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Ovarian Steroids Modulate Gonadotropin-releasing Hormone-induced Biphasic Luteinizing Hormone Secretory Responses and Inositol Phosphate Accumulation in Rat Anterior Pituitary Cells and αT3-1 Gonadotrophs

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The ovarian steroids estradiol and progesterone act as important modulators of GnRH-induced luteinizing hormone (LH) secretion from anterior pituitary cells. Recently, we demonstrated that the steroids are able to influence GnRH-stimulated Ca^{2+} mobilization from extra- and intracellular sources. Here we investigated the actions of estradiol and progesterone on GnRH-induced biphasic LH secretory responses in the model of perifused female rat pituitary cells. A 20 min GnRH stimulus elicited biphasic LH responses composed of an initial peak followed by a prolonged plateau phase. Both phases were equally enhanced by long-term (48 h) estradiol treatment. This action was facilitated by subsequent short-term progesterone treatment. In contrast, combined treatment with estradiol and progesterone for 48 h led to inhibited LH secretory profiles. To determine the steroid actions on the extracellular Ca^{2+} independent component of LH secretion we performed experiments using cells that were perifused with Ca^{2+} deficient medium. Under these conditions the cells responded exclusively with a single peak phase of LH secretion, which was augmented or inhibited by estradiol and progesterone treatment as described above. To test the hypothesis that an effect of estradiol and progesterone on GnRH-induced polyphophoinositide hydrolysis is responsible for their modulatory actions on Ca^{2+} signals and LH secretion we measured inositol phosphate (IP) accumulation after different steroid treatment paradigms in rat pituitary cells and αT3-1 immortalized gonadotrophs. GnRH-induced IP production was enhanced by long-term estradiol treatment. Short-term exposure of estradiol-primed cells to progesterone did not lead to significant changes of IP production. The long-term progesterone treatment paradigm enhanced GnRH-induced IP formation, while it decreased Ca^{2+} signals and LH secretion. αT3-1 cells were used to perform more detailed analysis of IP formation. The actions of estradiol and progesterone on the production of inositol mono-, bis-, and trisphosphates were similar to those observed in the mixed cell population. It is concluded that estradiol and progesterone modulate both peak and plateau phases of GnRH-stimulated LH secretory responses, effects which are associated with their impact on Ca^{2+} signals. Our findings argue against a role of IP modulation in the mechanism of progesterone actions on Ca^{2+} signaling and LH secretion in gonadotrophs. Such a mechanism might be involved in the positive effects of estradiol in these cells.

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INTRODUCTION

Ovarian steroids induce positive and negative feedback effects on gonadotropin secretion from the anterior pituitary gland. The direct actions of estradiol and progesterone on pituitary gonadotrophs lead to enhanced or inhibited responsiveness to hypothalamic gonadotropin-releasing hormone (GnRH) [1]. The model of cultured rat pituitary cells has been widely used to characterize the time- and dose-dependent

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actions of estradiol and progesterone on gonadotropin secretion. Prolonged exposure of such cells to estradiol leads to augmented luteinizing hormone (LH) responses to GnRH stimulation [2–4]. In contrast, long-term progesterone treatment results in reduced responsiveness of the gonadotroph [5, 6]. The acute exposure of estrogen-primed cells to progesterone exerts a pronounced facilitatory action on GnRH-induced gonadotropin secretion [7–9].

Several recent studies have provided experimental evidence for the hypothesis that the sensitizing and desensitizing effects of ovarian steroids on the responsiveness of gonadotrophs involve an action on different pathways of GnRH signal transduction [10–15]. The primary factor in agonist-stimulated LH secretion is the GnRH-induced rise in cytoplasmic calcium concentration ($[Ca^{2+}]_i$) [16, 17]. We were able to demonstrate steroid modulation of GnRH-stimulated cytoplasmatic Ca^{2+} signals in single gonadotrophs and clonal pituitary cells of the αT3-1 lineage [18,19]. The progesterone effects include actions on both Ca^{2+} mobilization and Ca^{2+} entry pathways. Previous studies have shown that GnRH-induced increases of the $[Ca^{2+}]_i$ are closely correlated to the exocytosis of LH [17, 20]. LH secretory responses to GnRH are biphasic with an initial spike and a secondary plateau phase. The early phase is dependent on the mobilization of intracellular Ca^{2+} while the sustained phase depends on Ca^{2+} influx [20]. Although sufficient information exists on the time- and dose dependence of steroid effects on gonadotropin secretion, there are no data which describe the influence of estradiol and progesterone on GnRH-induced LH secretory profiles. This is in part due to the experimental models employed (static cultures, insensitive superfusion systems) and also to experimental designs in which detailed analyses of LH secretory responses were not possible. In the present study we performed experiments using a sensitive perifusion system to examine the actions of ovarian steroids on GnRH-induced biphasic LH release. These effects were compared with our previous observations of steroid-modulated Ca^{2+} signals.

Furthermore, we investigated the hypothesis that estradiol and progesterone influence GnRH-induced inositol phosphate production, and subsequently, enhance or inhibit Ca^{2+} signals and the rapid phase of Ca^{2+}-mediated exocytosis.

MATERIALS AND METHODS

General

Pituitary glands obtained from adult female Wistar rats at random stages of the estrous cycle were dispersed into single cells by controlled trypsinization as described previously [21]. Dispersed pituitary cells were cultured in phenol-red free medium 199 (M199, Biochrom, Berlin, Germany) containing Hank's salts, 1.4% sodium bicarbonate, 10% horse serum that had been pretreated with 2% charcoal (Norit A) and 0.2% Dextran T 70 (Pharmacia, Uppsala, Sweden), 10 μg/ml streptomycin, and 100 U/l penicillin at 37°C under 5% CO_2–air and saturated humidity in multiwell culture dishes (2×10^5 cells/well) or Petri dishes [8, 22]. Under these conditions the presence of estrogens or estrogenic compounds could largely be excluded since the pure antiestrogen keoxifen did not alter GnRH-induced LH secretion [22]. αT3-1 immortalized gonadotrophs kindly provided by Dr P. M. Mellon (Department of Reproductive Medicine, University of California, San Diego) cells were grown in phenol red-free Dulbecco's modified Eagle's medium (Biochrom) supplemented with 10% fetal calf serum pretreated with 2% charcoal and 100 μg gentamycin, as described previously [23, 24]. The culture medium was changed every other day until the cells reached confluence. GnRH (Sigma, Deisenhofen, Germany) was dissolved in PBS containing 0.1% bovine serum albumine (BSA). Stock solutions of estradiol and progesterone (Sigma) were prepared in ethanol. The final concentration of ethanol in experimental media was 0.2%.

Effects of estradiol and progesterone on GnRH-induced LH secretory profiles

Rat pituitary cells were grown on Cytodex I microcarrier beads (Pharmacia) in four separate Petri dishes (I–IV) and treated for 48 h with vehicle (V, 0.2% ethanol, I), 1 nM estradiol (II, III), or 1 nM estradiol + 100 nM progesterone (IV). The beads and their attached cells were then transferred to four chambers of a perifusion system (Endotronics, Minneapolis, MN; 2×10^7 cells/chamber) and constantly perifused with medium (as described under General except that 0.1% BSA was used instead of horse serum and 25 mM HEPES instead of sodium bicarbonate) at a flow rate of 0.5 ml/min. During an equilibration period of 2 h chamber III (cells that had been pretreated with estradiol) was perifused with medium containing 100 nM progesterone in addition to 1 nM estradiol. Then, each of the chambers was challenged with a 20 min pulse of 1 nM GnRH. To examine the modulatory actions of steroids on the extracellular Ca^{2+} independent component of LH release another series of experiments was carried out using cells perifused with Ca^{2+} deficient medium (4 nM Ca^{2+}). Steroid pretreatments and GnRH stimulation were performed as described above. The effluent medium was collected in 1 min fractions which were analyzed for their LH content by RIA.

Effects of estradiol and progesterone on inositol phosphate (IP) production

Primary cultures of rat pituitary cells and αT3-1 cells were incubated for 48 h with vehicle (0.2% ethanol, V), 1 nM estradiol, 1 nM estradiol + 100 nM progesterone, or pretreated for 48 h with 1 nM estradiol and additionally with 100 nM progesterone during the last 4 h.

During the steroid treatment periods the cells were incubated for 48 h with inositol-free medium containing 10 μCi/ml myo-[^{3}H]inositol and 1% fetal bovine serum. The cells were washed three times and stimulated with 100 nM GnRH for 15 min in the presence of 10 mM LiCl. This stimulation period was chosen since agonist-induced IP formation was demonstrated to be maximal at 15 min in rat pituitary and αT3-1 cells [25–27]. The treatments were performed in duplicate. The reaction was stopped by the addition of 0.4 ml ice-cold 5% perchloric acid (PCA) after the culture media were removed. The culture dishes were then placed on ice for 30 min, and the cells were removed from each well by scraping, followed by washing with 0.1 ml water. The cell suspensions were transferred into glass tubes and placed on dry ice for 30 min. After thawing, the suspensions were centrifuged at 4 C (800 *g*; for 15 min) and the supernatants were extracted with 350 μl of a mixture of freon and tri-*n*-ocytlamine (1:1, v/v) and 100 μl 10 mM ethylenediamine tetraacetate (EDTA). The tubes were vortexed for 3 min and centrifuged for 5 min at 400 *g*, and the upper phases were transferred into Eppendorf tubes [28]. After neutralization with NaOH the samples from experiments with rat pituitary cells were analyzed for total IPs, those from experiments with αT3-1 cells for inositol monophosphates (IP_1), inositol bisphosphates (IP_2), and inositol trisphosphates (IP_3). Anion exchange chromatography on Dowex-1 columns (formate form, Sigma) was used to separate [^{3}H]IPs from free [^{3}H]inositol [29]. An IP marker set (Amersham, Braunschweig, Germany) was used to verify the IP separation. Radioactivity of the fractions collected was measured in a liquid scintillation counter. A separate set of cell cultures treated with the same combinations of steroids were incubated in the absence of myo-[^{3}H]inositol. At the end of the treatment periods cells were detached by trypsinization and cell number was determined.

RIA and data analysis

The LH content of the samples was determined by RIA using the reference preparation RP-3 rat LH provided by the National Pituitary Agency (Baltimore, MD). The LH responses in perifusion experiments were expressed as absolute values (nanograms of RP-3 per ml) during the spike and plateau phases of secretory responses or as the total mass of LH released by a 20 min pulse of GnRH in excess of basal LH secretion, as described previously [8, 30]. The data from 3 to 7 experiments (perifusion or IP experiments) were pooled and expressed in terms of the results obtained in the respective control cultures, which were defined as 100%. Statistically significant differences between treatments were determined by a paired *t*-test using the InStat program (Graph PAD software, San Diego, CA) and $P < 0.05$ was considered statistically significant.

RESULTS

Effects of estradiol and progesterone on GnRH-induced biphasic LH release

In perifused pituitary cells GnRH caused a biphasic LH secretory response with an initial peak and a secondary plateau phase. Long-term estradiol treatment led to enhanced LH responses. Both phases of the biphasic LH secretory response were augmented by 70 and 63% respectively (Fig. 1, Table 1). Short-term progesterone treatment of estradiol-primed cells induced a marked facilitatory action on both components of GnRH-induced LH secretion with increases of 196%. In contrast, the long-term progesterone treatment paradigm clearly inhibited the LH responses of the cells. Inhibitory and facilitatory actions of progesterone influenced the initial peak and the secondary plateau phase of the secretory response in a similar manner (Fig. 1, Table 1). The duration of the early and the sustained phase was not significantly affected. To demonstrate that the steroids act on the initial, extracellular Ca^{2+} independent component of GnRH-induced LH secretion we performed perifusion experiments using Ca^{2+} deficient medium. Under these conditions prolonged GnRH stimulation led to a single peak LH response without the plateau phase observed in the first series of experiments. The different steroid treatment paradigms induced inhibitory and stimulatory actions on GnRH-stimulated LH secretion as described above (Fig. 2, Table 2). The facilitatory amplitude changes after steroid treatments were even more pronounced than those seen in regular Ca^{2+} medium. None of the stimulatory steroid treatment paradigms could re-establish a secondary plateau phase of the LH response indicating the absolute requirement of extracellular Ca^{2+} for this phase. Prolonged treatment with progesterone reduced the amplitude of LH release by about 50% in Ca^{2+} deficient medium while it led to inhibition by 80% (compared to estradiol alone) in regular Ca^{2+} medium (Tables 1 and 2).

Effects of estradiol and progesterone on IP-accumulation

The initial phase of the LH response to GnRH is dependent on mobilization of Ca^{2+} from intracellular sources caused by the formation of inositol 1,4,5-trisphosphate. Since we have shown previously that steroids influenced GnRH-induced Ca^{2+} signals in gonadotrophs and demonstrated here modulation of the extracellular Ca^{2+} independent component of LH secretion, we investigated the actions of estradiol and progesterone on IP accumulation. In rat pituitary cells, treated with vehicle, GnRH induced an increase of IP production by 50%. Long-term treatment of rat pituitary cells with a combination of estradiol and progesterone had a small stimulatory action on IP accumulation in the absence of GnRH while the other treatment paradigms were ineffective (Fig. 3). Prolonged estradiol treatment enhanced GnRH-stimulated

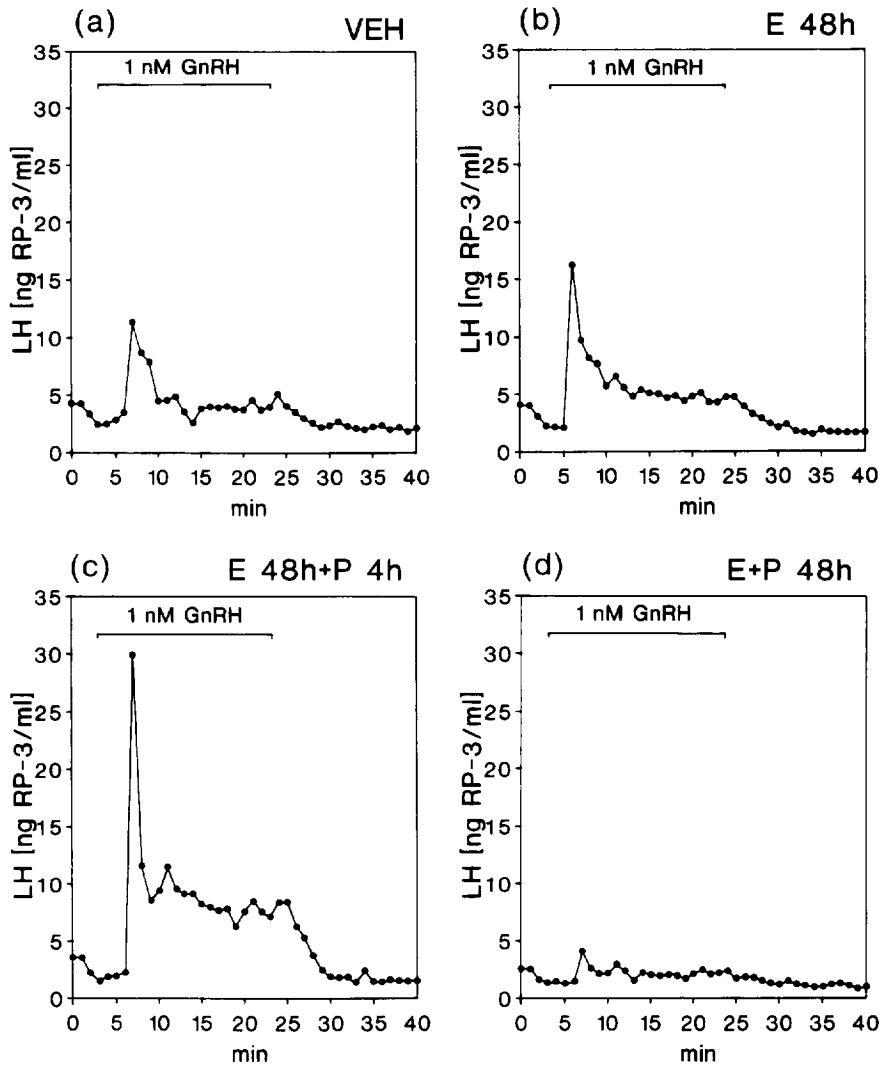

Fig. 1. Effects of estradiol and progesterone on LH secretory profiles (nanograms of RP-3 per fraction) in perifused rat pituitary cells in response to a 20 min pulse of GnRH. Before GnRH (1 nM) stimulation, the cells had been pretreated for 48 h with vehicle (V, 0.2% ethanol), 1 nM estradiol (E), 1 nM estradiol and 100 nM progesterone (P) or for 48 h with 1 nM estradiol and 2 h with 100 nM progesterone.

Table 1. Effects of estradiol and progesterone on peak and plateau phases of LH secretory responses to GnRH in perifused rat pituitary cells

	LH release (% of V)	
	Peak	Plateau
V (48 h)	100 ± 35	100 ± 22
E (48 h)	170 ± 25*	163 ± 16*
E (48 h) + P (2 h)	296 ± 40**	296 ± 50**
E + P (48 h)	32 ± 12**	45 ± 14**

Cells were pretreated with estradiol and progesterone and stimulated with GnRH as described in Fig. 1. Data are from 6 separate experiments and were presented as percentages (mean ± SE) of the total amount of LH secreted during peak and plateau phases of the secretory response to GnRH in vehicle treated cells (peak, 100% = 21 ng RP-3/ml; plateau, 100% = 32 ng RP-3 ml). *Indicates $P < 0.05$ vs V, **indicates $P < 0.05$ vs E (paired t-test).

IP production. Acute progesterone treatment, which had a facilitatory action on GnRH-induced LH secretion, did not lead to further enhancement of IP production, but a clear positive effect was observed after 48 h of combined treatment with estradiol. In the mixed population of primary pituitary cell cultures it is difficult to detect IP_3 after GnRH-stimulation. The use of αT3-1 cells permitted a more detailed analysis of IPs. GnRH stimulation led to 8-, 38- and 6-fold increases of IP_1, IP_2 and IP_3 accumulation, respectively. In non-stimulated cells estradiol alone or in combination with progesterone induced minor increases of IP_1 and IP_2 production which were statistically insignificant in most of the cases. Steroid-induced changes of IP_3 production were not detectable in these cells. In GnRH-stimulated cell cultures 48 h estradiol treatment enhanced IP_1, IP_2 and IP_3 production by

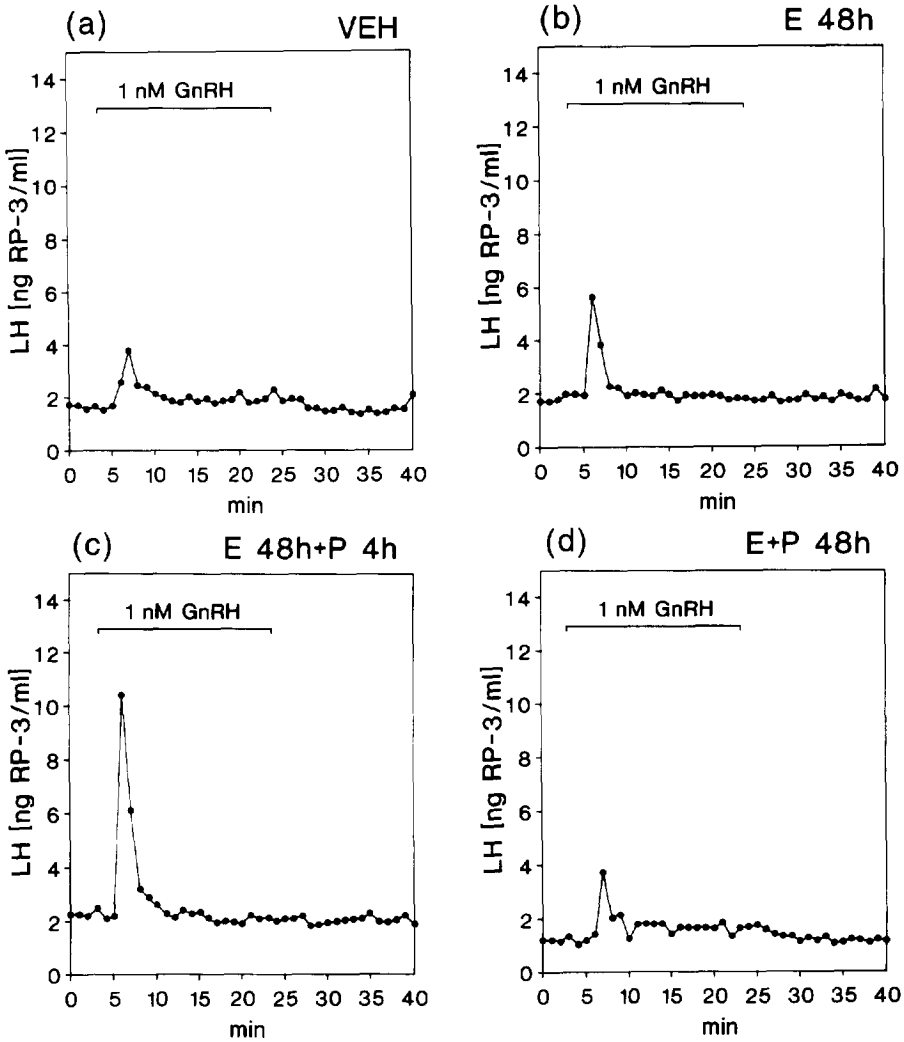

Fig. 2. Effects of estradiol and progesterone on extracellular Ca^{2+} independent LH secretion from perifused rat pituitary cells in response to a 20 min GnRH pulse. Before GnRH (100 nM) application the cells were pretreated with the steroids as described in Fig. 1.

37, 36 and 27%, respectively (Fig. 4). Short-term progesterone treatment of estradiol-primed cells had a weak stimulatory action. However, the results did not differ significantly from those obtained in cell cultures treated with estradiol alone. Long-term treatment with progesterone induced a clear augmentative effect on GnRH-induced IP_1, IP_2 and IP_3 accumulation with increases by 65, 57 and 56%, respectively (Fig. 4). None of the performed steroid treatments led to changes in the number of αT3-1 cells.

Table 2. Effects of estradiol and progesterone on GnRH-induced LH secretion from rat pituitary cells perifused with Ca^{2+} deficient medium

V (48 h)	E (48 h)	E (48 h) + P (2 h)	E + P (48 h)
100 ± 28	212 ± 44*	374 ± 49**	102 ± 31

Cells were pretreated with estradiol and progesterone and stimulated with GnRH as described in Fig. 2. Data are from 3 separate experiments and are presented as percentages (mean ± SE) of the total amount of LH secreted in response to GnRH in vehicle-treated cells (100% = 5.7 ng RP-3/ml). V, vehicle; E, estradiol; P, progesterone. *$P < 0.05$ vs V, **$P < 0.05$ vs E (t-test).

DISCUSSION

The results obtained from this study clearly demonstrate that estradiol and progesterone modulate GnRH-induced LH release from pituitary gonadotrophs with similar effects on peak and plateau phases of the secretory response. These findings support the significance of our previous observation that Ca^{2+} mobilization from intracellular stores and Ca^{2+} influx are markedly modulated by progesterone and less clearly by estradiol [18, 19]. Others have shown that the initial peak phase of LH release depends on mobilization of intracellular Ca^{2+} while the secondary plateau phase is associated with Ca^{2+} entry from the extracellular space [20]. Therefore it is suggested that the steroid actions on both Ca^{2+} mobilizing processes result in modulation

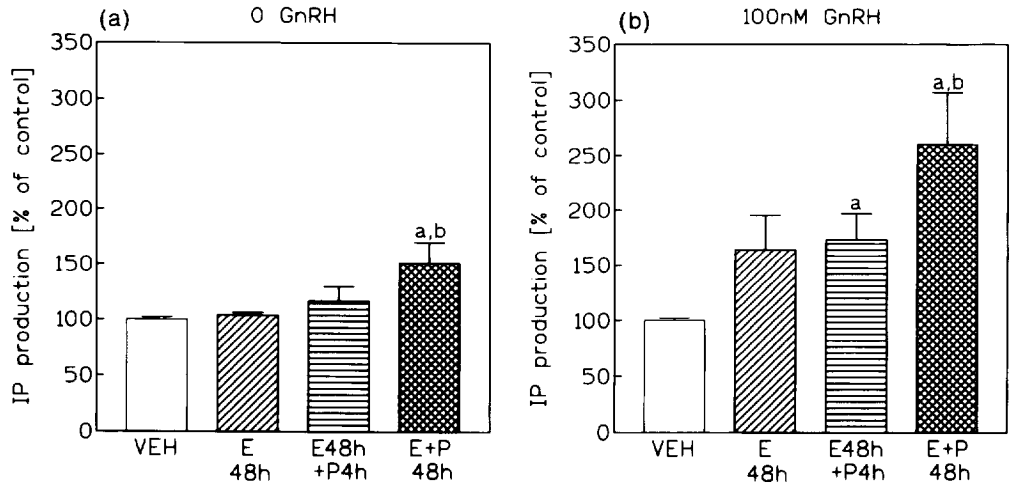

Fig. 3. Effects of estradiol and progesterone on total inositol phosphate (IP) production in rat anterior pituitary cells. Cells were treated for 48 h with vehicle (Veh, 0.2% ethanol), 1 nM estradiol (E), 1 nM estradiol plus 100 nM progesterone (P) or for 48 h with 1 nM estradiol and a further 4 h with 100 nM progesterone. After these steroid pretreatments the cells were incubated for 15 min in the absence or presence of GnRH (100 nM). Data are from 4 separate experiments and were presented as the percentage (mean + SE) of IP production in vehicle-treated cells (100%). (a) $P < 0.05$ vs Veh; (b) $P < 0.05$ vs E (by paired t-test).

of the two phases of LH secretory responses. Interestingly, this phenomenon is not restricted to GnRH-stimulated LH secretion. In a recent study we have described similar effects of estradiol and progesterone on endothelin-induced gonadotropin secretion [30]. Although we have not analyzed the actions of ovarian steroids on endothelin-stimulated Ca^{2+} signals in gonadotrophs it seems to be quite likely that these would be also affected. In the absence of extracellular Ca^{2+}, gonadotrophs responded with a single spike phase of LH secretion to GnRH stimulation. This type of response was also modulated by estradiol- and progesterone treatment in a time-dependent manner. Under these experimental conditions we could largely exclude actions of the steroids on GnRH-induced Ca^{2+} influx into gonadotrophs. Previously we demonstrated that progesterone was able to alter the initial extracellular Ca^{2+} independent phase of GnRH-stimulated Ca^{2+} signals in rat pituitary gondotrophs and αT3-1 cells [18, 19]. The rapid formation of inositol 1,4,5-trisphosphate results in mobilization of Ca^{2+} from intracellular sources [31, 32]. Therefore we investigated the actions of estradiol and progesterone on IP accumulation in such cells to test the hypothesis whether our present and previous observations could be explained by steroid effects on IP formation. In primary cell cultures GnRH-induced IP accumulation was enhanced by all treatment paradigms with marked effects after long-term progesterone incubation. The analysis of IP_3 is difficult in the mixed cell population because of the relatively small percentage (10%) of gonadotrophs. For this purpose we employed αT3-1 cells. We were able to demonstrate similar actions of estradiol and progesterone on agonist-stimulated IP_1, IP_2, and IP_3 formation. Unexpectedly, the steroid actions on IP accumulation do not necessarily correlate with the effects on Ca^{2+} signals and LH secretion. Long-term estradiol treatment was shown to have a moderate augmentative effect on IP production and had a weak sensitizing action on GnRH-induced Ca^{2+} signals reflected in increased frequency of Ca^{2+} oscillations in single gonadotrophs [18]. On the other hand, the positive action of estradiol on LH secretory responses is quite robust. In contrast, progesterone, which had marked facilitatory actions on GnRH-induced Ca^{2+} signals in estrogen-primed cells and gonadotropin secretion, did not increase IP accumulation compared to estrogen treatment alone. Quite unexpected were the effects of long-term progesterone treatment, which induced profound desensitization of LH secretory responses and Ca^{2+} signals while IP formation was clearly enhanced. It could be argued that the steroid actions might be different at early time points after GnRH stimulation. However, pilot experiments, in which we analyzed agonist-induced IP accumulation after 30 s, led to similar results. In the present study we have not examined the actions of estradiol and progesterone on the incorporation of myo-[^{3}H]inositol into membrane phospholipids, which may provide information on the mechanism that causes the observed changes of IP production under different steroid treatment paradigms. Taken together, our observations reveal a poor correlation of the amount of IP production with LH secretion and Ca^{2+} signals after steroid treatment.

In a previous study, others have shown augmentation of GnRH-induced IP formation in αT3-1 cells which had been pretreated for 4 days with estradiol [24]. However, when the authors corrected their data for cell numbers, they found an inhibitory action of the steroid.

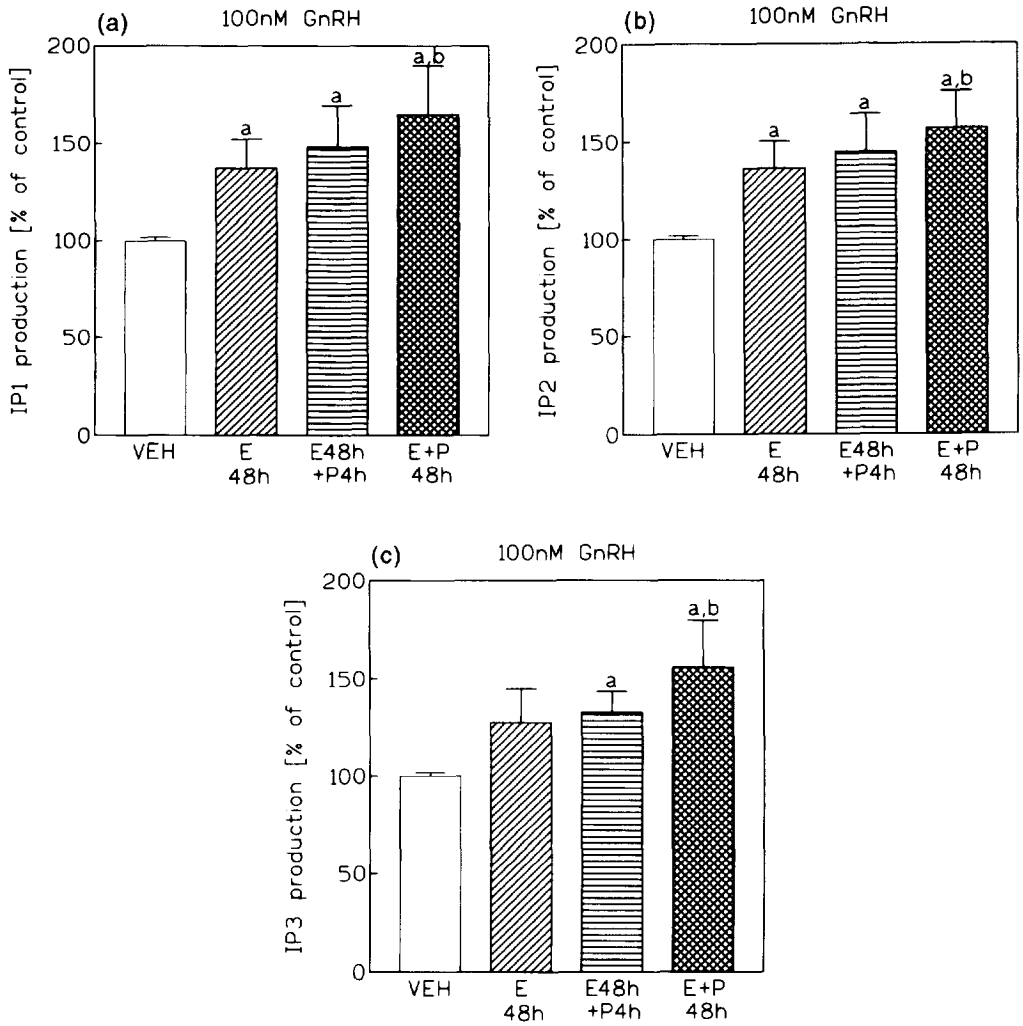

Fig. 4. Effects of estradiol and progesterone on IP production in αT3-1 cells. Cells were treated for 48 h with vehicle (Veh, 0.2% ethanol), 1 nM estradiol (E), 1 nM estradiol plus 100 nM progesterone (P) or for 48 h with 1 nM estradiol and a further 4 h with 100 nM progesterone. After these steroid pretreatments the cells were incubated for 15 min with GnRH (100 nM) and analyzed for accumulation of IP_1, IP_2 and IP_3. Data are from 6 separate experiments and were presented as the percentage (mean + SE) of IP_{1-3} production in vehicle-cells (100%). (a) $P < 0.05$ vs Veh; $P < 0.05$ vs E (by paired t-test).

In our experiments we did not observe changes in cell numbers after 48 h of estradiol treatment. The discrepancy between these findings might be due to the difference in the duration of steroid treatment (48 vs 96 h). On the other hand, in rat anterior pituitary cells, long-term estradiol treatment induced exclusively positive actions on gonadotropin secretion [1, 2, 4, 5].

Interestingly, steroid-induced changes of IP accumulation in response to GnRH are in accord with their actions on GnRH receptor numbers in rat pituitary gonadotrophs. These have been shown to respond with increases after long-term estradiol, short-term progesterone, and after long-term progesterone treatment [33]. This implicates that the regulatory processes at the peptide hormone receptor level and immediate post receptor mechanisms do not necessarily explain the effects on GnRH-induced exocytosis of LH. However, the observation that Ca^{2+} signaling is dependent on the steroid milieu to which the gonadotroph is exposed supports the hypothesis that this second messenger system is tightly coupled to the secretory machinery [17, 34]. The modulation of this system is certainly important for the mediation of sensitizing and desensitizing effects of ovarian steroids. At present, however, it is not clear which mechanism controls estrogen and progesterone modulation of GnRH-induced Ca^{2+} mobilization from the extracellular space or the endoplasmic reticulum.

In non-stimulated pituitary cells steroids exert relatively weak effects on basal gonadotropin secretion. In our hands, only short-term progesterone treatment of estrogen-primed cells leads to augmentation of LH secretion but does not consistently affect IP formation. The stimulatory actions of prolonged progesterone exposure on IP production reported here are in contrast to its weak negative actions on gonadotropin

secretion. Furthermore we were unable to demonstrate effects of estradiol or progesterone on $[Ca^{2+}]_i$ in quiescent gonadotrophs (unpublished observations). These data argue against a role of IPs in steroid regulation of basal hormone release from gonadotrophs.

Taking into account that changes of GnRH-stimulated IP_3 production did not result in consecutive changes of Ca^{2+} signals [18, 19], it could be speculated that steroids influence inositol 1,4,5-trisphosphate receptor channel activity. Although we were able to demonstrate that progesterone alters Ca^{2+} influx *via* depolarization-activated voltage sensitive Ca^{2+} channels there is no direct experimental evidence for the assumption that steroids act on the level of membrane Ca^{2+} channels in gonadotrophs [18]. However, such mechanisms of steroid actions have been shown in other cell types [35, 36]. Although our present data on steroid-influenced kinetics of LH release favour modulation of the Ca^{2+} messenger system as the predominant regulatory mechanism of these steroid actions, other parts of signal transduction might also be involved. There is experimental evidence that protein kinase C (PKC) and arachidonic acid or its metabolites may play a role as second messengers in gonadotrophs [16, 37]. It is possible that an influence on these two components of GnRH signal transduction leads to alteration of Ca^{2+} signaling and, in turn, of LH secretion. In the case of PKC, this could result in changes of Ca^{2+} channel phosphorylation. Total PKC activity has been shown to increase after estrogen treatment of rat pituitary cells [11]. This effect might facilitate LH secretion. At present it is not known whether progesterone acts on PKC in gonadotrophs. Furthermore, the role of PKC in the mechanism of GnRH-induced gonadotrophin secretion is still not fully clarified [38, 39]. Arachidonic acid and its lipoxygenase metabolites have been shown to act as Ca^{2+} mobilizing agents [40, 41]. Recent data from our laboratory demonstrated consistent actions of progesterone on GnRH-induced LH and arachidonic acid release from perifused rat pituitary cells [42]. If arachidonic acid acts as a Ca^{2+} mobilizing agent in gonadotrophs our observations might explain the progesterone effects on agonist-stimulated Ca^{2+} signals and LH secretion.

In conclusion, estradiol and progesterone exert coordinate actions on initial peak and prolonged plateau phases of GnRH-stimulated LH secretory responses of gonadotrophs. Based on these and previous observations, we propose that modulation of the Ca^{2+} messenger system is essential for steroid actions on exocytosis of LH. Our findings indicate that changes of IP production might be responsible for estradiol effects, but argue against a role of this second messenger in the marked actions of progesterone on GnRH-induced Ca^{2+} signals and LH secretion from gonadotrophs. Furthermore it is concluded that amplitude modulation of IP_3 signals does not necessarily lead to consistent changes of Ca^{2+} signals and LH secretory responses.

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