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# Participation of both adrenergic and opioidergic systems in the negative feedback of adrenal progesterone on LH secretion

A.M. Salicioni *, R.W. Carón, R.P. Deis

*Laboratorio de Reproducción y Lactancia, CONICET, Casilla de Correo 855, 5500 Mendoza, Argentina*

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**Abstract**

It has been shown that adrenal progesterone plays an important role in regulating the negative feedback of oestrogen on luteinizing hormone (LH) release in ovariectomized and oestrogen-treated rats. The purpose of the present study was to determine whether adrenal progesterone modulation of LH secretion is mediated by adrenergic and opioidergic systems in ovariectomized and oestrogen-treated rats. Oestradiol benzoate (20 μg/rat) was given s.c. to ovariectomized rats on day 0. Control animals were injected with the vehicle alone. The specific adrenoceptor antagonists prazosin (10 mg/kg), idazoxan (100 μg/kg), metoprolol (10 mg/kg) or ICI 118,551 (200 μg/kg) were injected at 12.00 and 20.00 h on day 2 and at 08.00 h on day 3 to oestrogen-primed rats treated or not with RU486. Control animals were injected with saline. RU486 (10 mg/kg) was administered s.c. at 08.00 h on day 3 to oestradiol-treated animals receiving adrenoceptor antagonists or saline. Naloxone (2 mg/kg) was administered i.p. 30 min before blood-sampling to oestrogen-primed rats treated or not with RU486. All groups were blood-sampled at 13.00 and 18.00 h on day 3, and LH concentration was measured by radioimmunoassay. The administration of oestradiol to ovariectomized rats decreased serum LH levels at 13.00 and 18.00 h on day 3. Prazosin or idazoxan partially prevented the effect of oestradiol at 13.00 h, while metoprolol, ICI 118,551 or naloxone totally blocked the inhibitory effect of oestradiol on LH secretion; both adrenoceptor and opioid receptor antagonists also prevented the effect of oestrogen on LH concentration at 18.00 h. RU486 increased serum LH concentration at 18.00 h in oestrogen-primed rats to values higher than in ovariectomized control rats, with no effect at noon. The administration of prazosin to ovariectomized and oestrogen-primed rats treated with RU486 prevented this increase while the other adrenoceptor antagonists or naloxone increased serum LH concentrations at 18.00 h. The present study shows that RU486 switches the feedback of oestradiol on LH secretion from negative to positive in ovariectomized and oestradiol-primed rats, activating a stimulatory $\alpha_1$-adrenergic pathway during the afternoon, and gives strong evidence about the participation of adrenal progesterone modulating neurotransmitter systems involved in the secretion of LH. It also supports the participation of endogenous opioid peptides in the negative feedback of oestradiol, suggesting that the inhibitory tone of endogenous opioid peptide is active regardless the action of adrenal progesterone. © 1997 Elsevier Science B.V.

*Keywords:* Mifepristone; Progesterone; Oestrogen; LH (luteinizing hormone); Adrenoceptor antagonist

## 1. Introduction

The effect of the ovarian steroids on luteinizing hormone (LH) secretion has been extensively studied (Fink, 1988). Recently, it has been shown that adrenal progesterone plays an important role in regulating the negative feedback of oestrogen on LH release in ovariectomized and oestrogen-treated rats, in a time-dependent manner (Salicioni et al., 1993). This study suggested the possibility of an inhibitory or stimulatory effect of oestrogen on serum LH concentration in ovariectomized rats according to the presence or absence of adrenal progesterone action.

Oestrogen can act on the brain to exert negative and positive feedback effects on the secretion of LH. Both oestrogen and progesterone receptors are reported not to be present on LH-releasing hormone (LHRH) neurons (Shivers et al., 1983; Fox et al., 1990), suggesting that another class of neurons must mediate their effects. It is likely that both steroids interact with other neurotransmitter-containing neurons in the brain that in turn regulate LHRH neuron activity. Since progesterone receptors are present in catecholaminergic neurons (Sar, 1988; Fox et al., 1990), a role for catecholamine neurotransmission in progesterone-induced LH release has been widely investigated (Brann and

* Corresponding author. Present address: Fox Chase Cancer Center, Room C-441, 7701 Burholme Avenue, Philadelphia, PA 19111, USA.


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Mahesh, 1991; Wilson et al., 1991). Noradrenergic systems have been shown either to stimulate (Gallo and Drouva, 1979; Taleisnik and Sawyer, 1986) or inhibit (Bergen and Leung, 1986; Coen and Gallo, 1986; Condon et al., 1986; Taleisnik and Sawyer, 1986) LH secretion in ovariectomized and steroid-primed rats, depending on the endocrine milieu. On the other hand, increasing amount of evidence shows that endogenous opioid peptides exert a tonic inhibitory influence on LH (for review, see Kalra, 1993).

The purpose of the present study was to determine whether adrenal progesterone modulation of LH secretion is mediated by adrenergic and opioidergic systems in ovariectomized and oestrogen-treated rats.

## 2. Materials and methods

### 2.1. Animals

Virgin female rats, 3–4 months old (200–220 g) bred in our laboratory and originally of the Wistar strain were used. The animals were kept in a light- (lights on 06.00–20.00 h) and temperature-controlled room; rat chow (Nutric, Córdoba, Argentina) and tap water were available ad libitum.

### 2.2. Surgical procedures

All the animals were ovariectomized when adult and used 14–16 days later. Ovariectomy was performed through two dorsolateral incisions under ether anaesthesia between 08.00 and 09.00 h. All rats were blood-sampled only once by cardiac puncture for samples collected at 13.00 h and by decapitation for samples at 18.00 h. In experienced hands, cardiac puncture takes only a few seconds, insufficient to produce significant changes in hormone release detectable in blood samples (Deis et al., 1989; Carón and Deis, 1996). 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 LH.

### 2.3. Drug treatments

Oestradiol benzoate (20 μg/rat; Schering, Buenos Aires, Argentina) was given s.c. in 0.2 ml purified sunflower seed oil at 13.00 h on day 0. Control animals were injected with the vehicle (oil) alone.

Adrenoceptor antagonists were administered to oestradiol-treated rats as follows: the specific $\alpha_1$-adrenoceptor antagonist prazosin (generously provided by Pfizer, Buenos Aires, Argentina) was dissolved in saline (10 mg/ml) and injected s.c. at a dose of 10 mg/kg. The specific $\alpha_2$-adrenoceptor antagonist idazoxan (Reckitt&Colman, Hull, UK) was dissolved in saline (0.1 mg/ml) and injected s.c. at a dose of 100 μg/kg. The specific $\beta_1$-adrenoceptor antagonist metoprolol (generously provided by Astra, Buenos Aires, Argentina) was dissolved in saline (10 mg/ml) and injected s.c. at a dose of 10 mg/kg. The specific $\beta_2$-adrenoceptor antagonist ICI 118,551 ((2*RS*,3*RS*)-3-isopropylamino-1-(7-methyl-indan-4-yloxy) butan-2-ol, ICI, Macclesfield, UK) was dissolved in saline (0.2 mg/ml) and injected s.c. at a dose of 200 μg/kg. All of the above antagonists were injected at 12.00 and 20.00 h on day 2 and at 08.00 h on day 3. Control animals were injected with saline with the same schedule.

Oestradiol-treated animals receiving adrenoceptor antagonists or saline were given an injection of the progesterone/glucocorticoid antagonist RU-38486 (Teutsch and Philibert, 1994) (RU486, mifepristone; 17$\beta$-hydroxy-11(4-dimethylamino-phenyl) 17$\alpha$-(prop-1-ynyl) oestra-4,9-dien-3-one, kindly provided by M. Garnier, Roussel-Uclaf, Romainville, France), which was dissolved in sunflower seed oil at 10 mg/ml and injected s.c. at a dose of 10 mg/kg at 08.00 h on day 3.

The $\mu$- and $\kappa$-receptor antagonist naloxone (Sigma, St. Louis, MO, USA) was dissolved in 0.9% saline and injected i.p. at a dose of 2 mg/kg at 12.30 h or at 17.30 on day 3; blood samples were taken 30 min later. Control animals were injected with the vehicle alone.

Both dosages and schedule of administration of antagonists have been previously used in our laboratory to study prolactin secretion (Jahn and Deis, 1991; Soaje and Deis, 1994; Carón and Deis, 1996).

### 2.4. Determination of LH

LH was measured by a double-antibody radioimmunoassay as previously described (Salicioni et al., 1993) using materials kindly provided by Dr. S. Raiti (NIADDK, Bethesda, MD, USA). Results are expressed in terms of the NIADDK rat LH-RP-3 standard.

### 2.5. Statistical analysis

This was 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. All values are expressed as means ± SEM.

## 3. Results

### 3.1. Effect of adrenoceptor antagonists or naloxone on serum LH levels at 13.00 h in ovariectomized and oestrogen-primed rats

The administration of oestradiol benzoate to ovariectomized rats on day 0 induced a significant decrease in serum LH levels measured at 13.00 h on day 3 (Fig. 1).

Serum LH values in oestrogen-primed rats receiving prazosin or idazoxan were significantly higher at 13.00 h but lower than in the ovariectomized and oil-treated animals. Oestrogen-primed groups given metoprolol or ICI 118,551 had LH levels not significantly different from those measured in oil-treated rats. Naloxone administration to oestrogen-primed rats elicited a clear increase in serum LH, significantly higher than in the vehicle-treated group. The administration of RU486 to oestrogen-primed rats did not modify significantly the LH values measured at 13.00 h, according to our previous study (Salicioni et al., 1993).

### *3.2. Effect of adrenoceptor antagonists or naloxone on serum LH concentration at 18.00 h in oestrogen-primed rats treated with RU486*

As we have previously reported (Salicioni et al., 1993), the decrease in serum LH induced by oestrogen at 18.00 h on day 3 was reverted by RU486 to values even higher than those observed in vehicle-treated animals (Fig. 2). RU486 had a specific antiprogesterone effect since similar results were obtained in oestrogen-primed animals treated with a progesterone antibody (Salicioni et al., 1993).

All the oestrogen-primed groups given adrenoceptor antagonists or naloxone had serum LH concentrations at 18.00 h significantly higher than in oestrogen-treated rats receiving saline.

The administration of prazosin to oestrogen-primed rats treated with RU486 decreased serum LH, while the other adrenoceptor antagonists or naloxone increased LH values at 18.00 h (Fig. 2).

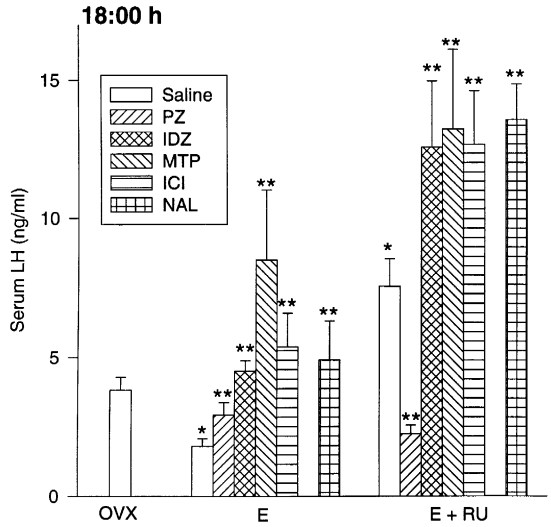


Fig. 2. Serum LH concentrations at 18.00 h on day 3. Ovariectomized rats received vehicle (OVX), estradiol benzoate (E, open bars), or estradiol benzoate on day 0 and RU486 on day 3 (E + RU, open bars). Ovariectomized and oestrogen-primed rats treated with or without RU486 also received one of the adrenoceptor antagonists (prazosin = PZ; idazoxan = IDZ; metoprolol = MTP; ICI 118,551 = ICI) or naloxone (NAL) (shaded bars). Data are means ± SEM from 6–8 animals per group. One-way analysis of variance followed by Tukey's test was used for multiple comparisons. * $P < 0.05$ compared to ovariectomized and vehicle-treated group. * * $P < 0.05$ compared to the corresponding group without antagonist.

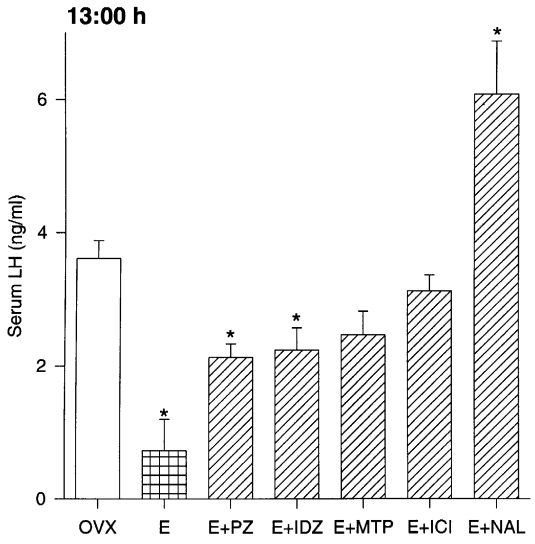


Fig. 1. Serum LH concentrations at 13.00 h on day 3. Ovariectomized rats received vehicle (OVX, open bars) or estradiol benzoate (E, shaded bar) at 13.00 h on day 0. Some ovariectomized and oestrogen-primed rats also received adrenoceptor antagonists (prazosin = PZ; idazoxan = IDZ; metoprolol = MTP; ICI 118,551 = ICI) or naloxone (NAL) (shaded bars). Data are means ± SEM from 6–8 animals per group. One-way analysis of variance followed by Tukey's test was used for multiple comparisons. * $P < 0.05$ compared to ovariectomized and vehicle-treated group.

## 4. Discussion

We have previously reported that oestrogen displays an inhibitory or stimulatory effect on LH secretion according to the presence or absence of adrenal progesterone action in ovariectomized rats (Salicioni et al., 1993). Our present results suggest the involvement of an $\alpha_1$-adrenergic pathway in the positive feedback provoked by oestrogen in absence of adrenal progesterone and that endogenous opioid peptides do not participate in this effect. However, both systems seem to be involved in the negative feedback of oestrogen.

It has been shown that a priming dose of oestradiol benzoate, that suppresses LH release in ovariectomized rats, decreases the turnover of norepinephrine in the hypothalamus suggesting that the inhibitory effect of oestradiol on LH secretion may be mediated by a decrement in adrenergic neurotransmission (Advis et al., 1980; De Paolo et al., 1982). Most evidence suggests that the stimulatory effects of norepinephrine on LHRH are mediated by $\alpha_1$-adrenoceptors (Sarkar and Fink, 1981; Drouva et al., 1982; Ojeda et al., 1982) and possibly $\alpha_2$-adrenoceptors (Estes et al., 1982), whereas the inhibitory effects are mediated by $\beta$-adrenoceptors (Condon et al., 1986; Taleisnik and

Sawyer, 1986). Our results support the view that adrenergic inputs to LHRH neurons mediate the negative feedback of oestrogen on LH secretion, since all the specific antagonists tested in our experiments were able to revert the inhibition evoked by oestradiol. The $\beta$-adrenoceptor antagonists completely prevented the effect of oestradiol at both 13.00 and 18.00 h on day 3. It has been shown that $\beta$-adrenergic pathways are involved in the inhibition of LH release evoked by electric stimulation of the locus ceruleus (Beltramino and Taleisnik, 1979) or the cingulate cortex (Caceres and Taleisnik, 1980). Inhibition of LH secretion by oestradiol might be the other situation in which $\beta$-adrenergic inhibitory inputs participate. On the other hand, in our ovariectomized and oestradiol primed rats, $\alpha$-adrenoceptor antagonists also prevented the negative feedback of oestrogen. This result is in line with the alteration induced by oestrogen of the diurnal rhythm demonstrated at least for $\alpha_1$-adrenoceptors in brain areas involved in the regulation of LH secretion in ovariectomized rats (Weiland and Wise, 1987). This suppressive effect of oestrogen on $\alpha_1$-adrenoceptors is more striking during the afternoon (Weiland and Wise, 1987) concomitant with the increase in the turnover of norepinephrine and the release of LH (Wise et al., 1981), and might explain the higher response to prazosin observed during the afternoon in our ovariectomized and oestrogen primed rats. Unfortunately, to our knowledge there is no similar study on $\alpha_2$-adrenoceptors to evaluate the unchanged response of LH to idazoxan in the present report. In addition, despite the fact of a diurnal rhythm in $\beta$-adrenoceptors in different brain areas has been described in ovariectomized rats, a suppressive effect of oestradiol on this rhythm has also been shown (Weiland and Wise, 1989). This effect might explain the lack of difference in the response to the $\beta$-adrenoceptor antagonist observed at 13.00 and 18.00 h in this study.

In the afternoon, when adrenal progesterone has a clear effect on enhancing the inhibition of LH release induced by oestrogen, the blockade of neither $\alpha_2$- nor $\beta$-adrenoceptors prevented the positive feedback of oestrogen in the absence of adrenal progesterone action. Furthermore, they increased LH concentration in ovariectomized and oestradiol-primed rats treated with RU486, suggesting that an $\alpha_2/\beta$-adrenergic inhibitory tone on the afternoon secretion of LH was still active in the absence of the progesterone effect. The response to those antagonists was comparable with or without previous RU486 administration, suggesting a lack of action of adrenal progesterone in activating the $\alpha_2/\beta$-adrenergic mechanism to inhibit LH secretion during the afternoon. In contrast, the results obtained with prazosin indicate that in the absence of adrenal progesterone oestradiol stimulates LH secretion at 18.00 h through an $\alpha_1$-adrenergic pathway. It is currently accepted that the steroid-induced rise in LH levels appears to involve a stimulatory action of norepinephrine or epinephrine via excitatory and presumably $\alpha_1$-receptors (Kalra, 1985; Coen and Gallo, 1986); however, an inhibitory effect of $\alpha_1$-adrenergic agonists on LH secretion in ovariectomized rats has also been described (Bergen and Leung, 1986). The apparently paradoxical ability of norepinephrine and $\alpha_1$-adrenergic agonists to either stimulate or inhibit LH release is probably due to dissimilar models. The relatively high amounts of adrenal progesterone (Feder et al., 1971) have not been considered in those models, albeit the influence of adrenal hormones in the regulation of the hypothalamic–pituitary function has been suggested (Wilson et al., 1978; Mahesh and Brann, 1992; Salicioni et al., 1993). Moreover, progesterone receptors are present in catecholaminergic neurons in ovariectomized rats (Sar, 1988; Fox et al., 1990), suggesting that adrenal progesterone has an important role in the absence of a further source of the steroid.

Morphine and endogenous opioid peptides have been shown to block the LH surge induced by ovarian steroids in ovariectomized rats (Kalra, 1993). Endogenous opioid peptides participate in the negative feedback of oestradiol on LH release in ovariectomized rats and, more interestingly, catecholamines may be the mediators of their influence on LHRH neurons (Kalra and Kalra, 1984; Akabori and Barraclough, 1986). Our results show that naloxone treatment enhanced the increase in serum LH concentration observed in the absence of adrenal progesterone action at 18.00 h on day 3. In both cases, with or without previous administration of RU486, endogenous opioid peptides showed a tonic inhibitory action on LH secretion. It has been shown that exogenous progesterone given to ovariectomized and oestrogen-primed rats abolishes LH secretion, and naloxone reverts this inhibition (Sylvester et al., 1982), even though a lack of effect of naloxone in ovariectomized and oestrogen plus progesterone treated rats has been also reported (Gabriel et al., 1983). Our results support the participation of endogenous opioid peptides in the negative feedback of oestradiol and suggest that the inhibitory tone of the endogenous opioid peptide is active regardless the action of adrenal progesterone.

In summary, the present study shows that RU486 switches the feedback of oestradiol on LH secretion from negative to positive in ovariectomized and oestradiol-primed rats, activating a stimulatory $\alpha_1$-adrenergic pathway during the afternoon. While additional studies are presently underway to further clarify the nature of this pathway, the present report gives strong evidence about the participation of adrenal progesterone modulating neurotransmitter systems involved in the secretion of LH.

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research fellows and R.P.D. is a career scientist from CONICET. For request of reprints, please contact R.P.D.

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