Role of the adrenal cortex in chronic stress-induced inhibition of prolactin secretion in male rats

A. López-Calderón, C. Ariznavarreta, M. D. Calderón, J. A. F. Tresguerres and M. I. Gonzalez-Quijano

Department of Physiology, Faculty of Medicine, University Complutense, 28040-Madrid, Spain

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ABSTRACT

The response of prolactin to chronic stress in intact, adrenalectomized and adrenomedullectomized male rats was studied. Immobilization stress in intact animals induced a significant increase in plasma concentrations of prolactin after 20 and 45 min and a significant decrease when the rats were submitted to chronic restraint (6 h daily for 4 days). Five weeks after adrenomedullectomy, plasma prolactin and corticosterone responses to chronic stress were not modified. In contrast, the inhibitory effect of chronic stress on prolactin secretion was totally suppressed by adrenalectomy. When treated with dexamethasone during the 4 days of restraint, adrenalectomized stressed rats showed similar plasma concentrations of prolactin to the intact stressed rats. These data indicate that the adrenal cortex is able to play an inhibitory role on prolactin secretion during stress only through a prolonged release of glucocorticoids.


INTRODUCTION

It is generally accepted that plasma concentrations of prolactin are increased during acute stress (Kruilich, Hefco, Illner & Read, 1974; Riegle & Meites, 1976). However, stress does not always result in stimulation of prolactin secretion, since a decrease in plasma concentrations of prolactin has been observed when concentrations have already been raised (Krieg, Lamberts & MacLeod, 1984; Gala & Haisenleder, 1986) or during chronic stress (Collu, Tache & Ducharme, 1979).

It has been suggested that adrenal secretion may be involved in the response of prolactin to stress. Adrenalectomy potentiated and glucocorticoid administration blunted the increase in plasma prolactin during acute stress (Euker, Meites & Riegle, 1975; Harms, Langlier & MacCann, 1975; Yelvington, Weiss & Ratner, 1984). However, its role in the paradoxical suppression of high afternoon plasma concentrations of prolactin induced by acute stress in female rats is controversial. Whereas Gala & Haisenleder (1982) found that the decrease in prolactin was similar in adrenalectomized rats to that observed in intact animals, Krieg et al. (1984) reported that paradoxical suppression of prolactin by acute stress was mediated by a catecholaminergic mechanism, the adrenal gland being essential for the effect.

Whether hypersecretion by the adrenal medulla or cortex is involved in prolactin inhibition during chronic stress has not been directly tested. This study was performed in order to elucidate the role played by the adrenal medulla and cortex in chronic stress-induced suppression of prolactin secretion.

MATERIALS AND METHODS

Animals and stress procedure

Male Wistar rats weighing around 350 g were housed three or four per cage under controlled conditions of light (lights on from 08.00 to 20.00 h) and temperature (22 ± 2 °C). They had free access to water and Panlab (Barcelona, Spain) rat chow pellets.

Rats subjected to chronic stress were removed from their cages and restrained in a small flexible wire mesh container for 6 h daily (from 10.00 to 16.00 h) over 4 consecutive days. Control animals were left in their cages, without food or water. At the end of the last immobilization period, rats were killed by decapitation between 16.00 and 16.35 h in a separate room (within 25 s after starting the procedure). Trunk blood
was collected into heparinized tubes and plasma was obtained after 10 min of centrifugation in a refrigerated centrifuge. Samples were stored at -20 °C until analysed.

**Experiment I**

Plasma concentrations of prolactin were studied in rats stressed for different periods of time. All rats were killed at 16.00 h, after being stressed for 0, 20, 45, 180 or 360 min or after 6 h of daily immobilization over 2, 3 or 4 days.

**Experiment II**

To determine the possible role of adrenal secretion in the prolactin response to stress, 40 rats were adrenalectomized or sham-operated under light ether anaesthesia. Adrenalectomized animals received 0.9% (w/v) NaCl in their drinking water. Four days later, half of the animals in each group were restrained over 4 consecutive days as described above.

**Experiment III**

Adrenomedullectomy was performed to analyse the role of the adrenal medulla and cortex on the response of prolactin to chronic stress. Twenty-two rats were adrenomedullectomized and 20 sham-operated. To perform the adrenomedullectomy, each adrenal gland was gently held and a small cut made in one of the poles with fine scissors. The medulla was then extruded through this cut by gentle compression of the adrenal body. Adrenomedullectomized animals were maintained with physiological saline instead of drinking water for 1 week. Six weeks after the operation, the adrenomedullectomized and the sham-operated rats were restrained over 4 days. Histological examination of the adrenal gland was carried out in order to verify the effectiveness of the adrenomedullectomy surgery, and the rats with evident remains of medullary tissue were eliminated from the experiment (14% of the whole adrenomedullectomized group).

**Experiment IV**

The effect of dexamethasone treatment in adrenalectomized animals was studied in order to elucidate whether the effect of adrenalectomy on the prolactin response to stress could be due exclusively to the absence of glucocorticoids. Forty rats were adrenalectomized and 20 sham-operated. Starting on day 4 after operation, the adrenalectomized animals were injected s.c. with 500 μg dexamethasone/kg (Decadran; Merck Sharp and Dohme, Madrid, Spain) or saline (250 μl) at 10.00 h. Sham-operated animals were injected with saline. Rats were submitted to chronic stress and dexamethasone treatment over 4 days.

**Assays**

Plasma concentrations of prolactin were measured by a double-antibody radioimmunoassay using reagents provided by the NIADDK and NHPP (School of Medicine, University of Maryland, Baltimore, MD, U.S.A.). Results are expressed as rat-PRL-RP-3; sensitivity of the assay was 0.05 ng/ml and the intra-assay coefficient of variation was 6%. Plasma concentrations of corticosterone were determined by a competitive protein-binding assay as described previously (Mancheño, Duran & Oriol Bosch, 1975).

**Statistical analysis**

Student's *t*-test was performed to compare control and stressed but otherwise identically treated groups (prolactin levels in experiments II and III), and one-way analysis of variance followed by Tukey's multiple comparison test when more than two experimental groups were compared among themselves (prolactin levels in experiments I and IV and corticosterone in experiments I and III).

**RESULTS**

**Experiment I**

The stress of immobilization caused a significant increase in plasma concentrations of both prolactin and corticosterone by 20 and 45 min respectively (Fig. 1). Plasma concentrations of prolactin began to decline after 90 min of restraint, and at 360 min they were lower than basal values. Plasma concentrations of corticosterone maintained a significant (*P* < 0.01) increase over basal values throughout the period studied. In contrast, chronic immobilization for 2, 3 and 4 days lowered plasma prolactin (Fig. 2).

**Experiment II**

The effect of adrenalectomy on the prolactin response to chronic stress is shown in Fig. 3a. Chronic stress decreased plasma concentrations of prolactin in sham-operated rats (*P* < 0.05) but had no effect in adrenalectomized rats.

**Experiment III**

The effect of adrenomedullectomy on plasma prolactin response to chronic stress can be seen in Fig.
3b. Both adrenomedullectomized and sham-operated groups showed a similar decrease in plasma prolactin when they were submitted to chronic stress. Plasma concentrations of corticosterone were also statistically equivalent in adrenomedullectomized and sham-operated rats, both basally (443 ± 67 vs 550 ± 58 (S.E.M.) nmol/l) and after chronic stress (1160 ± 84 vs 1040 ± 110 nmol/l).

**FIGURE 1.** Plasma concentrations of prolactin (●) and corticosterone (○) in male rats during the first 360 min of restraint. Values are means ± s.e.m., n = 10.

**P < 0.01** compared with control levels (one-way analysis of variance).

**FIGURE 2.** Plasma concentrations of prolactin (open bars) and corticosterone (stippled bars) in male rats subjected to restraint stress (6 h daily) for 0 (unstressed), 2, 3 and 4 days. Values are means ± s.e.m., n = 8.

**P < 0.05,**

**P < 0.01** compared with unstressed rats (one-way analysis of variance).
Experiment IV

Dexamethasone administration to adrenalectomized rats significantly ($P<0.01$) reduced plasma prolactin in unstressed rats (Fig. 4). In stressed groups, adrenalectomized saline-treated rats had higher concentrations of prolactin than sham-operated saline-treated rats ($P<0.01$). The effect of adrenalectomy on the prolactin response to stress was reversed by dexamethasone treatment, since adrenalectomized dexamethasone-treated rats had similar plasma prolactin levels to those of sham-operated saline-injected rats but significantly ($P<0.01$) lower concentrations than adrenalectomized saline-treated rats (Fig. 4).

DISCUSSION

In accordance with previous reports (Krulich et al. 1974; Kawakami, Higuchi & Matsuura, 1979), this study suggests that prolactin is secreted during restraint stress in a biphasic pattern, with an early stimulatory phase, followed by a decrease. Restrain-induced prolactin decrease seems not to be due to simple habituation or tolerance of neural mechanisms as has been postulated (Fekete, Kanyicska, Szentendrei et al. 1984), since a significant further reduction in plasma prolactin is observed when the period of stress is prolonged. In addition, the decrease in plasma prolactin after chronic restraint disappears when the animals are adrenalectomized. This finding suggests

**FIGURE 3.** Effect of (a) adrenalectomy (ADX) and (b) adrenomedullectomy (AMEX) and sham-operation (SHAM) on plasma concentrations of prolactin in response to immobilization stress (shaded bars) (6 h daily over 4 days). Values are means $\pm$ s.e.m., $n=8$.

$^*P<0.05$ compared with respective unstressed groups (open bars) (Student's $t$-test).

**FIGURE 4.** Effects of adrenalectomy and dexamethasone treatment on plasma concentrations of prolactin in control and stressed rats. Rats were sham-operated and treated with saline (stippled bars), adrenalectomized and treated with saline (open bars) or adrenalectomized and treated with 500 $\mu$g dexamethasone (hatched bars). Values are means $\pm$ s.e.m., $n=10$.

$**P<0.01$ compared with adrenalectomized saline-treated group (one-way analysis of variance).
that stress-induced increase in adrenal secretion has an active suppressive effect on plasma concentrations of prolactin.

Adrenomedullectomy alone did not modify either prolactin inhibition or the increase in plasma corticosterone during chronic stress. Our results therefore indicate that the chronic stress-induced decrease in plasma prolactin is not mediated by adrenal catecholamines. This hypothesis is supported by in-vitro studies showing that adrenaline and noradrenaline do not have an inhibitory effect; moreover, they can increase prolactin release by acting directly on pituitary β-adrenergic receptors (Baes & Denef, 1982). All these data indicate that peripheral catecholamines from the adrenal do not seem to be involved in chronic stress-induced prolactin inhibition.

The blockade of prolactin inhibition induced by adrenalectomy must be due to the lack of glucocorticoids, since adrenalectomized dexamethasone-treated rats showed similar plasma prolactin levels to intact stressed rats.

The inhibitory effect of glucocorticoids on prolactin secretion is well documented. Glucocorticoids inhibit basal plasma prolactin secretion both in vivo (Bratusch-Marrain, Vierhapper, Waldhausl & Nowotny, 1982; López-Calderón, Esquifino & Tresguerres, 1984; Oosterom, Verleunt, Zuiderwijk et al. 1985) and in vitro (Leung, Chen, Verkaik et al. 1980; Naes, Haug & Gautvik, 1980) as well as the increase in plasma prolactin during acute stress (Euker et al. 1975; Harms et al. 1975; Jobin, Ferland & Labrie, 1976; Rossier, French, Rivier et al. 1980). The observed decrease in plasma prolactin during stress must be due to the maintenance of enhanced corticosterone levels as have been confirmed in this study.

In conclusion, these data indicate that chronic restraint has an inhibitory effect on prolactin secretion due to the increase in glucocorticoid secretion.

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