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COORDINATE ACTIONS OF ARACHIDONIC ACID AND PROTEIN KINASE C IN GONADOTROPIN-RELEASING HORMONE-STIMULATED SECRETION OF LUTEINIZING HORMONE

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SUMMARY: The relative contributions of arachidonic acid and protein kinase C during GnRH-stimulated LH release were investigated in cultured rat anterior pituitary cells. Maximal or near-maximal concentrations of arachidonic acid or the phorbol ester, 12-O-tetradecanoylphorbol 13-acetate, were less effective than a maximal dose of GnRH in stimulating LH release. However, the effect of a combination of arachidonic acid and phorbol ester was equivalent with that of GnRH. The protein kinase C inhibitor, retinal, significantly reduced GnRH- and phorbol-induced, but not arachidonic acid-stimulated, LH release. The lipoxygenase inhibitors, 5,8,11,14-eicosatetraynoic acid and nordihydroguaiaretic acid, partially inhibited GnRH- and arachidonic acid-stimulated, but not phorbol-induced, LH secretion. Simultaneous addition of retinal and either lipoxygenase inhibitor completely abolished LH responses elicited by GnRH, as well as by combined treatment with arachidonic acid and the phorbol ester. These results suggest that hormone release is mediated by phospholipid-dependent mechanisms that are coordinated during the stimulation of LH secretion by GnRH.

The requirement for Ca^{2+} during GnRH-stimulated LH release from rat gonadotropes has been extensively documented (for reviews see 1,2). More recent evidence has implicated the activation of protein kinase C, a Ca^{2+}- and phospholipid-dependent enzyme, in the mechanism of GnRH-induced LH secretion. The phorbol ester, TPA, stimulated LH release and also activated cytosolic protein kinase C in cultured rat gonadotropes (3-5). Both TPA and GnRH also caused a redistribution of protein kinase C activity from the cytosol to membrane fractions of enriched rat gonadotropes; the time-course as well as the dose-dependence of protein kinase C translocation parallelled those of LH release (5,6).

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Abbreviations: AA, arachidonic acid; ETYA, 5,8,11,14-eicosatetraynoic acid; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; NDGA; nordihydroguaiaretic acid; TPA, 12-O-tetradecanoylphorbol 13-acetate.

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Furthermore, retinal, an inhibitor of protein kinase C, suppressed both the GnRH-induced increase in LH release as well as protein kinase activity in pituitary cytosol (5). Arachidonic acid (AA) and its lipoxygenase products have also been implicated as potential mediators of GnRH actions. LH release in vitro is stimulated by AA and its lipoxygenase metabolite, 5-hydroxy-6,8,11, 14-eicosatetraenoic acid, and the LH response to GnRH can be suppressed by the addition of ETYA, NDGA and 3-amino-1-[m-(trifluoromethyl)-phenyl]-2-pyrazoline (BW755c), inhibitors of the lipoxygenase pathway (7). In this study, the relative contributions and interactions of AA and protein kinase C in the control of GnRH-stimulated LH secretion were investigated by monitoring the LH release of cultured rat anterior pituitary cells exposed to AA, TPA, GnRH, retinal, NDGA, ETYA, or a combination of these agents.

MATERIALS AND METHODS

Anterior pituitary glands were removed from female Sprague-Dawley rats and dispersed by controlled trypsinization as previously described (8). GnRH (Peninsula Laboratories Inc.) was dissolved in distilled water, while AA (Nu-Chek) was dissolved in ethanol. TPA and retinal (Sigma) were dissolved in dimethyl sulfoxide. Aliquots of these reagents were stored at -20°C until use. ETYA (Hoffman-La Roche) and NDGA (Sigma) were made up in dimethyl sulfoxide and ethanol, respectively, on the day of use. All drugs were diluted to their respective working concentrations with test medium before use (see below). Statistical analysis were performed by Student's 2-tailed t-test.

Dispersed anterior pituitary cells (10^5 cells/well, 12-well plates) were cultured in Medium-199 containing Earle's salts, sodium bicarbonate, 10% horse serum, 10 µg/ml streptomycin, and 100 U/ml penicillin at 37°C under 5% CO_2 and saturated humidity for 3-7 days before use. Prior to each experiment, cells were washed twice with Medium-199 containing Hank's salts, sodium bicarbonate, and 25 mM Hepes and allowed to rest in the incubator for 1-2 h. The washing medium was then replaced by fresh medium containing 0.01% bovine serum albumin, and stimuli and drugs were added in 1 µl-aliquots to a total incubation volume of 1 ml. After incubation for 3 h, the media were transferred to glass tubes and stored at -20°C until analyzed for LH content by radioimmunoassay using RP-2 rat LH standard provided by the National Pituitary Agency, Baltimore, MD (9). Four to six wells were used for each drug treatment in all experiments, and all samples were assayed in duplicate. Results were expressed as ng/ml/10^5 cells.

RESULTS AND DISCUSSION

In cultured pituitary cells, both AA and TPA stimulated LH release during the 3-h incubation period, as shown in Figures. 1 and 2. As noted above, such responses have indicated that AA and/or its metabolites as well as protein kinase C may act as mediators of GnRH action. Although AA at 5×10^{-5} or 10^{-4}

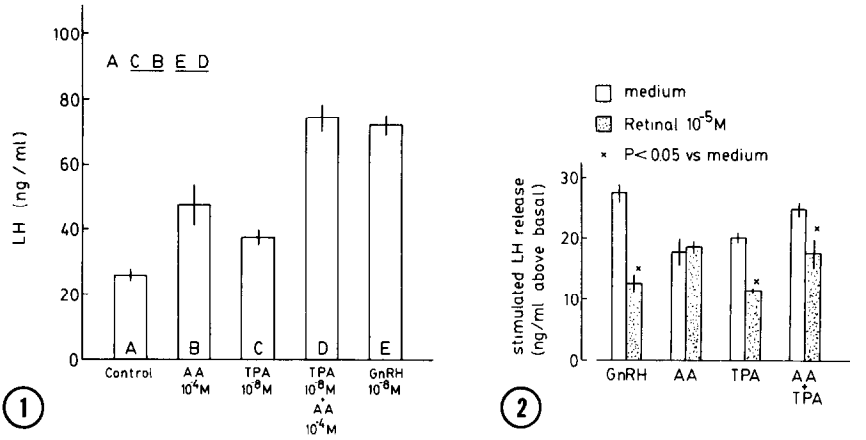


Figure 1. Comparison of the relative potencies of AA, TPA, GnRH and AA + TPA in stimulating LH release. LH values (mean ± SE) are expressed as ng/ml/10^5 cells in this and subsequent figures. Groups joined by the same underline are not different from one another ($P > 0.05$). Results from one of six separate experiments using AA at 5 x 10^{-5} or 10^{-4} M were presented.

Figure 2. Differential inhibition by 10^{-5} M retinal of LH responses to 10^{-8} M GnRH, 5 x 10^{-5} M AA, 10^{-7} M TPA and AA + TPA. LH values for medium and retinal controls were 7.0 ± 0.5 and 10.4 ± 0.9 ng/ml/10^5 cells, respectively. Similar results were observed in three other separate experiments.

M and TPA at 10^{-8} or 10^{-7} M were potent secretagogues for LH release, these maximal or near maximal doses of AA and TPA were not as effective as a maximal dose (10^{-8} M) of GnRH in causing LH release (Figure 1). These results suggest that although AA or TPA can partially mimic the action of GnRH, AA or protein kinase C alone cannot account for all the actions of GnRH. Hirota et al (6) and Iwashita et al (4) also reported that the maximal TPA-stimulated LH release was only 40-60% of that elicited by maximal doses of GnRH. Hirota et al (5) showed that whereas maximal inhibition of rat gonadotrope protein kinase C activity was obtained with 10^{-5} M retinal, the same concentration of retinal only partially reduced the GnRH-stimulated LH response. Also, Naor et al (7) found that although ETYA and NDGA were effective in reducing GnRH-induced LH secretion, even high contributions (10^{-4} M) of these inhibitors did not abolish GnRH action.

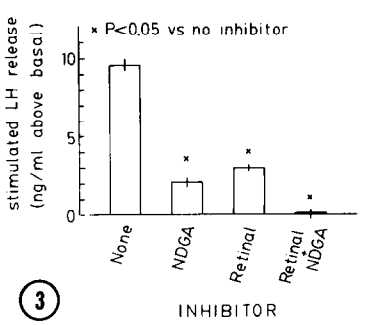
In the present study, the LH response elicited by simultaneous addition of AA and TPA was similar to that observed with 10^{-8} M GnRH (Figure 1). This result suggests that GnRH-mediated LH release may be dependent upon at least two major mechanistic components, namely those of an AA-mediated pathway and a

protein kinase C-mediated pathway. The hypothesis that AA and/or its metabolites, and protein kinase C are major components of the GnRH mechanism of action was further tested by the use of retinal (a known inhibitor of protein kinase C activation) and the lipoxygenase inhibitors, ETYA and NDGA.

Treatment with 10^{-5} M retinal reduced GnRH- as well as TPA-stimulated LH release, but retinal did not decrease the effect of AA on LH secretion (Figure 2). Retinal also decreased the amount of LH released by the combination of AA and TPA to levels similar to those observed with AA alone (Figure 2). These inability of retinal to interfere with the action of AA on LH release indicated that the secretory response to AA is not mediated by activation of protein kinase C. AA has been reported to activate protein kinase C in human neutrophils (10), but does not appear to have this effect in pituitary cytosol (Hirota, K. and Catt, K.J., unpublished data).

Retinal, ETYA, or NDGA, at the concentrations used in this study, significantly reduced but did not abolish GnRH-stimulated LH release (Figures 3,4A) as in previous reports (5,7). However, addition of retinal together with either ETYA or NDGA completely inhibited GnRH-induced LH secretion (Figures 3,4A). Treatment with retinal + ETYA also completely abolished the LH increase induced by the combined actions of AA and TPA (Figure 4B). In three preliminary experiments (results not shown), ETYA and NDGA at the concentrations used in this study reduced the amount of LH released by 5×10^{-5} M AA but did not significantly alter the LH response to 10^{-8} M TPA. Since retinal also did not interfere with AA action (see previous paragraph), these findings supported the idea that GnRH stimulation of LH release includes an AA as well as a protein kinase C component. Since ETYA and NDGA are selective inhibitors of the lipoxygenase pathway, these results are also consistent with the view that the AA action on LH release is, at least in part, mediated by its lipoxygenase metabolites.

It is probable that phorbol esters can exert actions in addtion to stimulation of protein kinase C. For example, TPA has been reported to increase the production of [^{3}H]AA metabolites in [^{3}H]AA prelabeled rat gonadotropes (2). Since ETYA and NDGA did not alter the TPA-induced LH response, it is unlikely



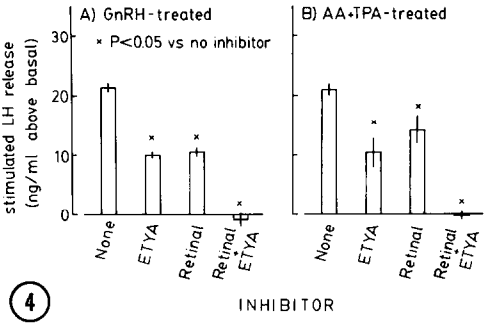


Figure 3. Combined actions of 10^{-5} M retinal and/or 4 x 10^{-5} M NDGA on 10^{-8} M GnRH-stimulated LH release. Basal LH values for medium control = 2.5 ± 0.5; retinal = 3.8 ± 0.4; NDGA = 3.1 ± 0.8; and retinal + NDGA = 5.0 ± 0.4. Similar results were observed in two other separate experiments.

Figure 4. Effects of 10^{-5} M retinal and/or 2 x 10^{-5} M ETYA on A) 10^{-8} M GnRH-, and B) 5 x 10^{-5} M AA + 10^{-7} M TPA-stimulated LH release. Basal values for medium control = 9.4 ± 0.6; ETYA = 18.2 ± 1.8; retinal = 11.6 ± 0.6; and ETYA + retinal = 21.4 ± 1.4. Results presented are representative of two separate experiments.

that TPA stimulation of LH release is also mediated by AA metabolites, at least those of the lipoxygenase pathway.

The results of this study are consistent with the view that GnRH-stimulation of LH release is mediated by protein kinase C as well as by AA and/or its metabolites. In accordance with recent evidence for the involvement of phospholipids in mediating peptide hormone actions, GnRH has been shown to stimulate the turnover of phosphatidylinositides in pituitary cells (11-13), with increased production of inositol phosphates and diglyceride (14,15). Inositol triphosphate is an important potential second messanger in signal transduction and diglyceride is a major activator of protein kinase C (for reviews see 16-19). Diglyceride may also facilitate the actions of phospholipases, including phospolipase A_2 (20); also, diglyceride can itself be acted upon by diglyceride lipase resulting in the production of AA (21). Experiments are in progress to investigate the manner in which inositol-phospholipid metabolism and the mobilization and metabolism of AA, as well as activation of protein kinase C, are coordinated during the temporal and quantitative actions of GnRH on gonadotropin release.

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