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Luteinizing hormone-releasing hormone (LHRH)-induced arachidonic acid release in rat granulosa cells

Role of calcium and protein kinase C

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In rat granulosa cells prelabeled with [^{3}H]arachidonic acid, addition of LHRH, A23187 or 12-0-tetradecanoyl phorbol-13-acetate (TPA) enhanced the release of [^{3}H]arachidonic acid into the culture medium. The effect of A23187 was significant as early as 5 min and the lowest effective dose was 5×10^{-8}/M. On the other hand, TPA was effective only at dosages greater than 10^{-6}M. These results suggest that the stimulatory effect of LHRH on arachidonic acid release is coupled more tightly to a Ca^{2+}-dependent rather than a protein kinase C-mediated pathway.

Luteinizing hormone-releasing hormone; Arachidonic acid; Phorbol ester; Ca^{2+}; Protein kinase C; (Ovary)

1. INTRODUCTION

It is becoming clear that similarities exist between luteinizing hormone-releasing hormone (LHRH) actions in the pituitary and gonads, and identical receptors for LHRH are presumably present in both tissues [1]. Increase in inositol lipid metabolism has been observed within minutes following the exposure of anterior pituitary cells [2,3] or ovarian cells [4–12] to LHRH, suggesting that stimulation of polyphosphoinositide metabolism may be intimately involved in the mechanism of LHRH at both the pituitary and gonadal levels. Furthermore, in cultured anterior pituitary and ovarian cells prelabeled with [^{3}H]arachidonic acid, LHRH caused a significant increase in the level of arachidonic acid release from phospholipids, which also could be observed within minutes following LHRH addition [9,13].

Several reports have emphasized the importance of calcium in LHRH action [14], and arachidonic acid and its metabolites have been implicated as intracellular messengers in calcium-mediated processes [15]. Also, a calcium-activated phospholipid-dependent protein kinase (protein kinase C) [16] may be involved in the action of LHRH in ovarian cells [17]. Therefore, the present study was designed to determine whether arachidonic acid release in granulosa cells could be affected by a calcium ionophore, A23187, which alters the intracellular concentrations of Ca^{2+}, as well as by a known activator of protein kinase C, TPA.

2. EXPERIMENTAL

Granulosa cells were harvested from PMSG-primed immature female rats, as in [8,9]. The cells were suspended in MEM (modified) with Eagle's salts and supplemented with 5% fetal calf serum, glutamine, antibiotics, nonessential amino acids, and including 0.1 μCi/ml [5,6,8,9,11,12,14,15-

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[3]H]arachidonic acid (60 Ci/mmol, New England Nuclear). Aliquots of the cell suspension (5×10^5/ml) were added to 24-well culture plates (Falcon) and cultured at 37°C under an atmosphere of 5% CO_2 in air. 2 days after plating, the cells were washed thoroughly and incubated for a further 60 min in MEM containing 0.2% bovine serum albumin. At this time hormone additions were performed and the incubations continued for various time intervals as required. The following were purchased from Sigma: A23187, TPA, 4α-phorbol 12,13-didecanoate and synthetic LHRH. At the end of the experiment, fatty acids in the culture medium were extracted by the method of Borgeat and Samuelsson [18] and the ^{3}H-labeled arachidonic acid, $PGF_{2\alpha}$ and PGE_2 were isolated by thin-layer chromatography as described [9]. Data are expressed as mean ± SE ($n = 4$); statistical significance was determined by analysis of variance.

3. RESULTS

As indicated in table 1, the addition of either LHRH, A23187 or TPA caused a significant enhancement of arachidonic acid release from prelabeled cells ($p < 0.01$). The newly synthesized prostaglandins (i.e. ^{3}H-labeled PGE_2 and $PGF_{2\alpha}$), which represented less than 1% of the total radioactivity in the incubation medium, were not affected. It appeared that LHRH was less effective when compared with A23187 or TPA ($p < 0.05$), whereas the maximal levels of [^{3}H]arachidonic acid release induced by A23187 or TPA were not significantly different from each other. By contrast, addition of a phorbol derivative which does not activate protein kinase C, 4α-phorbol 12,13-didecanoate [21], failed to affect [^{3}H]arachidonic acid release in similar cell cultures (not shown).

Table 1

Effects of LHRH, calcium ionophore and phorbol ester on [^{3}H]arachidonic acid release in rat granulosa cells

Treatment	n	Arachidonic acid (% control ± SE)
LHRH (10^{-6} M)	6	146 ± 6
A23187 (5×10^{-7} M)	4	210 ± 18
TPA (5×10^{-5} M)	4	211 ± 14

n, number of separate experiments (each with 4 replicates)

As illustrated in fig.1, the effects of 4×10^{-7} M A23187 on arachidonic acid release from prelabeled granulosa cells were observed as early as 5 min following addition of the calcium ionophore ($p < 0.05$), and continued to increase to approx. 180% of control levels at 15 min. In the same experiment, stimulation of [^{3}H]arachidonic acid release by 10^{-5} M TPA was slower, a significant increase being found at approx. 15 min after addition of the phorbol ester.

The effect of increasing concentrations of A23187 was determined 60 min after addition of the calcium ionophore (fig.2). A23187 significantly stimulates [^{3}H]arachidonic acid release in granulosa cells at a minimal effective concentration of about 5×10^{-8} M ($p < 0.01$). A 2.2-fold maximal enhancement of [^{3}H]arachidonic acid

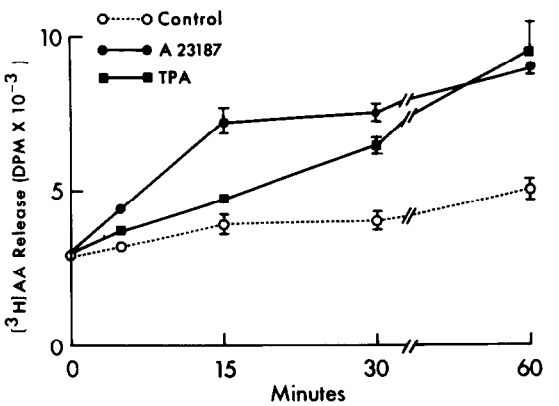

Fig.1. Time course of arachidonic acid release induced by calcium ionophore or phorbol ester.

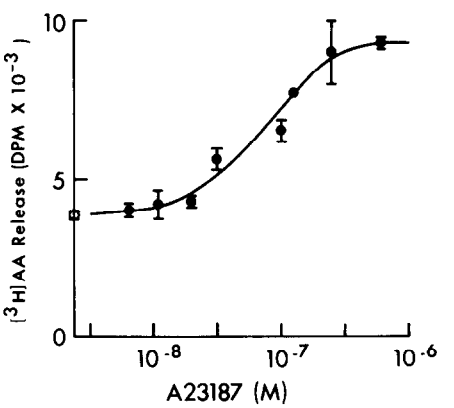

Fig.2. Effect of increasing concentrations of A23187 on arachidonic acid release in granulosa cells in culture.

release in granulosa cells was observed at concentrations of A23187 above 4×10^{-7} M. On the other hand, TPA was effective only at concentrations greater than 10^{-6} M and caused a maximal 2.1-fold increase at 10^{-4} M (fig.3).

To determine the possible interaction between the calcium ionophore and phorbol ester, prelabeled granulosa cells were treated with A23187 and TPA, either alone or in combination in a 2×2 factorial experiment. As illustrated in fig.4, following a 60 min incubation, 10^{-7} M A23187 caused significant stimulation of [^{3}H]arachidonic acid release (180% of control, $p < 0.05$), but the same concentration of TPA was not effective. Interestingly, when both A23187 and TPA were present concomitantly, the effect of A23187 was potentiated ($p < 0.05$), with the level of [^{3}H]arachidonic acid release reaching 230% of control levels.

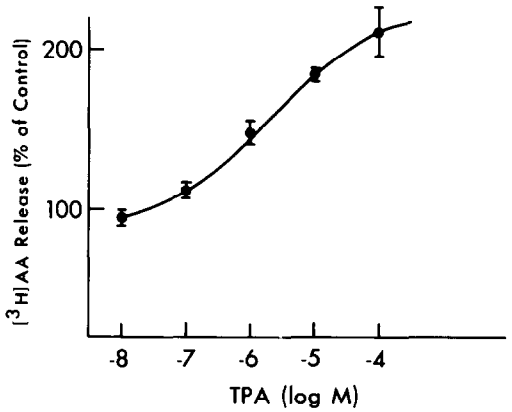

Fig.3. Effect of increasing concentrations of TPA on arachidonic acid release in granulosa cells.

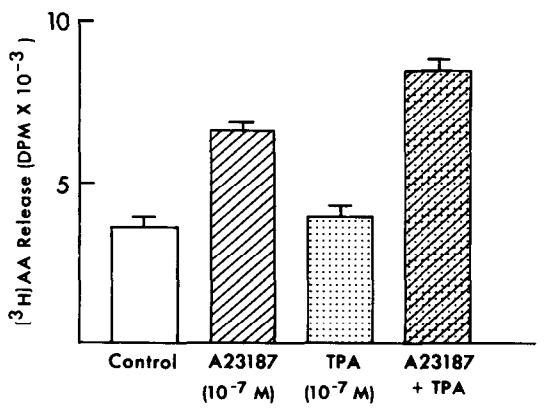

Fig.4. Interaction of calcium ionophore and phorbol ester on arachidonic acid release.

4. DISCUSSION

In ovarian cells, we and others have obtained evidence that an initial action of LHRH following receptor binding is the breakdown of polyphosphoinositides into 1,2-diacylglycerol (DG) and inositol phosphates [11,19]. The latter compounds, especially inositol 1,4,5-trisphosphate (IP_3), are known to mobilize calcium from intracellular stores [20], while DG is now widely accepted to be a potent activator of protein kinase C [21]. Recently, we have reported that LHRH enhances arachidonic acid release in rat granulosa cells [9]. Whereas the precise relationship between polyphosphoinositide breakdown and arachidonic acid release is not clear, it is possible that one or both of the breakdown products (i.e. calcium and DG) may mediate the arachidonic acid response. Hence in the present study, we investigated the effects of the calcium ionophore A23187 and TPA on arachidonic acid release from prelabeled granulosa cells. Our results indicate that while both A23187 and TPA are potent stimulators of arachidonic acid release from granulosa cells, the effect of LHRH in this regard is coupled more tightly to a Ca-dependent rather than a protein kinase C-mediated pathway.

The manner by which arachidonic acid is liberated from cellular phospholipids has been a subject of intense research. One possibility is that arachidonic acid is derived from inositol lipids through two consecutive reactions catalyzed by phospholipase C followed by DG lipase [22]. Alternatively, arachidonic acid may be released from the *sn*-2 position of several phospholipids by phospholipase A_2. It appears that in many tissues a single extracellular messenger induces the activation of both phospholipase C and phospholipase A_2 reactions [22]. Here, the time course of effect of A23187 in enhancing arachidonic acid release appears to be somewhat slower than the time course of LHRH stimulation of polyphosphoinositide breakdown in granulosa cells, which was observed within the first minute of LHRH addition [11,19]. This temporal relationship can be taken to support the notion that phosphodiesteratic cleavage of the inositides (by phospholipase C) precedes possible activation of phospholipase A_2 that induces the liberation of arachidonic acid.

Our present results show that A23187 caused a

significant increase in arachidonic acid release from prelabeled granulosa cells at 5×10^{-8} M, suggesting that the ovarian cells are rather sensitive to calcium changes in terms of the activity of phospholipase A_2 and/or phospholipase C when compared to other cellular systems [22]. The effective doses of A23187 on arachidonic acid release correlate well with the effectiveness of this calcium ionophore in affecting progesterone production in ovarian cells [23]. In contrast, the present data involving TPA indicate that it affects arachidonic acid effective release only at excessively high concentrations, when compared with the doses of TPA that have been reported to affect hormone production in granulosa cells [24,25]. Thus, the possibility of a nonspecific or cytotoxic effect cannot be ruled out. On the other hand, it is interesting that 10^{-7} M TPA failed to affect arachidonic acid release by itself but potentiated the stimulatory action of 10^{-7} M A23187, suggesting a possible synergistic role of calcium and protein kinase C for eliciting full cellular responses [21,26].

In anterior pituitary cells, recent studies have suggested that LHRH-stimulated LH release may be associated with the production of oxidized arachidonic acid metabolites [2,13,27]. One or more of the epoxygenated and/or lipoxygenated metabolites of arachidonic acid might be a component of the cascade of reactions initiated by LHRH that ultimately results in secretion of LH. Whether or not arachidonic acid itself or its metabolites mediate the actions of LHRH in ovarian cells remains to be elucidated.

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