Acute effects of oestradiol and progesterone on melittin- and gonadotrophin-releasing hormone-induced LH secretion

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ABSTRACT

It is well established that oestradiol and progesterone modulate gonadotrophin-releasing hormone (GnRH)-induced LH secretion from cultured rat pituitary cells. Short-term oestradiol and long-term progesterone treatment exert inhibition, while short-term progesterone and long-term oestradiol treatment lead to enhancement of GnRH-stimulated LH secretion. There are several lines of evidence to suggest that the steroid effects might be mediated via a mechanism involving modulation of the GnRH signal-transduction system. To evaluate the role of arachidonic acid, which serves as an intracellular signal transducer by itself or its lipoxygenase metabolites, in the mediation of oestradiol and progesterone actions, we examined their effects on melittin (activator of phospholipase A₂)-stimulated LH secretion. When pituitary cells from adult female rats were treated for 48 h with 1 nmol oestradiol/l or 1 nmol oestradiol/l plus 100 nmol progesterone/l, GnRH (1 nmol/l)-induced LH secretion was stimulated or inhibited respectively. However, melittin (10–300 nmol/l)-stimulated LH secretion remained unaffected after such treatment. Short-term treatment with oestradiol inhibited GnRH-induced LH secretion while progesterone treatment of oestradiol-primed cells led to a stimulatory effect. Interestingly, melittin-stimulated LH secretion was influenced in the same way after the short treatment paradigm. Perfusion studies were performed to assess the kinetics of these acute steroid actions further. Four separate perfusion chambers were continuously perfused with medium and stimulated for 2 min with 1 nmol GnRH/l or 1 μmol melittin/l every 50 min in a pulsatile fashion. When 1 nmol oestradiol/l was added to the perfusion medium after the application of an initial control pulse, GnRH- and melittin-stimulated LH secretion were inhibited by 69 and 61% respectively. This effect was present after 50 min. When oestradiol-primed cells were treated with 100 nmol progesterone/l starting after the initial GnRH or melittin pulse, an acute stimulatory effect was observed in response to both stimuli after 50 min. LH release was enhanced by up to 279 (GnRH) or 419% (melittin) compared with the control pulse. The kinetics of inhibited or stimulated pulsatile LH secretion were virtually identical when GnRH or melittin were used as stimuli. These results demonstrate that short-term oestradiol or progesterone treatment modulate arachidonic acid-mediated LH secretion in a similar fashion to GnRH-induced LH secretion, while long-term oestradiol or progesterone treatment only affected GnRH-induced LH secretion.

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INTRODUCTION

The secretory response of the pituitary gonadotroph to gonadotrophin-releasing hormone (GnRH) is initiated by rapid increases in polyphosphoinositide hydrolysis (Guillemette, Balla, Baukal & Catt, 1987). The cleavage of phosphatidylinositol 4,5-bisphosphate (PIP₂) by phospholipase C produces inositol 1,4,5-trisphosphate and diacylglycerol (DG), which respectively mobilize Ca²⁺ from intracellular sources and activate protein kinase C (Hiroti, Hiroti, Aguilera & Catt, 1985; Stojilkovic, Chang, Ngo & Catt, 1988; Chang, Morgan & Catt, 1988). Arachidonic acid is liberated from DG by DG-lipase or from membrane phospholipids by phospholipase A₂, and serves as an intracellular signal transducer by itself or via its lipoxygenase metabolites (Naor & Catt, 1981; Naor, Kiesel, Vanderhoek & Catt, 1985; Kiesel, Przylipiak, Emid et al. 1987b).

There is evidence that oestradiol and progesterone, which exert well-established inhibitory and stimulatory
effects on GnRH-induced gonadotrophin secretion (Lagace, Massicotte & Labrie, 1980; Drouin & Labrie, 1981; Frawley & Neill, 1984; Emons, Knuppen, Ball & Catt, 1984; Emons, Ortman, Fingscheidt et al. 1986; Ortman, Emons, Knuppen & Catt, 1988; Ortman, Emons, Knuppen & Catt, 1989a; Ortman, Wiese, Knuppen & Emons, 1989b) might mediate their effects by an influence on different pathways of the GnRH signal transduction system (Kiesel, Helm, Berges et al. 1987a; Liu & Jackson, 1988; Emons, Frevert, Ortman et al. 1989; Audy, Boucher & Bonnin, 1990; Drouva, Gorene, Laplante et al. 1990). As a number of studies demonstrated that arachidonic acid and certain lipoxygenase metabolites are involved in GnRH signal transduction, we intended to clarify the role of this second-messenger system for the regulation of steroid effects in the gonadotroph. We investigated whether oestradiol and progesterone could modulate arachidonic acid-induced luteinizing hormone (LH) secretion from cultured rat pituitary cells. As pilot experiments had shown that the effective concentrations of exogenous arachidonic acid have toxic effects in these cells, we employed melittin, an activator of phospholipase A2, to generate arachidonic acid from cell membrane phospholipids (Camoratto & Grandison, 1985; Kiesel, Rabe, Hauser et al. 1987c). Experiments with rat pituitary cells in static culture were carried out to analyse the long-term and short-term actions of oestradiol and progesterone on melittin-induced LH release and to compare them with those after GnRH stimulation. Further experiments were performed using a perfusion system to investigate the kinetics of the short-term effects of the steroids on GnRH- or melittin-induced LH secretion. This study has been presented in part at the 34th symposium of the Deutsche Gesellschaft für Endokrinologie (Ortmann, Johannsen, Knuppen & Emmons, 1990).

**MATERIALS AND METHODS**

**Hormones**

Oestradiol, progesterone and GnRH were obtained from Sigma Chemical Co. (Deisenhofen, Germany). Melittin was purchased from Peninsula Laboratories, Inc. (Belmont, CA, U.S.A.). The steroids were prepared in ethanol, and GnRH and melittin were dissolved in phosphate-buffered saline (PBS) containing 1 g bovine serum albumin (BSA)/l.

**Pituitary cell preparation and culture conditions**

Pituitary glands obtained from 3-month-old female Wistar rats (Winkelmann, Borchen-Kirchborchen, Germany) were used for the preparation of primary cell cultures (Hyde, Childs, Wahl et al. 1982). Cells were cultivated on multiwell culture dishes (200 000 cells/well) or on Cytodex 1 microcarrier beads (Pharmacia, Uppsala, Sweden) in medium 199 without phenol red with Hank’s salts and l-glutamine (Biochrom, Berlin, Germany) supplemented with 1.4 g sodium bicarbonate/l, 10 μg streptomycin/ml, 100 U penicillin/ml and 10% horse serum (Biochrom) pretreated with 2% charcoal (Norit A) and 0.2% Dextran T70 (Pharmacia) for experiments under static or dynamic conditions (Ortmann et al. 1989b; Ortman, Sturm, Knuppen & Emmons, 1990a).

**Experiments in static culture**

After preparation, pituitary cells were maintained in a water-saturated atmosphere of 95% air–5% CO₂ at 37 °C for 48 h before experiments were started. To evaluate the long-term effects of ovarian steroids on LH secretion, cell cultures were treated for 52 h with 1 nmol oestradiol/l or 1 nmol oestradiol/l plus 100 nmol progesterone/l. To assess the short-term effects, another group of cell cultures was treated for 4 h with 1 nmol oestradiol/l or 100 nmol progesterone/l. The cells exposed to short-term progesterone treatment had been preincubated for 48 h with 1 nmol oestradiol/l. Control cultures received vehicle (0.2% ethanol). During the last 3 h of the indicated treatment periods, the cells were stimulated with 1 nmol GnRH/l or 10–300 nmol melittin/l. At the end of the 3 h stimulation period, media were collected and analysed for their LH content by radioimmunoassay (RIA). The experiments were carried out three to five times.

**Perifusion experiments**

Four perifusion chambers containing 20 × 10⁶ cells each were used for the first experiment. The cells had been pretreated with 1 nmol oestradiol/l for 48 h in Petri dishes before the perifusion was started. The medium used for perifusion was composed as the incubation medium, but contained 1 g BSA/l instead of horse serum and 25 nmol Hepes/l instead of sodium bicarbonate. Two chambers (1 and 2) were challenged with an initial 2 min pulse of 1 nmol GnRH/l while the two other chambers (3 and 4) received a 2 min pulse of 1 μmol melittin/l. These initial applications of melittin or GnRH were defined as control pulses. After the administration of the control pulse, cells in chambers 2 and 4 were treated with 100 nmol progesterone/l. Three further pulses of GnRH or melittin were applied to each of the chambers at 50 min intervals.

In the second experiment, the cells did not receive any steroid treatment before the start of the perifusion. As described above, cells in four perifusion
chambers were treated with an initial pulse of 1 nmol 
GnRH/l or 1 μmol melittin/l. Then chambers 2 and 
4 were perfused with medium containing 1 nmol 
oestradiol/l. Further pulses of GnRH (chambers 1 
and 2) or melittin (chambers 3 and 4) were adminis-
tered as described above. In both experiments, 
complete effluent medium was collected in 1-min 
fractions (0.5 ml/fraction) which were analysed for 
their LH content by RIA.

RIA and data analysis

The LH content of the samples was determined by 
RIA using the reference preparation RP-2 rat LH 
(AFP-5666 C) provided by the National Pituitary 
Agency, Baltimore, MD, U.S.A. (Solano, Dufau & 
Catt, 1979). The data from three to five experiments 
with pituitary cells in static culture were pooled and 
expressed in terms of percentage of the respective 
control cultures (100%). Vehicle served as control (100%) 
when oestradiol treatment was evaluated, whereas 
oestradiol served as control (100%) when progester- 
one treatment was evaluated. Data were analysed for 
statistically significant differences between treatments 
by using the Mann–Whitney U test.

The LH responses in perfusion experiments were 
expressed as the total mass of LH released by a 2-min 
pulse of GnRH or melittin in excess of basal LH 
secretion as described previously (Ortmann et al. 
1989b), except that basal LH secretion was deter-
mined by calculating the mean of basal secretion 
before and after the LH peaks. The response to the 
first GnRH or melittin stimulus (control pulse) was 
defined as 100%, and the subsequent LH responses 
after steroid or vehicle treatment were expressed in 
terms of percentage of the first LH response of 
the respective chamber. The data obtained in three 
independent experiments were pooled and the results 
analysed for statistically significant differences between 
oestradiol and oestradiol plus progesterone treatment 
by the Mann–Whitney U test.

RESULTS

Effects of progesterone and oestradiol on GnRH- or 
melittin-induced LH secretion by rat pituitary cells in 
static culture

Pituitary cells in static culture that had been treated 
for 52 h with 1 nmol oestradiol/l showed enhanced 
responsiveness to the GnRH stimulus. When these 
cells were co-incubated with 1 nmol oestradiol/l 
plus 100 nmol progesterone/l the augmentative effect 
of oestradiol on GnRH-induced LH secretion was 
reversed (Fig. 1). When melittin was used to induce LH 
secretion neither oestradiol nor combined treatment 
with oestradiol and progesterone showed statistically 
significant modulatory effects on LH secretion (Fig. 
1). Absolute LH values (ng RP-2/ml) corresponding 
to 100% were 3.7±0.9 at 0, 3.8±0.8 at 10−8, 
4.9±1.0 at 3×10−8, 5.4±0.8 at 6×10−8, 33.7±3.8 at 10−7, 
66.1±6.2 at 3×10−7 mol melittin/l and 22.5±3.8 at 
10−9 mol GnRH/l for vehicle and 2.9±0.7 at 0, 
3.6±1.0 at 10−8, 4.0±0.9 at 3×10−8, 6.4±0.9 at 
6×10−8, 28.9±2.3 at 10−7, 67.4±5.7 at 3×10−7 mol 
melittin/l and 30.6±2.5 at 10−9 mol GnRH/l for 
oestradiol-treated cells.

Short-term treatment of pituitary cells with 1 nmol 
oestradiol/l reduced GnRH-stimulated LH secretion, 
while short-term progesterone treatment of oestra-
diol-primed cells led to enhanced LH release. Both the 
short-term inhibitory effect of oestradiol and the 
short-term stimulatory effect of progesterone also 
occurred when pituitary cells were stimulated with 
melittin instead of GnRH (Fig. 2). Maximal inhibi-
tory or stimulatory effects of the steroids were 
observed at submaximal concentrations of melittin 
(30–100 nmol/l). Absolute LH values (ng RP-2/ml) 
corresponding to 100% were 4.1±0.8 at 0, 3.5±0.9 
at 10−8, 5.0±1.1 at 3×10−8, 5.6±0.6 at 6×10−8, 
29.6±6.3 at 10−7, 65.7±6.2 at 3×10−7 mol melittin/l 
and 18.9±1.3 at 10−9 mol GnRH/l for vehicle (4 h) 
and 2.9±0.7 at 0, 3.6±1.0 at 10−8, 4.0±0.9 at 
3×10−8, 6.4±0.9 at 6×10−8, 28.9±2.3 at 10−7, 
67.4±5.7 at 3×10−7 mol melittin/l and 30.6±2.5 at 
10−9 mol/l GnRH for oestradiol-treated (52 h) cells.

Short-term effects of oestradiol and progesterone on 
GnRH- or melittin-induced LH secretion by perfused 
rat pituitary cells

Pituitary cells that were perfused with medium 
containing 1 nmol oestradiol/l after the application of 
an initial 2 min GnRH (1 nmol/l) pulse showed 
reduced responsiveness to subsequent GnRH stimuli. 
This effect was present after 50 min of perfusion. 
Maximal suppression of LH release was observed 
after 150 min when the LH response was only 31% of 
that in the controls. Cells that received vehicle showed 
invariable responses to repeated GnRH stimuli (Fig. 3 
and Table 1). These responses consisted of an initial 
rapid increase in LH release followed by a more 
prolonged decrease before baseline levels were reached. 
When melittin was used instead of GnRH to induce 
LH secretion the secretory profiles of the LH peaks 
were also biphasic but generally wider than those 
after GnRH stimulation. However, almost identical 
kinetcs of inhibition of LH release after oestra-
diol treatment were observed. Again there was no signifi-
cant change in the LH responses to the melittin pulses 
when the perfusion medium contained vehicle (Fig. 3 
and Table 1).

In the second perifusion experiment cells were primed for 48 h with 1 nmol oestradiol/l and then treated with 100 nmol progesterone/l after application of the initial pulse of GnRH. Such treatment led to pronounced enhancement of the subsequent LH responses to the three further GnRH pulses. This stimulatory effect was present after 50 min of progesterone treatment and was fully expressed after 150 min (Fig. 4 and Table 2). Pituitary cells that had been treated with oestradiol or oestradiol and progesterone in the same way but received melittin to induce LH secretion also showed enhanced responsiveness after progesterone treatment compared with oestradiol treatment alone (Fig. 4 and Table 2). Although the shape of the individual secretory responses were different when melittin or GnRH were administered, the time course of progesterone-induced enhancement of LH secretion was quite similar.

DISCUSSION

In the present study we have evaluated the effects of short-term and long-term oestradiol or progesterone treatment on melittin and GnRH-induced LH secretion from rat pituitary cells in culture. We have confirmed the results of previous studies showing that oestradiol induces a short-term inhibitory and a long-term stimulatory effect on GnRH-stimulated LH secretion (Frawley & Neill, 1984; Emons et al. 1986). Progesterone, in contrast, facilitated GnRH-induced LH secretion after short-term incubation of
Melittin (mol/l)

Effects of short-term (4 h) treatment of rat pituitary cells in static culture with (a) 1 nmol oestradiol/l (●) or (b) 100 nmol progesterone/l (◆) on melittin (10–300-nmol/l) or GnRH (1 nmol/l)-induced LH release. Cells that were treated with progesterone had been primed with 1 nmol oestradiol/l for 52 h (b). Results of three (oestradiol) or five (progesterone) independent experiments are expressed as percentage of vehicle treatment (■ in a) or oestradiol treatment (▲ in b). See Results for absolute LH values. *P < 0.05 vs vehicle (a) or oestradiol (b) (Mann–Whitney U test).

Oestradiol-primed cells and inhibited LH secretion after long-term incubation (Lagace et al. 1980; Ortmann et al. 1989a,b; Turgeon & Waring, 1990). When melittin was used as the secretagogue there were no changes in the secretory responses after prolonged oestradiol or progesterone treatment. However, when short-term treatment was carried out oestradiol and progesterone modulated melittin-stimulated LH secretion in the same manner as GnRH-induced LH release. These results from experiments in static culture, which demonstrated a specific effect of the short-term steroid treatment regimen, led us to investigate the kinetics of this effect in a dynamic culture system. The perifusion system employed enabled us to run four separate perifusion chambers simultaneously and therefore represented an adequate model for the comparison of rapid steroid actions on pulsatile gonadotrophin secretion under controlled conditions. Interestingly, the time course of the inhibitory and facilitatory actions of oestradiol and progesterone on LH secretion were similar when GnRH or melittin were used as stimuli. As melittin is able to induce LH release from pituitary gonadotrophs via activation of phospholipase A₂ leading to liberation of arachidonic acid, the results of this study indicate that arachidonic acid-activated LH secretion can be modulated by steroid treatment under certain conditions and that this can occur independently of GnRH receptor activation. Although numerous studies support a role for arachidonic acid and/or its metabolites in the mechanism of action of...
FIGURE 3. Release of LH (ng RP-2/fraction) in response to 2-min (a), (b) GnRH (1 nmol/l) or (c), (d) melittin (1 μmol/l) pulses administered every 50 min (arrows) to rat pituitary cells cultured on microcarrier beads. After an initial control pulse, the cells were perifused with medium containing (b), (d) 1 nmol oestradiol/1 or (a), (c) vehicle (0·1% ethanol).

TABLE 1. Effects of short-term oestradiol (1 nmol/l) treatment on LH release induced by pulses of gonadotrophin-releasing hormone (GnRH; 1 nmol/l) or melittin (1 μmol/l) in perifused rat pituitary cells. Values are means ± s.e.m. for data from four separate perifusion experiments that were combined and expressed as a percentage of a control pulse (= 100%).

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<tr>
<th></th>
<th>Vehicle</th>
<th>Oestradiol</th>
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<tr>
<td>Pulse 1</td>
<td>92 ± 2</td>
<td>73 ± 2*</td>
<td>Pulse 1</td>
<td>102 ± 1</td>
<td>73 ± 7*</td>
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<tr>
<td>Pulse 2</td>
<td>99 ± 5</td>
<td>56 ± 2*</td>
<td>Pulse 2</td>
<td>99 ± 10</td>
<td>51 ± 1*</td>
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<tr>
<td>Pulse 3</td>
<td>103 ± 4</td>
<td>31 ± 2*</td>
<td>Pulse 3</td>
<td>98 ± 10</td>
<td>39 ± 6*</td>
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*P < 0·05 vs vehicle (Mann-Whitney U test).

Mean absolute LH (ng RP-2) values of the control pulses were 134 ± 2 ng/peak for vehicle (GnRH), 146 ± 8 ng/peak for oestradiol (GnRH), 422 ± 95 ng/peak for vehicle (melittin), and 550 ± 74 ng/peak for oestradiol (melittin).

GnRH in the pituitary gonadotrophin (Naor et al. 1985; Chang, Graeter & Catt, 1986; Kiesel et al. 1987a; Przylipiak, Kiesel, Habenicht et al. 1990), there is only limited information about a modulatory action of ovarian steroids on this signal transduction pathway. Liu & Jackson (1988) were unable to demonstrate an effect of long-term oestradiol treatment on arachidonic acid- or melittin-stimulated LH or FSH

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secretion from rat pituitary cells in static culture. Their findings have been confirmed by the results from similar experiments in this study and extended by the observation that long-term progesterone treatment was also without any effect on melittin-induced LH secretion, while short-term treatment with both steroids resulted in marked inhibitory or stimulatory actions. Therefore it could be speculated that acute exposure of pituitary cells to oestradiol or progesterone might have an influence on the arachidonic acid pathway in the gonadotroph. Although further investigations are necessary to reveal direct evidence for this assumption, our results support the hypothesis that modulation of GnRH signal transduction could be involved in the mechanism of action of oestradiol and progesterone in the gonadotroph. Other studies have demonstrated that these steroids affect the activity of protein kinase C and the hydrolysis of PIP₂, suggesting a multistep mechanism (Kiesel et al. 1987a; Emons et al. 1989; Audy et al. 1990; Drouva et al. 1990; Ortmann, Tilse & Emons, 1990b). It has been shown that phorbol ester-stimulated LH secretion is enhanced by short-term progesterone treatment, while long-term progesterone treatment was without an effect (Ortmann et al. 1990; Krey & Kamel, 1990). These findings are consistent with the actions of progesterone on arachidonic acid-activated LH secretion. As arachidonic acid is able

**FIGURE 4.** Release of LH (ng RP-2/fraction) in response to 2-min (a), (b) GnRH (1 nmol/l) or (c), (d) melittin (1 μmol/l) pulses administered every 50 min (arrows) to rat pituitary cells cultured on microcarrier beads. Prior to perifusion the cells had been pretreated for 48 h with 1 nmol oestradiol/l. After an initial control pulse, cells of chambers 1 and 3 (a and c) were further perifused with medium containing 1 nmol oestradiol/l, cells of chambers 2 and 4 (b and d) with medium containing 1 nmol oestradiol/l plus 100 nmol progesterone/l.
TABLE 2. Effects of short-term progesterone (100 nmol/l) treatment on LH release induced by pulses of gonadotrophin-releasing hormone (GnRH; 1 nmol/l) or melittin (1 μmol/l) in oestradiol (1 nmol/l) primed perfused rat pituitary cells. 

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<th>GnRH</th>
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<td></td>
<td>Oestradiol:</td>
<td>Oestradiol:</td>
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<tr>
<td>Pulse 1</td>
<td>116 ± 8</td>
<td>164 ± 17*</td>
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<tr>
<td>Pulse 2</td>
<td>119 ± 11</td>
<td>241 ± 30*</td>
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<tr>
<td>Pulse 3</td>
<td>123 ± 16</td>
<td>279 ± 31*</td>
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<td>Oestradiol + progesterone:</td>
<td>Oestradiol + progesterone:</td>
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<td>109 ± 14</td>
<td>220 ± 39*</td>
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<td>126 ± 10</td>
<td>319 ± 106*</td>
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<td>132 ± 16</td>
<td>419 ± 162*</td>
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*P < 0.05 vs oestradiol (Mann-Whitney U test).

Mean absolute LH (ng RP-2) values of the control pulses were 106 ± 14 ng/peak for oestradiol (GnRH), 89 ± 18 ng/peak for oestradiol plus progesterone (GnRH), 138 ± 19 ng/peak for oestradiol (melittin), and 142 ± 83 ng/peak oestradiol plus progesterone (melittin).

to activate protein kinase C subspecies differentially (Naor, 1990), it might be possible that ovarian steroids act as modulators of one signal transduction pathway (arachidonic acid) to exert an effect on a second one (protein kinase C).

In conclusion the study presented here has shown that acute oestradiol or progesterone treatment of cultured pituitary cells modulated melittin-induced LH secretion. The results indicate that such steroid treatment modulates arachidonic acid-activated LH secretion in the same manner as GnRH-induced LH secretion. It needs to be determined whether the arachidonic acid pathway as a part of the GnRH signal transduction system might have a regulatory function in the mechanism of action of these rapid steroid effects on gonadotrophin secretion.

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REFERENCES


