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Modulation of Calcium Signaling and LH Secretion by Progesterone in Pituitary Gonadotrophs and Clonal Pituitary Cells

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In estradiol-treated pituitary cells, progesterone enhances gonadotropin-releasing hormone (GnRH)-induced LH secretion from cultured rat pituitary cells during short-term treatment but attenuates this response during prolonged treatment. In the present study, the effects of gonadal steroids on GnRH-induced cytoplasmic calcium ($[Ca^{2+}]_i$) responses in gonadotrophs were analyzed in rat pituitary cells and immortalized (αT3-1) murine gonadotrophs. Ca^{2+} responses were measured in cell suspensions and single gonadotrophs, loaded with Fura-2 or Indo-1, respectively, and pretreated for 48 h with 1 nM estradiol with or without 100 nM progesterone, or for 48 h with 1 nM estradiol and then for 3 h with 100 nM progesterone. In cells of the αT3-1 gonadotroph lineage, GnRH elicited biphasic Ca^{2+} signals composed of an initial peak response followed by a prolonged plateau phase. The amplitudes of both the extracellular Ca^{2+}-independent spike phase and the extracellular Ca^{2+}-dependent plateau phase were enhanced or inhibited by short- or long-term progesterone treatment, respectively. In single pituitary gonadotrophs, GnRH (0.5 nM) elicited oscillatory responses due to intermittent release and uptake of Ca^{2+} from intracellular stores. Treatment with progesterone shifted the oscillatory signal toward biphasic (3 h) or subthreshold (48 h) response profiles, revealing a steroid-induced change in the pattern of Ca^{2+} mobilization. In addition to these agonist-induced responses, the transient $[Ca^{2+}]_i$ responses of pituitary cells and individual gonadotrophs to high K^+ were enhanced or inhibited after short- or long-term progesterone treatment, respectively. These actions were correlated with the effects of progesterone on K^+-induced LH secretion. The $[Ca^{2+}]_i$ and LH secretory responses to phorbol ester treatment were also enhanced by short-term exposure of the cells to progesterone. The results demonstrate that the stimulatory and inhibitory effects of progesterone on agonist-induced Ca^{2+} signaling result from changes in Ca^{2+} mobilization and entry, and contribute to the modulatory actions of the steroid on GnRH-induced LH secretion.

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INTRODUCTION

Estradiol and progesterone are important ovarian modulators of gonadotropin secretion from the anterior pituitary gland. The positive and negative feedback effects of these steroids include both sensitizing and inhibitory actions on the responsiveness of pituitary gonadotrophs to hypothalamic gonadotropin-releasing hormone (GnRH). In cultured pituitary cells from female rats, long-term (>12 h) estradiol treatment leads to enhanced LH responses to GnRH stimulation [1–3]. In such estrogen-primed cells, short-term (>45 min) progesterone treatment exerts an acute facilitatory effect on GnRH-stimulated gonadotropin secretion [4–6]. Conversely, prolonged exposure (>16 h) of these cells to progesterone leads to inhibition of LH secretory responses [1, 5].

Although the effects of gonadal steroids on gonadotropin release are well characterized, the underlying mechanisms of these actions are poorly understood. Several lines of evidence support the hypothesis that modulation of gonadotropin secretion by estradiol and progesterone includes an action on the GnRH signal

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transduction system [7–9]. GnRH stimulates polyphosphoinositide hydrolysis and the formation of inositol 1,4,5-trisphosphate and diacylglycerol, which respectively mobilize calcium (Ca^{2+}) from intracellular sources and activate protein kinase C (PKC) [10–13]. In single gonadotrophs, the GnRH-induced increase of $[Ca^{2+}]_i$, which is the primary signal for exocytosis, exhibits dose-related response profiles that range from subthreshold (low doses) to oscillatory (intermediate doses) and biphasic (high doses) patterns [14]. The initial oscillatory or spike response is independent of Ca^{2+} influx while the plateau phase requires Ca^{2+} entry into the cell [14–16].

In the present study, we investigated the ability of ovarian steroids to modulate agonist-induced cytoplasmic Ca^{2+} signals and Ca^{2+}-mediated exocytosis in rat pituitary cells. $[Ca^{2+}]_i$ measurements were performed in cultured pituitary gonadotrophs and cells of the αT3-1 gonadotroph lineage [17]. Based on $[Ca^{2+}]_i$ measurements in single gonadotrophs and cell suspensions, stimulated by GnRH and agents that promote Ca^{2+} entry through voltage-sensitive calcium channels (VSCC), we conclude that both Ca^{2+} mobilization and Ca^{2+} entry are bidirectionally modulated by progesterone, depending on the duration of steroid action.

MATERIALS AND METHODS

General

Pituitary glands obtained from intact or 2–3 week ovariectomized adult female Sprague Dawley rats were dispersed into single cells by controlled trypsinization as described previously [18]. Dispersed pituitary cells were cultured in medium 199 (M199, Biofluids Inc., Rockville, MD, U.S.A.) containing Earle'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. αT3-1 cells were grown in Dulbeco's modified Eagle's medium supplemented with 10% fetal calf serum pretreated with 2% charcoal and 100 μg gentamycin, as described previously [19]. GnRH (Peninsula Laboratories Inc., Belmont, CA, U.S.A.) was dissolved in distilled water; phorbol 12-myristate-13-acetate (PMA) in dimethylsulfoxide, and estradiol and progesterone in ethanol (all from Sigma, St Louis, MO, U.S.A.). All drugs were diluted to their respective working concentrations before use.

Effects of estradiol and progesterone on cytoplasmic Ca^{2+} signals

$[Ca^{2+}]_i$ measurements were performed either in cell suspensions (pituitary cells from ovariectomized rats or αT3-1 cells) or in single gonadotrophs. When suspensions were used the cells were cultured on 6-well plates (2×10^6 cells/well) and pretreated for 48 h with 1 nM estradiol, or 1 nM estradiol + 100 nM progesterone, or for 48 h with estradiol and during the last 3 h with 100 nM progesterone. The cells then were detached by pretreatment with 0.02% EDTA in Ca^{2+}-free M199 for 10 min at 37°C, and centrifuged at 100 *g* for 5 min. The cells were resuspended in M199 containing Hank's salts, 0.1% bovine serum albumin (BSA) and 2 μM Fura-2 AM (Calbiochem, San Diego, CA, U.S.A.) and incubated for 30 min at 37°C. After a further centrifugation step (100 *g* for 5 min) the cells were resuspended in phenol red-free M199 containing Hank's salts, 1.2 mM $CaCl_2$ and 0.1% BSA. Spectrofluorometric analyses of $[Ca^{2+}]_i$ were performed in a Delta Scan Spectrofluorometer (Photon Technology Inc., Princeton, NJ, U.S.A.) [20]. The $[Ca^{2+}]_i$ values calculated from emission data at 500 nM were corrected for dye leakage and autofluorescence as described by Grynkiewicz *et al.* [21]. To investigate the effects of estradiol and progesterone on Ca^{2+} influx through PKC-activated VSCC, pituitary cells from ovariectomized rats were stimulated with 40–60 mM K^+ or 100 nM PMA. Since αT3-1 cells respond exclusively with biphasic Ca^{2+} signals [20], they were utilized to determine whether estradiol and progesterone exert specific actions on the spike and plateau phases of the Ca^{2+} response.

For $[Ca^{2+}]_i$ measurements in single gonadotrophs, pituitary cells were plated in 35 mm Petri dishes (Falcon, Oxnard, CA, U.S.A.) containing 25 mm round glass coverslips coated with 0.01% poly-L-lysine (Sigma). Steroid pretreatments were performed as described above. After a culture period of 2–3 days the incubation medium was replaced with 2 ml M199 (Hank's salts, 0.1% BSA, 25 mM HEPES, 12.5 mM $NaHCO_3$, 100 U/l penicillin and 10 μg/ml streptomycin) containing 2 μM Indo-1 AM (Molecular Probes, Eugene, OR, U.S.A.). After incubation for 60 min at 37°C the coverslips were washed twice with fresh incubation medium containing 1.2 mM $CaCl_2$ and maintained in the same medium at room temperature before fluorescence measurements. Individual coverslips were transferred into a Leiden coverslip dish (Medical Systems Co., Greenvale, NY, U.S.A.) with 1 ml phenol red-free M 199, mounted on the stage of an inverted Diaphot microscope attached to a dual emission microscopic fluorometer (Nikon, Garden City, NY, U.S.A.) and examined under a ×40 oil immersion fluorescence objective. Fluorescence measurements and calculation of $[Ca^{2+}]_i$ values were performed as described previously [14]. An intermediate dose of GnRH (0.5 nM) or high $[K^+]_e$ was used to stimulate the cells.

Effects of estradiol and progesterone on LH secretion

To test the effects of estradiol and progesterone on secretory responses to GnRH, PMA or depolarization induced by high extracellular K^+ anterior pituitary cells were cultured in multiwell culture dishes (200,000 cells/well). The cells were pretreated for 48 h with vehicle (V, 0.2% ethanol), 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. Stimulation with 1 nM GnRH, 10 nM PMA, or 60 mM K^+ was performed during the last 3 h of the indicated treatment periods. Media were collected and analyzed for their LH content by radioimmunoassay using the RP-3 rat LH standard provided by the National Hormone and Pituitary Agency (Baltimore, MD, U.S.A.).

The effect of acute progesterone treatment on LH secretion induced by Ca^{2+} influx through VSCC was analyzed in cell perifusion experiments. For this purpose, pituitary cells were cultured for 48 h in Petri dishes on Cytodex I microcarrier beads in the presence of 1 nM estradiol. The beads and their attached cells were then transferred to two perifusion chambers (Endotronics, Minneapolis, MN, U.S.A.; 2×10^7 cells/chamber) and constantly perifused with medium (as described under *General* except that 1% BSA was used instead of horse serum and 25 mM HEPES instead of sodium bicarbonate) containing 1 nM estradiol. After an equilibration period of 90 min each of the chambers was challenged with a 2-min pulse of 60 mM K^+ (control pulse). One of the chambers was then perifused with medium containing 100 nM progesterone in addition to 1 nM estradiol. Three further K^+ pulses were administered to each of the chambers at 40-min intervals. The effluent medium was collected in 1-min fractions (0.5 ml/fraction) which were analyzed for their LH content by RIA.

RESULTS

Effects of estradiol and progesterone on cytoplasmic Ca^{2+} signals

It has been shown previously that suspensions of αT3-1 cells respond exclusively with biphasic $[Ca^{2+}]_i$ profiles to GnRH (100 nM), with a spike phase predominantly dependent on Ca^{2+} mobilization, and a sustained plateau phase that is dependent on Ca^{2+} entry through VSCC [20]. In estradiol-primed cells, short-term exposure of these cells to progesterone caused marked enhancement of the spike and plateau phases, while long-term application of the steroid had an inhibitory action (Fig. 1, Table 1). These data indicate that progesterone modulates both Ca^{2+} mobilization and Ca^{2+} entry pathways.

More detailed examination of the modulatory effects of progesterone on Ca^{2+} mobilization pathways was performed in single gonadotrophs after steroid treatment of cultured pituitary cells. For this purpose, individual gonadotrophs were stimulated with a submaximal dose (0.5 nM) of GnRH to elicit oscillatory cytoplasmic Ca^{2+} signals that are predominantly dependent on Ca^{2+} mobilization during the first 5 min of stimulation [14–16]. In long-term estradiol-treated cells, this dose of GnRH induced oscillatory $[Ca^{2+}]_i$ response profiles in about 80% of the cells. Progesterone application for 3 h switched the subthreshold to oscillatory responses and oscillatory to biphasic responses. In contrast, long-term progesterone treatment shifted oscillatory to subthreshold response profiles (Fig. 1,

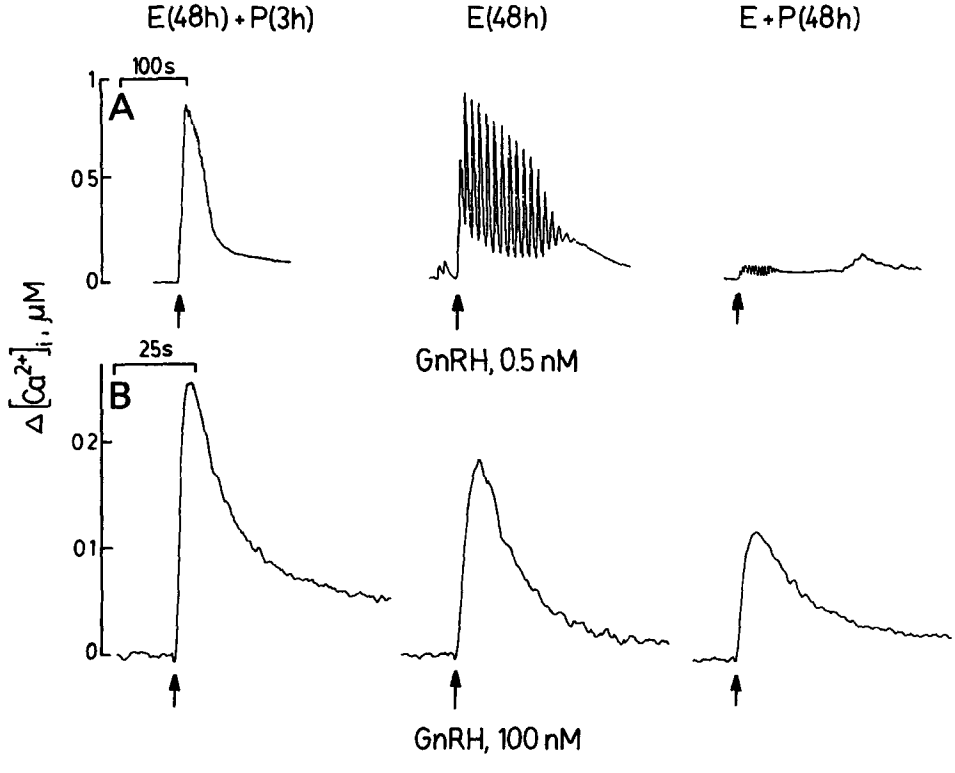

Fig. 1. Short- and long-term effects of progesterone on GnRH-induced $[Ca^{2+}]_i$ signals in single gonadotrophs (A) and suspensions of αT3-1 cells (B). Cells were treated for 48 h with 1 nM estradiol or 1 nM estradiol + 100 nM progesterone, or for 48 h with 1 nM estradiol and 3 h with 100 nM progesterone before stimulation with 0.5 nM (single gonadotrophs) or 100 nM GnRH (αT3-1 cells).

Table 1. Effects of progesterone on GnRH-induced Ca^{2+} responses in αT3-1 cells

Response	E 48 h	E + P 48 h	E 48 h + P 3 h
Spike amplitude (nM)	205.6 ± 14 3	175.3 ± 13 3	345.6 ± 56 4*
Plateau amplitude (nM)	26.4 ± 3.6	10.8 ± 3.1*	60.2 ± 11 8*

$[Ca^{2+}]_i$ changes (nM) in suspended αT3 cells that had been pretreated for 48 h with 1 nM estradiol (E), 48 h with 1 nM estradiol and 3 h with 100 nM progesterone (P), or 48 h with 1 nM estradiol + 100 nM progesterone and then stimulated with 100 mM K^+ Data of 6–8 independent experiments were analyzed for statistically significant differences of net $[Ca^{2+}]_i$ increases during the spike and plateau phases of the responses. *Indicate $P < 0.05$ or higher vs estradiol (ANOVA + Fisher's test).

Table 2). None of the steroid treatments influenced the amplitude of the oscillatory or biphasic Ca^{2+} signals, while long-term progesterone exposure of the cells reduced spike frequency [22].

In addition, depolarization-induced Ca^{2+} signals elicited by high $[K^+]_e$ were analyzed to evaluate the modulatory effects of progesterone on the voltage-sensitive Ca^{2+} entry pathway. In suspensions of cultured rat pituitary cells, K^+ (60 mM) elicited typical biphasic $[Ca^{2+}]_i$ responses with an initial spike and a subsequent plateau phase (Fig. 2). Short-term (left panels) and long-term (right panels) progesterone treatment clearly enhanced or reduced the $[Ca^{2+}]_i$ responses, respectively. These treatments influenced both the spike and the plateau phase of the Ca^{2+} signal (Table 2). Since the above experiments were performed in suspensions of unfractionated pituitary cells, it is possible that these findings could result from actions of estradiol and progesterone on cell types other than gonadotrophs. However, studies performed in single gonadotrophs demonstrated that progesterone affected the depolarization-induced Ca^{2+} signal in the same biphasic fashion as in suspended cells, i.e. with enhancement after short-term and inhibition after long-term treatment (Fig. 3).

Phorbol esters are known to exert modulatory effects on VSCC status in pituitary gonadotrophs [15] and other types of pituitary cells [23]. In long-term estradiol-treated pituitary cells in primary culture, PMA induced immediate and relatively small Ca^{2+} signals. Short-term progesterone treatment in such estradiol-primed cells resulted in clearly enhanced $[Ca^{2+}]_i$ responses to PMA (Table 3). However, PMA response in long-term estradiol + progesterone treated cells was similar to that observed in cells treated with estradiol alone (Table 3).

Table 2. Effects of progesterone on depolarization-induced increases of $[Ca^{2+}]_i$ in rat pituitary cells

	Spike	Plateau
E 48 h	124 ± 29	51 ± 14
E 48 h, P 3 h	195 ± 21*	78 ± 6
E + P 48 h	85 ± 22*	26 ± 7*

For details of steroid treatments and data analysis see legend of Table 1.

Effects of estradiol and progesterone on GnRH-, phorbol ester-, and depolarization-induced LH secretion

When cultured pituitary cells were treated for 48 h with estradiol, GnRH-stimulated LH secretion was significantly enhanced. Coincubation with progesterone for the same period reduced GnRH-stimulated LH secretion. However, short-term (4 h) progesterone treatment led to enhanced LH responses (Fig. 4, left panel). Similar effects were observed when cells were stimulated with PMA, except that the long-term inhibitory action of progesterone did not occur (Fig. 4, middle panel). When depolarizing concentrations of K^+ were used to induce gonadotropin secretion both inhibitory and stimulatory effects of the steroids were observed, similar to those seen in GnRH-stimulated cells (Fig. 4, right panel).

Perifused rat pituitary cells were used to investigate the kinetics of the acute progesterone effect on depolarization-induced LH secretion. As shown in Fig. 5, the LH response to K^+ was augmented by 33% compared to estradiol treatment alone within 40 min after the onset of progesterone treatment. This increase in the K^+-induced secretory response (+123%, $P < 0.05$ vs estradiol, Mann-Whitney U-test, $n = 3$) was statistically significant after 80 min, and was fully expressed after 120 min, resulting in a 3-fold enhancement of LH release. The LH responses of cells in the control chamber, which received estradiol alone, showed only a small increase after repeated K^+ pulses. The kinetics of the progesterone-induced augmentation of depolarization-stimulated LH responses were similar to those in GnRH-stimulated cells [6].

DISCUSSION

The present study extends earlier reports on the stimulatory and inhibitory effects of progesterone on agonist-induced gonadotropin secretion in cultured pituitary gonadotrophs [22]. Since gonadotropin secretion is initiated by increases of $[Ca^{2+}]_i$ that result from mobilization of intracellular Ca^{2+} and Ca^{2+} entry through VSCC [23], we investigated the ability of progesterone to modulate these mechanisms. Measurements of $[Ca^{2+}]_i$ responses were performed in suspensions of αT3-1 cells, which respond to GnRH stimulation with prominent $[Ca^{2+}]_i$ responses

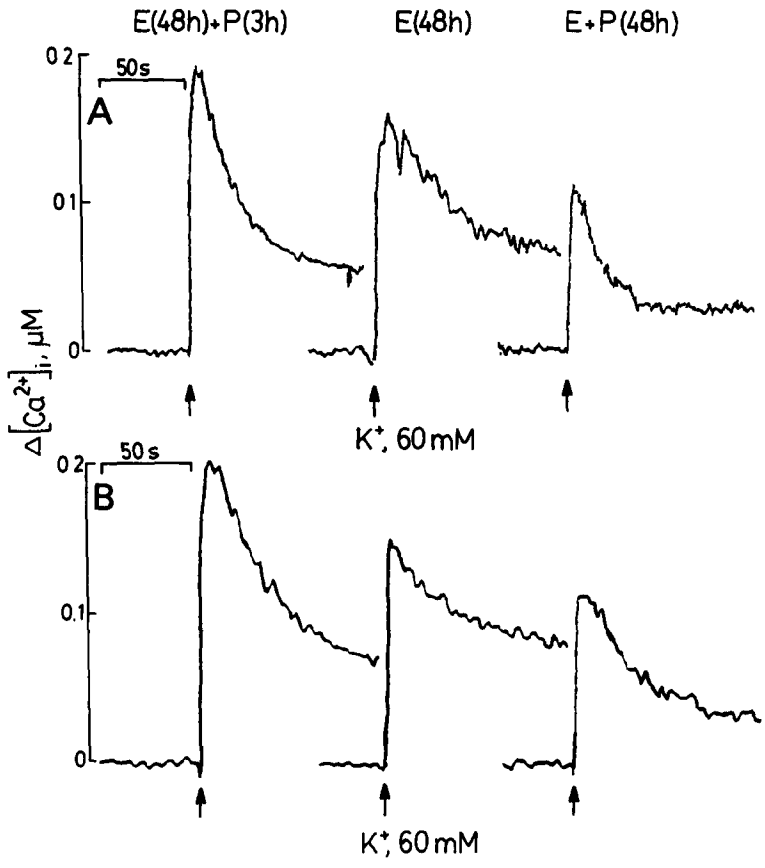

Fig. 2. Short- and long-term effects of progesterone on depolarization (60 mM K^+)-induced $[Ca^{2+}]_i$ responses in suspensions of pituitary cells. Before $[Ca^{2+}]_i$ measurements, pituitary cells from ovariectomized female rats were treated with estradiol and progesterone as described in the legend of Fig. 1. Two representative experiments (A and B) are shown.

comparable to those observed in single cell recordings [20, 24]. In these cells of the gonadotroph lineage, short- and long-term treatment with progesterone enhanced or reduced the amplitude of the Ca^{2+} signal, respectively. Furthermore, both the extracellular Ca^{2+}-independent spike phase and the extracellular Ca^{2+}-dependent plateau phase were similarly affected.

The modulatory effects of progesterone on GnRH-induced Ca^{2+} mobilization pathways were further characterized in single gonadotrophs from primary pituitary cell cultures. Such cells show a complex pattern of Ca^{2+} responses to increasing agonist concentrations, composed of subthreshold amplitude-modulated signals, threshold-oscillatory responses, with dose-dependent modulation of frequency but not amplitudes of Ca^{2+} spiking, and threshold-biphasic responses [23]. We observed bidirectional regulation of such $[Ca^{2+}]_i$ response profiles by progesterone during the first 5 min after agonist application, when the response is predominantly dependent on Ca^{2+} mobilization from intracellular stores [14]. Short-term progesterone treatment shifted subthreshold responses to oscillatory responses, and oscillatory to biphasic responses. In contrast, long-term progesterone treatment shifted biphasic to oscillatory and oscillatory to subthreshold $[Ca^{2+}]_i$ response profiles.

These data clearly indicate that progesterone increases or decreases the sensitivity of the intracellular events responsible for intracellular Ca^{2+} mobilization. The step at which progesterone influences the signal transduction system to modulate Ca^{2+} signaling and GnRH-induced gonadotropin secretion is not yet clear. Among the possible progesterone-regulated targets within this system are the GnRH receptor, the transducing G protein, and the polyphosphoinositide hydrolysis process. In contrast to the marked inhibitory and stimulatory actions of progesterone on Ca^{2+} signaling in estrogen-primed cells, estradiol itself, which augments agonist-induced gonadotropin secretion, did not significantly alter the $[Ca^{2+}]_i$ responses to GnRH or stimulators of Ca^{2+} entry. The absence of such an action demonstrates that modulation of LH secretory responses can be mediated by mechanisms other than regulation of Ca^{2+} signaling.

The data from experiments with αT3-1 gonadotrophs indicate that not only GnRH-induced Ca^{2+} mobilization, but also Ca^{2+} entry, is affected by progesterone treatment. In accord with this, receptor-independent activation of VSCC by K^+-mediated depolarization of the cell membrane is also modulated by progesterone. Mixed cell populations prepared from pituitaries of ovariectomized rats that were stimulated with K^+ showed rapid increases of $[Ca^{2+}]_i$ and responded with bursts of LH release when K^+ was applied to perifused cells in a pulsatile fashion. Long-term progesterone treatment, which had an inhibitory

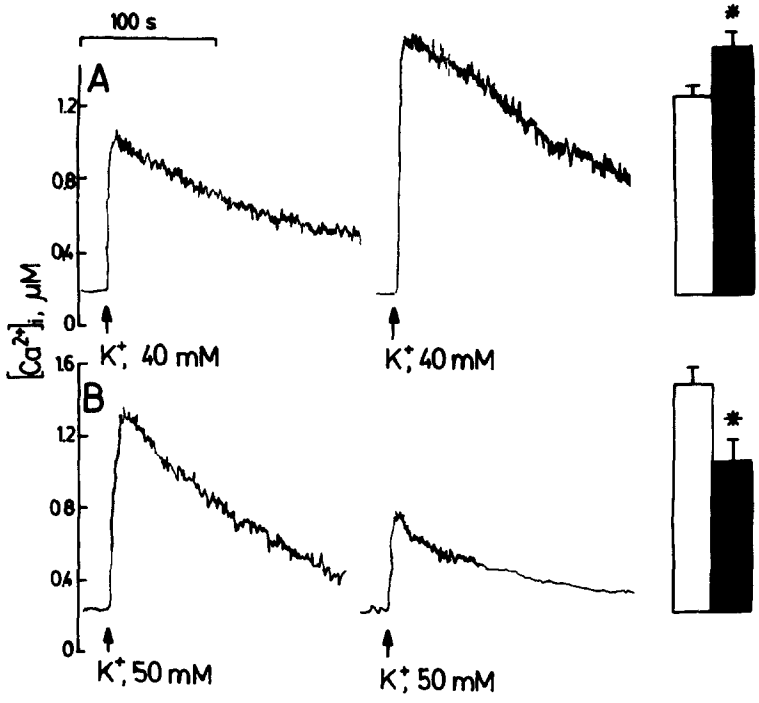

Fig. 3. Short- and long-term effects of progesterone on depolarization-induced $[Ca^{2+}]_i$ responses in single gonadotrophs. (A) Short-term effects: cells were treated with 1 nM estradiol for 48 h (left panel), or for 48 h with 1 nM estradiol and 3 h with 100 nM progesterone (right panel) before stimulation with 40 mM K^+ (B) Long-term effects: cells were treated for 48 h with 1 nM estradiol (left panel) or for 48 h with 1 nM estradiol and 100 nM progesterone (right panel) before stimulation with 50 mM K^+. Open or closed bars indicate mean ± SE of $[Ca^{2+}]_i$ amplitudes in estradiol or estradiol + progesterone treated cells, respectively. (*Indicates $P < 0.01$ vs estradiol, t-test, $n = 9$–15.)

action on GnRH-induced LH secretion, also reduced LH and $[Ca^{2+}]_i$ responses to high $[K^+]_e$. On the other hand, we observed a stimulatory action of short-term progesterone treatment on K^+-induced Ca^{2+} signals and LH secretion. The kinetics of the facilitatory action of progesterone on the secretory response of the gonadotroph to K^+ were similar to those previously described in GnRH-stimulated cells [6, 25]. In this regard, we also performed experiments in which $[Ca^{2+}]_i$ was measured in single gonadotrophs. This experimental approach confirmed our finding that progesterone regulates depolarization-induced Ca^{2+} signals in a biphasic manner. These data strongly suggest that progesterone modulation of Ca^{2+} entry through VSCC represents one possible mechanism by which this steroid influences Ca^{2+} signaling in the gonadotroph.

To further examine this mechanism, we analyzed the effects of progesterone treatment on phorbol ester-stimulated Ca^{2+} signals. In previous studies, phorbol esters were shown to promote Ca^{2+} entry mainly through VSCC in quiescent gonadotrophs [15]. The present finding that PKC-mediated LH secretion and $[Ca^{2+}]_i$ responses are enhanced by short-term progesterone treatment suggests that progesterone might affect Ca^{2+} channel activity via an action on PKC. On the other hand, long-term administration of progesterone did not significantly alter secretory and $[Ca^{2+}]_i$ responses to PMA, indicating that the steroid does not inhibit PKC-mediated Ca^{2+} influx after prolonged exposure. Recent studies in estradiol-treated pituitary cells have shown increased activity of PKC and enhanced LH responses to phorbol ester [8, 26] Since this effect was also observed under extracellular Ca^{2+} deficient conditions, and the present data have shown that estradiol does not alter PMA-induced Ca^{2+} signals, it is unlikely that modulation of PKC-induced Ca^{2+} entry is involved in the mechanism of the positive effect of estradiol on gonadotropin secretion. However, it is possible that the long-term action of estradiol is regulated via another PKC-dependent mechanism, since such treatment modulates phorbol ester-induced LH secretion.

Table 3. Effects of progesterone on PMA-induced increases of $[Ca^{2+}]_i$ in rat pituitary cells

	Spike	Plateau
E 48 h	12 ± 3	8 ± 3
E 48 h, P 3 h	37 ± 3*	18 ± 3
E + P 48 h	25 ± 5	12 ± 7

For details of steroid treatments and data analysis see legend of Table 1

Since progesterone receptors are found predominantly in the gonadotroph fraction of rat pituitary cells [27], it is possible that the modulation of agonist- and K^+-induced Ca^{2+} signals observed in the present study results from a direct action of progesterone on the gonadotroph. An important aspect of the interaction

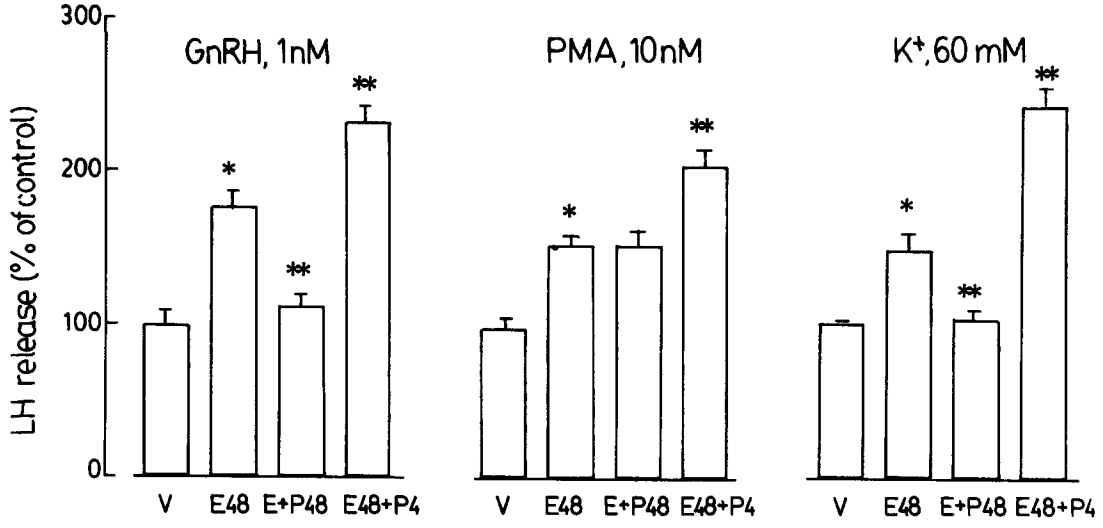

Fig. 4. Effects of estradiol and progesterone on GnRH, PMA, and depolarization-induced LH secretion from cultured rat pituitary cells. Cells were cultured for 48 h with vehicle (V, 0.2% ethanol), 1 nM estradiol (E), 1 nM estradiol + 100 nM progesterone (P), or for 48 h with 1 nM estradiol and 4 h with 100 nM progesterone. During the last 3 h of the indicated incubation periods the cells were stimulated with 1 nM GnRH (left panel), 10 nM PMA (middle panel), or 60 mM K^+ (right panel). Data from 3–7 independent experiments are presented as percentage ± SE of the respective controls (V = 100%). *Indicates $P < 0.05$ vs V, **indicates $P < 0.05$ vs estradiol (ANOVA and Newman-Keuls test).

between progesterone receptors and Ca^{2+} signaling is related to the estradiol-dependence of short- and long-term effects of progesterone on Ca^{2+} signaling and secretory response. It has been shown that the priming effect of estradiol on gonadotroph responsiveness to progesterone is coincident with the increased expression of progesterone receptors [28]. Such estradiol-induced increases in progesterone receptor

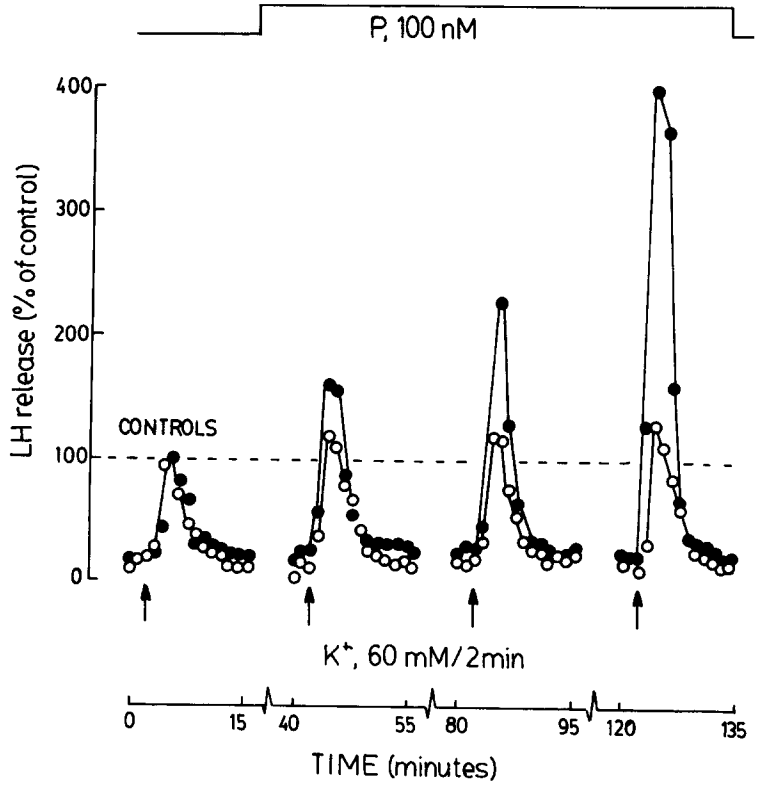

Fig. 5. Stimulation of LH release by episodic depolarization during column purification. After pretreatment with 1 nM estradiol for 48 h, perifused rat pituitary cells cultured on microcarrier beads were exposed to repeated 2 min K^+ (60 mM, arrows) pulses. Following an initial control pulse, cells in one chamber (○) were perifused with medium containing 1 nM estradiol, and those in the second chamber (●) with medium containing 1 nM estradiol + 100 nM progesterone (P). Data from a representative experiment are shown and expressed in percentage of maximal LH responses (100%) to the initial control pulses.

levels are abolished by concomitant treatment with the anti-estrogen, tamoxifen [29]. It is likely that the acute actions of progesterone on calcium signaling are attributable to the increased number of its receptors, while the long-term inhibitory effects of progesterone treatment probably reflect down-regulation of receptors.

Together with the findings in single gonadotrophs and suspended pituitary cells, these data suggest a complex mechanism of Ca^{2+} signal modulation by progesterone in estradiol-primed gonadotrophs: (1) agonist-stimulated mobilization of Ca^{2+} from intracellular sources can be inhibited or enhanced as shown by shifts of the $[Ca^{2+}]_i$ response profiles in single gonadotrophs and spike amplitudes in αT3-1 cells; (2) Ca^{2+} entry provoked by membrane depolarization is bidirectionally modulated by the steroid; and (3) the latter mechanism might involve PKC-mediated Ca^{2+} channel phosphorylation, which could serve as an amplifier in the regulation of the facilitatory action of progesterone on Ca^{2+} influx. Since Ca^{2+} signaling is the primary factor leading to exocytosis in the gonadotroph, it is concluded that these multiple mechanisms of progesterone action would contribute to its enhancing and attenuation effects on GnRH-induced LH secretion.

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