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Mifepristone Treatment Demonstrates the Participation of Adrenal Glucocorticoids in the Regulation of Oestrogen-induced Prolactin Secretion in Ovariectomized Rats

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Accumulated evidence indicates that the adrenal cortex is able to regulate prolactin (PRL) secretion in rats. The aim of this study was to determine the participation of adrenal steroids on the regulation of PRL release in ovariectomized (OVX) and oestrogen-treated rats, by using mifepristone or a specific progesterone antiserum. Blood samples were obtained at 13:00 and 18:00 h 3 days after priming with oestradiol benzoate (OB). A significant increase in serum PRL at 13:00 and 18:00 h was induced by OB treatment. The administration of mifepristone to OVX and oestrogen-primed rats enhanced serum PRL increase at 13:00 h, without modifying the values at 18:00 h; while the administration of progesterone antiserum did not modify PRL levels, indicating that the effect of mifepristone on PRL secretion is due to its antiglucocorticoid action. Adrenalectomy induced a release of PRL at 13:00 h similar to that observed in the OVX and oestrogen-primed rats after mifepristone administration. Treatment with a low dose of progesterone (0.1 mg/rat) to OVX, adrenalectomized and oestrogen-primed rats did not modify the effect of adrenalectomy in serum PRL. Progesterone (2 mg/rat) given at 08:00 h to OVX and oestrogen-primed rats increased serum PRL 5 h later. Mifepristone treatment partially reverted the PRL increase induced by progesterone. These results suggest that after a previous sensitization of the pituitary by oestrogen, circulating glucocorticoids may exert a direct inhibitory effect on PRL release. This inhibition takes place at 13:00 h on day 3. On the other hand, the lack of effect of mifepristone or adrenalectomy on the PRL release at 18:00 h may also indicate that neither progesterone nor glucocorticoids modify PRL release induced by oestrogen at this time.

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INTRODUCTION

The participation of ovarian steroids in the release of gonadotrophins and prolactin (PRL) has been well established [1–3]. Caligaris *et al.* [4] showed that in long-term ovariectomized (OVX) virgin rats, the administration of oestradiol benzoate (OB) was capable of inducing PRL release in a circadian rhythm, with high levels in the afternoon and discrete changes in the morning. A dual action of progesterone on PRL secretion has been shown in OVX and oestrogen-treated rats [4], in pseudopregnant [5] and in pregnant rats [6].

Most studies on the hormonal regulation of PRL release have focused on the role of the ovarian steroids. However, accumulated evidence indicates that the rat adrenal cortex is able to regulate PRL secretion [7, 8]. Thus, serum PRL increases after adrenalectomy in female rats [9–11]. It has been suggested that the rise of PRL after adrenalectomy might be due to the removal of a direct inhibition by corticosterone [10]. On the other hand, it was recently found that changes in circulating progesterone, mostly of adrenal origin, modulate PRL secretion in response to stress [12, 13].

The aim of the present study was to determine the participation of adrenal steroids on the regulation of PRL release in OVX and oestrogen-primed rats by using mifepristone and a specific progesterone antiserum.

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EXPERIMENTAL

Animals

Virgin female rats, 3–4 months old (200–220 g) bred in our laboratory and originally of the Wistar strain were used. The rats were kept in a light- (lights on 06:00–20:00 h) and temperature-controlled room; rat chow (Nutric, Cordoba, Argentina) and tap water were available *ad libitum*.

Surgical procedures

All the animals were OVX when adult (3 months old) and used 14–16 days later. A group of rats was also adrenalectomized 2 days after priming with oestradiol. All surgical procedures were performed under ether anaesthesia between 08:00 and 10:00 h. Ovariectomy and adrenalectomy were performed through two dorsolateral incisions. The adrenalectomized rats were provided with an ample supply of 0.9% (w/v) NaCl as drinking water after the operation. Blood samples were obtained by a single cardiac puncture of the conscious animals or by decapitation. In experienced hands, cardiac puncture is a simple method that takes only a few seconds, insufficient to produce significant changes in hormone release detectable in the blood sample [12–14]. All the rats were accustomed to being handled daily to minimize the effect of handling stress. All work was in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH publication No. 86-23, revised 1985). Blood was allowed to clot at room temperature and serum was separated and stored frozen (−30°C) until assayed for PRL.

Drugs

OB (20 μg/rat: Schering, Buenos Aires, Argentina) and progesterone (2 mg/rat: Roussel-Uclaf, Romaineville, France) were given s.c. in 0.2 ml of purified sunflower seed oil at the times indicated in the text. Mifepristone (RU-38486: 17β-hydroxy-11β-(4-dimethylamino-phenyl)17α-(prop-1-ynyl)oestra-4,9-dien-3-one, kindly provided by M. Garnier, Roussel-Uclaf, Romaineville, France) was dissolved in sunflower seed oil at 10 mg/ml and injected s.c. at a dose of 10 mg/kg. Control rats were injected with the vehicle alone.

OVX animals received a single s.c. injection of vehicle (oil) or OB at 13:00 h. This day was designated as day 0. Two or 3 days later the rats were bled or subjected to different treatments according to the time-schedule given in the Results and figures. A group of 7 OVX and oestrogen-treated animals was adrenalectomized at 08:00 h on day 2 and received a single s.c. injection of progesterone (0.1 mg/rat in oil) at 08:00 h on day 3. Another group of 8 OVX and oestrogen-primed rats was injected i.p. with two doses of 50 μl each of a specific progesterone antiserum, raised in our laboratory [13, 15], at 08:00 and 13:00 h on day 3. In preliminary experiments we measured the capacity of the antiserum to bind [^{3}H]progesterone and found that 1 μl antiserum could bind approx. 60 ng (0.19 nmol) progesterone and 0.3 ng (0.86 pmol) corticosterone in a volume of 1 ml. Thus, the quantity of antiserum injected (100 μl) was more than enough to neutralize essentially all circulating progesterone without interfering with the action of corticoids. Control animals received the same quantity of normal rabbit serum (NRS).

Determination of PRL

PRL was measured by a double-antibody radioimmunoassay (RIA) using materials kindly provided by NIADDK. PRL was radioiodinated using the chloramine T method. Results were expressed in terms of the rat PRL RP-3 standard preparation. Assay sensitivity was 1 μg/l serum and the inter- and intra-assay coefficients of variation were 8 and 3%, respectively.

Statistical analysis

Results are expressed as means ± SEM. Statistical comparisions were performed using one-way analysis of variance followed by Tukey's multiple range test. When variances were not homogeneous, logarithmic transformation of data was applied.

RESULTS

Effect of mifepristone or a specific progesterone antiserum on serum PRL concentrations in OVX and oestrogen-treated rats (Fig. 1)

Oestrogen treatment to OVX rats induced a significant increase in serum PRL concentration at 13:00 ($P < 0.05$) and 18:00 h ($P < 0.01$) on day 3 compared with the control group.

Mifepristone administration to OVX and oestrogen-primed animals enhanced significantly ($P < 0.01$) the effect of OB on serum PRL at 13:00 h, without modifying PRL values at 18:00 h.

On the contrary, the administration of the progesterone antiserum to OVX and oestrogen-primed rats had no effect on serum PRL at 13:00 or at 18:00 h. Treatment with NRS showed a response similar to that obtained in the group injected with the progesterone antibody (results not shown).

Effect of adrenalectomy and progesterone administration on serum PRL concentration in OVX and oestrogen-primed rats (Fig. 2)

Adrenalectomy performed on day 2 to OVX and oestrogen-treated rats induced a significant ($P < 0.01$) increase in serum PRL at 13:00 but not at 18:00 h on day 3. However, it had no effect in OVX and vehicle-treated animals. Administration of progesterone (0.1 mg/rat in oil) to OVX and adrenalectomized rats treated with OB did not prevent the increase of serum PRL induced by adrenalectomy.

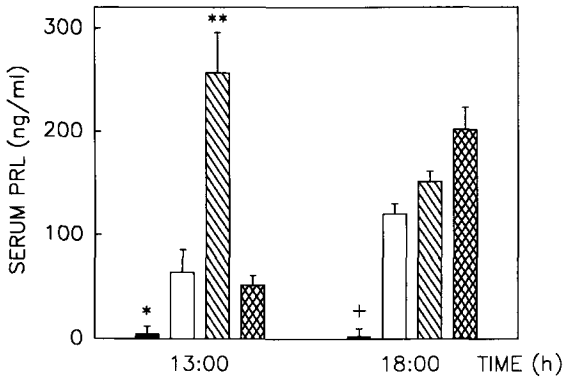

Fig. 1. Effect of mifepristone and progesterone antiserum on serum PRL concentration at 13:00 and 18:00 h on day 3 in OVX and oestrogen-treated rats. OVX rats were treated s.c. with vehicle or OB (20 μg/rat) at 13:00 h on day 0. ■, OVX and vehicle-treated rats ($n = 6$); □, OVX and oestrogen-treated rats ($n = 6$); ▨, OVX and oestrogen-primed rats treated s.c. with mifepristone (10 mg/kg) at 08:00 h on day 3 ($n = 7$); and ▩, OVX and oestrogen-primed rats injected i.p. with progesterone antiserum (50 μl) at 08:00 and bled at 13:00 h ($n = 8$), a second dose was injected at 13:30 h and the animals were decapitated at 18:00 h on day 3. Results are means ± SEM. *$P < 0.05$ compared with OVX and oestrogen-treated rats receiving oil or progesterone antiserum. **$P < 0.01$ compared with OVX rats treated with oil, oestrogen or oestrogen plus progesterone antiserum. $^{+}P < 0.01$ compared with OVX and oestrogen-primed rats treated with oil, mifepristone or progesterone antiserum. (Analysis of variance followed by Tukey's test.)

Effect of mifepristone on serum PRL concentration induced by oestrogen plus progesterone in OVX rats

Progesterone administration (2 mg/rat in oil) to OVX and oestrogen-primed rats increased dramatically ($P < 0.001$) serum PRL levels at 13:00 h compared with those obtained in OVX and oestrogen-treated animals injected with vehicle. Mifepristone treatment partially reverted the PRL increase induced by progesterone [Fig. 3(A)], but it was not able to diminish PRL concentration to values similar to those from OVX and oestrogen-treated group ($P < 0.01$).

Serum PRL at 18:00 h on day 3 in OVX and oestrogen-primed rats was not modified significantly by the administration of progesterone (2 mg/rat) at 13:00 h alone or in combination with mifepristone injected at 08:00 h [Fig. 3(B)].

DISCUSSION

Mifepristone is a synthetic 19-nor steroid with high binding affinity for progesterone and glucocorticoid receptors; it also binds to androgen receptor but with a much lower affinity [16, 17]. This ability of the drug makes it difficult to be precise about which of the two actions is predominant in the regulation of PRL secretion. However, in the present work the significant increase in serum PRL at 13:00 h in OVX and oestrogen-primed rats after treatment with mifepristone was not reproduced when a specific progesterone antibody was administered instead of mifepristone. This response indicates that the effect of mifepristone on PRL secretion is due to its antiglucocorticoid action and not to its capacity to block progesterone receptors.

The present results show that PRL secretion in OVX and oestrogen-primed rats may be under the inhibitory influence of adrenal glucocorticoids. This inhibition takes place at 13:00 h on day 3 since rats treated with mifepristone, but not with a progesterone antibody, showed a significant enhancement of oestrogen-induced PRL increase at this time and no alteration at 18:00 h. Adrenalectomy on day 2 also induced a significant rise in serum PRL at 13:00 h on day 3 which was not different from the rise induced by treatment with mifepristone. On the other hand, adrenalectomy did not modify the basal serum PRL concentration of OVX animals not treated with OB. Thus, the initial stimulatory action of oestrogen on the PRL response to ovariectomy may be facilitated by the absence of adrenal glucocorticoids, at least at 13:00 h. Moreover, this increase in PRL due to adrenalectomy was not modified by administration of low doses of progesterone (0.1 mg/rat), indicating that glucocorticoids rather than progesterone exert the negative feedback on PRL secretion. Recently, Nishino *et al.* [18] have shown a serum PRL rise in OVX rats treated with oestradiol and onapristone for 5 days. The authors, attending to indirect evidence, have suggested that this increase might be partly due to the antiglucocorticoid effect of the compound. Unfortunately, we do not have further details regarding the daily variations of that PRL response.

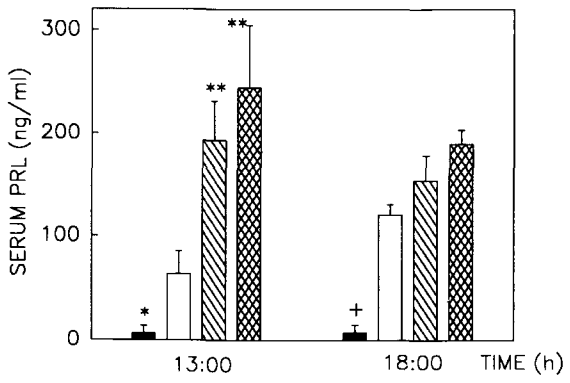

Fig. 2. Effect of adrenalectomy alone or in combination with progesterone administration on serum PRL at 13:00 and 18:00 h on day 3 in OVX and oestrogen-treated rats. OVX rats were treated s.c. with vehicle or OB (20 μg/rat) at 13:00 h on day 0. Adrenalectomy was performed at 08:00 h on day 2. ■, OVX and adrenalectomized rats receiving vehicle ($n = 6$); □, OVX and oestrogen-treated rats ($n = 6$); ▨, OVX and adrenalectomized animals receiving OB ($n = 7$); and ▩, OVX and adrenalectomized oestrogen-primed rats treated s.c. with 0.1 mg/rat progesterone ($n = 7$). Results are means ± SEM. *$P < 0.05$ compared with OVX and oestrogen-treated rats. **$P < 0.01$ compared with OVX and adrenalectomized rats receiving vehicle or OVX and oestrogen-treated animals. $^{+}P < 0.01$ compared with the other three groups. (Analysis of variance followed by Tukey's test.)

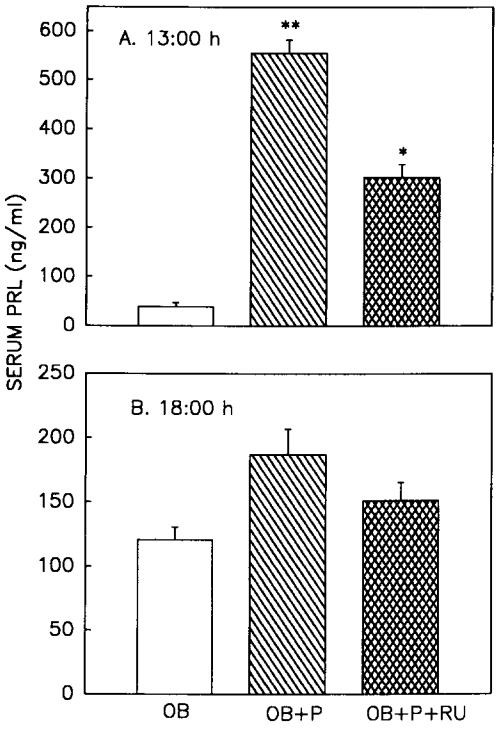

Fig. 3. Effect of progesterone alone or combined with mifepristone on serum PRL concentration in OVX and oestrogen-primed rats at 13:00 (A) and 18:00 h (B) on day 3. (A) OVX rats were injected s.c. with OB (20 μg/rat) at 13:00 h on day 0 and with vehicle □, progesterone (2 mg/rat) ▨ or progesterone plus mifepristone (10 mg/kg) ■ at 08:00 h on day 3. (B) OVX rats were injected s.c. with OB (20 μg/rat) at 13:00 h on day 0 and with vehicle □ at 08:00 and 13:00 h on day 3. Progesterone (2 mg/rat) alone ▨ or combined with mifepristone ▩ was administered s.c. at 13:00 h on day 3. Mifepristone (10 mg/kg) was given s.c. at 08:00 h on day 3. Results are means ± SEM of groups of 6 to 8 rats. *$P < 0.01$ compared with OB or OB + P groups. **$P < 0.001$ compared with OB group. (Analysis of variance followed by Tukey's test.)

On the other hand, exogenous treatment with a higher dose of progesterone (2 mg/rat) in the morning of day 3 to OVX and oestrogen-primed animals provoked a noteworthy increase of serum PRL at 13:00 h, as shown by Caligaris *et al.* [4]. This increase was partially prevented by mifepristone administration. These results may suggest that glucocorticoids along with progesterone are participating in this event. Since the administration of mifepristone reverted the progesterone-induced PRL increase to values similar to those observed in OVX and oestrogen-treated rats receiving mifepristone, we can assume that this treatment blocks both progesterone stimulatory and corticosterone inhibitory effects on PRL secretion at noon.

It is interesting to highlight that the participation of corticosterone on the regulation of PRL secretion induced by oestrogen in OVX rats takes place only at noon. It has been demonstrated previously that the proestrus surge of PRL occurs when corticosterone secretion is at its daily peak, indicating that this steroid is not exerting a negative feedback on PRL secretion at this time [19]. Furthermore, according to our findings, the lack of effect of mifepristone on the release of PRL at 18:00 h may also indicate that neither progesterone nor glucocorticoids are affecting PRL release induced by oestrogen at this time. Gaillard *et al.* [20] have observed that the antiglucocorticoid effect of mifepristone treatment is only apparent during the morning hours of the human circadian rhythm, when cortisol levels are rapidly increasing. Moreover, dexamethasone is a potent inhibitor of stress-induced PRL release, and adrenalectomy enhances stress-induced PRL release [21]. Both effects are observed at 13:00 h. According to these reports and our own findings, we can assume that the inhibitory effect of adrenal corticoids on PRL release may be evident only in the morning.

The present results suggest that after a previous sensitization of the pituitary by oestrogen, circulating glucocorticoids may exert a direct inhibitory effect on PRL release or may modulate the positive feedback of oestrogen. Moreover, the synergistic effect of oestradiol and mifepristone increasing PRL release described by Van der Schoot *et al.* [22] may be due to the antiglucocorticoid action of mifepristone and not to its antiprogesterone effect.

Additional studies are needed to clarify the precise mechanisms involved in the facilitation of PRL release after mifepristone treatment. While such studies are presently under way, the results presented here allow us to conclude that adrenal glucocorticoids inhibit PRL secretion only in the morning, while neither progesterone nor glucocorticoids affect PRL release in the afternoon in OVX and oestrogen-primed rats.

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