS AND METHODS

### Animals and experimental design

Adult male Wistar rats weighing 200 g were housed in individual cages (30 x 19 x 13 cm), in a room provided with temperature ( $23 \pm 1^\circ\text{C}$ ) and light control (12h light – 12h dark cycle) with free access to pelleted food and tap water. All experimental protocols were performed according to the guidelines of the Ethics Committee for Animal Use of the School of Medicine of Ribeirao Preto, Universidade de Sao Paulo (protocol no. 056/2005). Bilateral ADX and Sham surgery were performed under ether anesthesia using the dorsal approach. ADX rats received oral saline (0.9% NaCl) *ad libitum*. Animals were studied in basal condition at different times (3h, and 1, 3, 7, and 14 days) after Sham surgery or ADX. Animals were killed by decapitation between 8:30 and 10:30 a.m. Blood was collected from the trunk into heparinized plastic tubes, and centrifuged at  $4^\circ\text{C}$ . Plasma samples were frozen at  $-20^\circ\text{C}$  until ACTH, corticosterone (B), and prolactin (PRL) determinations.

### Assays and methods

Plasma corticosterone and ACTH were determined by radioimmunoassay (RIA), after plasma extraction using ethanol or silicic acid, respectively (25). Plasma PRL was determined using the NIDDK RIA reagents, and expressed in terms of RP-1 reference preparations (26). Mean assay sensitivity was  $0.4 \mu\text{g/dL}$  for corticosterone,  $7.7 \text{ pg/mL}$  for ACTH, and  $0.4 \text{ ng/mL}$  for PRL. The intra- and inter-assay coefficients of variation (CV) were 4.8% and 6.7% for corticosterone, 6.3% and 14.0% for ACTH, and 11.7% and 5% for PRL. All samples were analyzed in duplicate.

### Statistical analysis

Data are expressed as means  $\pm$  SEM. Data were compared using the Mann-Whitney test or analysis of variance (Kruskal-Wallis test and Dunn's post-test). Significance was set at  $p < 0.05$ .

## RESULTS

### Corticosterone concentration

There was a significant increase in plasma corticosterone levels 3h after Sham surgery compared with 1, 3, 7, and 14 days ( $p < 0.05$ ), with no difference in plas-

ma corticosterone levels between 1, 3, 7, and 14 days (Figure 1A). Sham groups showed higher corticosterone levels than ADX groups throughout the study ( $p < 0.004$ ). ADX groups showed undetectable corticosterone levels at all periods studied.

### ACTH concentration

ACTH plasma levels after 3h, 1 and 3 days of Sham surgery were similar, and all of them were higher than those observed after 7 and 14 days ( $p < 0.05$ ; Figure 1B). Plasma ACTH showed a triphasic response to bilateral ADX, with a transient increase after 3h ( $p < 0.0001$ ), followed by a decrease after 1 day ( $p < 0.0001$ ), and a further increase after 3, 7, and 14 days ( $p < 0.001$ ). Plasma ACTH levels were higher in ADX groups compared with the Sham group in all periods studied ( $p < 0.008$ ).

### Prolactin (PRL) concentration

The Sham group did not show difference in PRL levels after 3 hours ( $2.8 \pm 0.9$ ), 1d ( $5.9 \pm 3.2$  ng/mL), 3d ( $3.8 \pm 1.7$  ng/mL), 7d ( $4.3 \pm 2.9$  ng/mL), and 14d ( $3.2 \pm 1.6$  ng/mL). The same was also observed in plasma PRL levels in ADX rats after 3h ( $4.9 \pm 4.1$  ng/mL), 1d ( $5.3 \pm 2.3$  ng/mL), 3d ( $3.9 \pm 1.9$  ng/mL), 7d ( $5.2 \pm 3.5$  ng/mL), and 14 days ( $3.3 \pm 1.9$  ng/mL) (Figure 1C). No difference was observed between ADX and Sham groups at the same moment, at all time-points of the study.

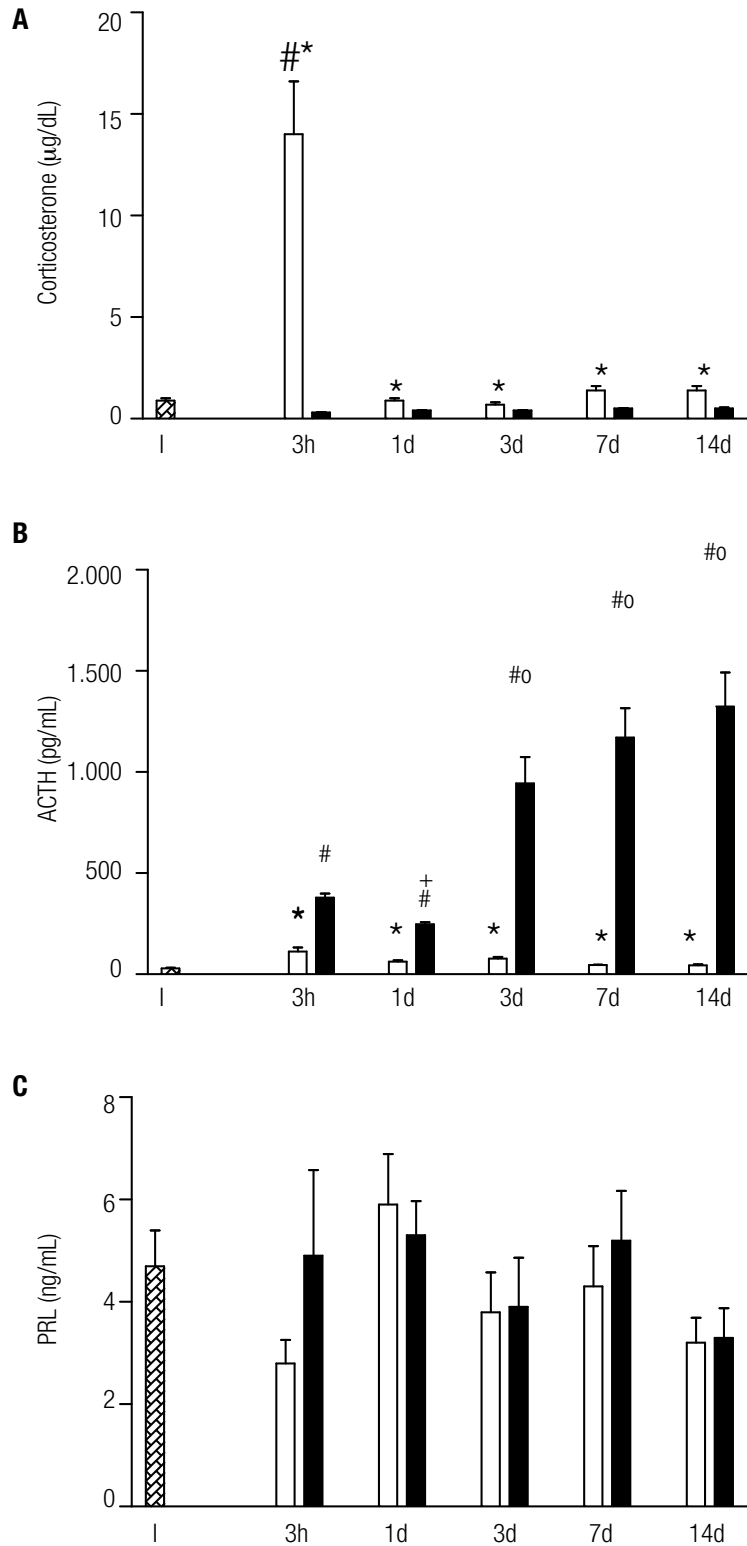
## DISCUSSION

The hypothalamic paraventricular nucleus (PVN) has been shown to be important for PRL secretion, and may play a role in the suppression of tuberoinfundibular dopamine neurons (27,28). Furthermore, the presence of CRH receptors have been demonstrated in dopamine neurons, suggesting a possible role of CRH in the regulation of PRL secretion (29). Stress mediators – CRH and vasopressin – are essential not only for ACTH release in response to stress, but also in ACTH release after ADX. Therefore, we hypothesized that prolactin secretion could be affected in the short- and long-term after ADX. In the present study, we observed no changes in plasma prolactin concentrations in the short- and long-term after ADX. As expected, these animals showed undetectable corticosterone levels, and the classic ACTH triphasic response to the removal of glucocorticoid feedback.

ADX is an interesting experimental model, since it alters CRH/vasopressin expression in the PVN, induces hydroelectrolytic imbalance, and represents a stress model that may modulate PRL secretion. Our data demonstrated a triphasic response pattern of ACTH levels induced by ADX: at first, there was a marked increase in ACTH release, leading to depletion of pituitary ACTH storage, which explains the decrease in circulating ACTH levels during the second phase. Finally, compensatory changes occur, the most important being an increase in ACTH synthesis, as confirmed by increased pituitary ACTH content and pro-opiomelanocortin mRNA expression (19,24,25). These findings indicate a profound alteration in the HPA axis induced by ADX in the short- and long-term.

Different stress models might determine the role of hypothalamic factors in ACTH and/or prolactin responses. Previous data have demonstrated increased serum PRL stimulated by ether stress, with maximum effect one minute after the onset of stress (30). In addition, reports have shown that ADX potentiates prolactin release in response to ether stress (31); this increment usually occurs after 5 to 10 minutes, and remains significantly higher until 30 min after stress compared with controls (32). In the present study, prolactin levels did not differ between intact animals and those in the short-term after ADX (3 hours). However, as mentioned, physiological responses to ether stress are usually observed within few minutes (30,31). In this context, the lack of PRL response 3 hours after ADX may not reflect PRL immediate response. In addition, we cannot rule out that, in our model, PRL levels had already returned to basal levels. Previously, our group described an increase in OT concentration 3 hours after ADX (19,24,25). It is important to point out that subcutaneous administration of oxytocin induces a reduction in basal, as well as in stress-induced prolactin secretion, in male rats (33-35). Therefore, increased OT levels observed at this moment could also compensate the expected increase of prolactin plasma secretion after ether and surgical stress (18).

We also demonstrated that prolactin plasma levels did not change in the long-term after removal of the glucocorticoid negative feedback, suggesting that basal prolactin secretion is not modulated by long-term ADX or by glucocorticoids. However, we cannot rule out the effect of plasma hypoosmolality observed in the long-term after ADX. Indeed, we previously reported a significant decrease in plasma osmolality associated



# p < 0.01 (ADX vs. intact group), \* p < 0.008 (Sham vs. ADX); + p < 0.0001 (ADX 1D vs. ADX 3H, 3D, 7D, and 14D); p < 0.0001 (ADX 3H vs. ADX 3D, 7D, and 14D).

**Figure 1.** Plasma corticosterone (A), ACTH (B), and prolactin (C) levels in intact animals (I; hatched boxes), 3 hours (h); 1, 3, 7, and 14 days (d); after sham surgery (empty boxes) or adrenalectomy (ADX; filled boxes). The numbers of animals in each group varied from 5 to 25.

with severe dehydration in rats that underwent ADX after 3, 7, and 14 days (24). Moreover, chronic hypoosmolality inhibits PRL secretion induced by the stress of restraint, indicating the involvement of magnocellular AVP neurons in these responses (36).

Although AVP induces prolactin release *in vivo* (37), we did not observe changes in plasma AVP throughout the time course after ADX surgery (24), suggesting that this peptide might not have a role in prolactin secretion in this experimental model. We and others have shown an increase in plasma PRL induced by increased water and salt ingestion or after hypertonic EVE infusion (21,38). However, the increase in PRL and OT levels after isotonic or hypertonic EVE were blocked by glucocorticoid treatment. These results are similar to those of a previous report that showed that glucocorticoids exert an inhibitory effect on parvocellular OT-expressing neurons (39), PRL and OT secretion induced by volume and concentration changes.

Lesions in the PVN of rats strongly inhibited ether-induced ACTH secretion. In contrast, PVN lesion failed to inhibit ether-induced PRL release, suggesting that the peptidergic neurons essential for stress-induced PRL release are modulated by neurons other than PVN neurons (7). In addition, prolactin-releasing peptide (PrRP), expressed in the nucleus *tractus solitarii* and in the ventrolateral medulla oblongata, has been shown to induce prolactin secretion and plays an important role in the stress response (40).

In conclusion, there are different HPA axis points of equilibrium after the glucocorticoid negative feedback is removed. Although prolactin is a hormone responsive to different stress factors, it does not seem to be altered in the short or long-term after ADX, suggesting that the peptidergic neurons essential for basal prolactin release are not activated during time course after ADX.

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