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Hormone-Stimulated Redistribution of Gonadotrope Protein Kinase C *In Vivo:* Dependence on Ca^{2+} Influx

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SUMMARY

In the present study we show that natural sequence gonadotropin-releasing hormone (GnRH) and a high affinity, metabolically stable agonist (Buserelin) promote redistribution of protein kinase C (PKC) activity to a particulate fraction prepared from anterior pituitary. The action of the agonists, administered *in vivo* to ovariectomized rats, is both time and dose dependent. GnRH antagonist alone does not measurably alter distribution of this enzymatic activity but inhibits the ability of GnRH to do so and to stimulate luteinizing hormone release. This finding indicates that receptor occupancy alone is insufficient to cause PKC redistribution. Redistribution of PKC in response to Buserelin is inhibited by the calcium ion channel antagonist methoxyverapamil (D600), suggesting that redistribution of PKC activity, like GnRH-stimulated gonadotropin release, requires the influx of extracellular calcium.

GnRH is a hypothalamic decapeptide which stimulates the release of LH and follicle-stimulating hormone from pituitary gonadotropes. Several observations indicate a second messenger role for Ca^{2+} in this system (1, 2). First, agents which elevate intracellular Ca^{2+} also provoke LH release with the same efficacy as does GnRH (3). Second, GnRH-stimulated LH release is inhibited by Ca^{2+} channel blockers (4) or by removal of extracellular Ca^{2+} (5). Finally, GnRH increases the cytoplasmic Ca^{2+} concentration in dispersed pituitary cells (6). Calmodulin has been implicated as an intracellular receptor for Ca^{2+} in this system since GnRH provokes the redistribution of calmodulin from the cytosol to membrane patches containing the GnRH receptor (7, 8), and calmodulin inhibitors of several different classes inhibit GnRH-stimulated LH release (9–11).

In many systems which use Ca^{2+} as a second messenger, its mobilization is associated with hydrolysis of polyphosphoinositides leading to the production of metabolic intermediates including diacylglycerol and inositol 1,4,5-trisphosphate (12–14). These metabolites have potential informational roles since inositol 1,4,5-trisphosphate appears to release Ca^{2+} from intracellular pools (15) and diacylglycerol is an activator of the Ca^{2+}- and lipid-dependent kinase, PKC (16).

Considerable evidence exists to suggest that phosphatidylinositol metabolism and PKC activation play a role in signal transduction in the gonadotrope. For example, GnRH has been shown to cause the rapid and specific stimulation of phosphatidylinositol metabolism (17–19) with consequent production of inositol phosphates and diacyglycerol (20, 21). Diacylglycerols have been shown to activate pituitary PKC *in vitro* (22), to provoke LH release (23), and to synergistically enhance the stimulatory effect of a Ca^{2+} ionophore on LH release (24). The specificity of the effects of diacylglycerol was demonstrated in structure-activity studies which revealed an excellent correlation between the potency of a range of diacylglycerols in stimulation of PKC and of LH release from gonadotropes (23).

Redistribution of PKC from the cytosolic to the particulate fraction of homogenates has been shown to occur following stimulation in several systems (25–28). The potential of this event to alter cellular function prompted us to examine the effect of secretagogues on the distribution of anterior pituitary PKC in the context of the established role for Ca^{2+} in signal transduction at the gonadotrope.

Materials and Methods

Treatment of animals. In order to increase the proportion of gonadotrope tissue in the pituitary, ovariectomized animals were used in these studies. Female Sprague-Dawley rats were bilaterally ovariectomized under light ether anesthesia at 21–25 days of age and were used in experiments 14–21 days later. Animals were killed at the indicated times after the administratin of test compounds or vehicle. Trunk blood was collected for determination of serum LH by RIA, and anterior pituitaries were removed for determination of kinase activity.

Preparation of particulate and cytosolic fractions. Anterior pituitaries were removed to ice-cold homogenization medium immediately after death. Cytosolic and particulate fractions were obtained as

ABBREVIATIONS: GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; PKC, protein kinase C; PMA, 4β-phorbol-12-β-myristate-13α-acetate; RIA, radioimmunoassay; PBS/BSA, Dulbecco's phosphate-buffered saline containing 0.3% Cohn fraction V bovine serum album; D600, methoxyverapamil; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid.

previously described (29). Briefly, the tissues were homogenized (Dounce tissue grinder, 20 strokes pestle B) in 5 ml of 25 mM Tris-HCl, pH 7.5, containing 0.25 M sucrose, 2.5 mM $MgCl_2$, 2.5 mM EGTA, 50 mM 2-mercaptoethanol, and 0.1 mM phenylmethylsulfonyl fluoride. After centrifugation at 100,000 × *g* for 60 min, the supernatant (cytosol) was retained and the pellet was resuspended in 5 ml of homogenization buffer containing 0.3% Triton X-100 (Sigma). This suspension was shaken for 30 min, then centrifuged at 100,000 × *g* for 10 min and the supernatant (particulate fraction) was retained. All of these procedures were performed at 4°.

Partial purification of PKC by DEAE-cellulose chromatography. The fractions were applied to DEAE-cellulose columns (bed volume approximately 1 ml, Bio-Rad) which had been equilibrated with homogenization buffer. The columns were washed with 10 ml of homogenization buffer followed by 2 ml of this buffer containing 20 mM NaCl. Samples were then eluted from the column with 5 ml of homogenization buffer containing 100 mM NaCl. In preliminary experiments in which identical samples were purified by DEAE-cellulose chromatography, 95% of the eluted PKC was recovered in the 100 mM NaCl fraction, and the variability between columns was less than 5%. The majority of cAMP-dependent protein kinase activity (assessed in the presence of 15 μM cAMP) was retained on the column with this procedure, but it could be eluted with homogenization buffer containing 250 mM NaCl.

Determination of PKC activity. Protein kinase activity was assayed by determination of the rate of transfer of ^{32}P from [γ-^{32}P] ATP to histone at 30°C as previously described (30). The standard assay mixture contained, in a total volume of 250 μl, 5 μmol Tris-HCl, pH 7.5, 1.25 μmol of magnesium nitrate, 50 μg of histone (Sigma type III-S), 2.5 nmol of [γ-^{32}P]ATP, and 50 μl of DEAE-cellulose column eluate containing 0.25–2 μg of protein [assayed according to the method of Bradford (31), using bovine serum albumin as the protein standard]. The standard assay mixture was supplemented either with: EGTA (1 mM), $CaCl_2$ (1 mM), or $CaCl_2$ and lipids (10 μg of phosphatidylserine and 1 μg of 1,2-diolein, both from Sigma). The reaction was started by addition of the [γ-^{32}P]-ATP [500–2000 dpm/pmol, prepared according to the method of Glynn and Chappell (32)] and was stopped after 3–4 min by spotting an aliquot (100 μl) of the reaction mixture onto filter paper (Whatman 3 MM) and rapidly immersing this paper in ice-cold 10% trichloroacetic acid. The filter papers were then washed by continuous stirring for 30 min in 10% trichloroacetic acid (three changes of washing solution). The papers were then transferred sequentially through methanol and ether before drying and determination of radioactivity by scintillation counting. For the purpose of these experiments PKC activity was defined as that seen in the presence of $CaCl_2$ and lipids, minus that seen in the presence of $CaCl_2$ alone. The concentrations of $CaCl_2$, phosphatidylserine, and 1,2-diolein described were found to be maximally effective for stimulation of PKC, and the kinase activity in the presence of $CaCl_2$ alone did not consistently differ from that obtained with 1 mM EGTA. PKC activity was linearly dependent on both incubation time and protein concentration under the conditions used.

Radioimmunoassay. The RIA for LH was performed as recommended in the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (NIADDK) kit instructions except that antiserum (LH 102) prepared and characterized in our laboratory was used (33). Highly purified rat LH (NIADDK LH I-5) was iodinated by a modification of the method of Hunter and Greenwood (34) and rat LH (NIADDK RP-1) was used as the reference protein. Intra- and inter-assay variances were less than 5 and 7%, respectively.

Drug sources. Natural sequence GnRH was provided by the National Pituitary Agency (Baltimore, MD). Buserelin (pyroGlu^1-His^2-Trp^3-Ser^4-Tyr^5-D(tertBu)Ser^6-Leu^7-Arg^8-Pro^9-ethylamide) was provided by Hoescht-Roussel Pharmaceuticals, Inc. (Sommerville, NJ) and the GnRH antagonist D-pyroGlu^1-D-Phe^2-D-Trp^3-Ser^4-Tyr^5-D-Trp^6-Leu^7-Arg^8-Pro^9-Gly^{10}-amide was purchased from Peninsula Laboratories (Torrance, CA). These peptides were injected subcutaneously in 0.1 or 0.2 ml of PBS/BSA. The Ca^{2+} antagonist, D600 was obtained from Knoll Pharmaceuticals (Ludwigshafen am Rhein, FRG) and was administered intraperitoneally in 125 μl of Me_2SO. PBS (Dulbecco's) was from Gibco Laboratories (Grand Island, NY). All other reagents were of the highest degree of purity available.

Data analysis. Data are expressed as the mean ± SE of 3–15 separate observations and the values shown are representative of those obtained in 2–4 similar experiments. Differences between control and treatment groups were assessed using the Student's *t* test, $p < 0.05$ being considered significant.

Results

DEAE-cellulose purification of pituitary PKC. PKC activity was observed in cytosolic fractions both before and after DEAE-cellulose chromatography (Table 1). In contrast, particulate PKC activity was measurable only after chromatographic purification. The kinase activity seen in the presence of 1 mM EGTA or 1 mM $CaCl_2$ alone was greatly reduced in column eluates, suggesting the removal of endogenous activators of PKC activity (lipids, proteins, etc.) or removal of lipid-independent kinases. A combination of $CaCl_2$, phosphatidylserine, and 1,2-diolein was routinely used to stimulate PKC activity, although the enzyme could also be stimulated by PMA or *sn*-1,2-dioctanoylglycerol (EC_{50} values were approximately 1 nM and 10 μM, respectively, in the presence of 10 μM $CaCl_2$ and 1 μg of phosphatidylserine).

In control groups, particulate and cytosolic PKC activities were 1.65 ± 0.12 and 1.32 ± 0.10 pmol of ^{32}P incorporated/min/μg of protein, respectively (mean ± SE, data pooled from 20 separate experiments), and 23.7 ± 0.8% of the total cellular PKC activity was recovered in the particulate fraction.

Effect of GnRH on serum LH and pituitary PKC distribution. Administration of 2 μg of GnRH to ovariectomized rats 30 min before death caused a rapid increase in both serum LH and in the proportion of cellular PKC recovered in the particulate fraction (Fig. 1). As no consistent change in total cellular PKC activity was observed, these results are compatible with the redistribution of PKC from cytoplasm to cellular membranes. The demonstration that GnRH-stimulated redistribution of PKC was greatly reduced in intact (i.e., not ovariectomized) rats and in ovariectomized rats pretreated with a

TABLE 1

Kinase activity of pituitary cytosolic and particulate fractions before and after DEAE-cellulose chromatography

Particulate and cytosolic fractions from a homogenate of three anterior pituitaries in 5 ml of extraction buffer were obtained as described in Materials and Methods. Kinase activity was determined either before (crude sample) or after (column eluate) extraction and DEAE-cellulose purification. Kinase activity was determined in the presence of either EGTA (1 mM), $CaCl_2$ (1 mM), or $CaCl_2$ plus lipid (10 μg of phosphatidylserine, 1 μg of 1,2-diolein) and is expressed as pmol of ^{32}P incorporated/min/mg of tissue. For the purpose of the experiments reported herein, PKC has been defined as the kinase activity seen in the presence of $CaCl_2$ and lipid minus that seen in the presence of $CaCl_2$ alone. Values shown are the mean ± SE of four observations and are representative of those obtained in two similar experiments.

	Kinase activity		
	EGTA	$CaCl_2$	$CaCl_2$ + lipid
	pmol of ^{32}P incorporated/min/mg		
Cytosol			
Crude sample	15.0 ± 0.7	28.3 ± 0.7	42.1 ± 4.1
Column eluate	2.3 ± 0.4	4.0 ± 0.3	27.1 ± 2.7
Particulate			
Crude sample	10.6 ± 0.6	9.6 ± 0.4	9.6 ± 0.6
Column eluate	1.4 ± 0.3	1.2 ± 0.2	8.7 ± 0.3

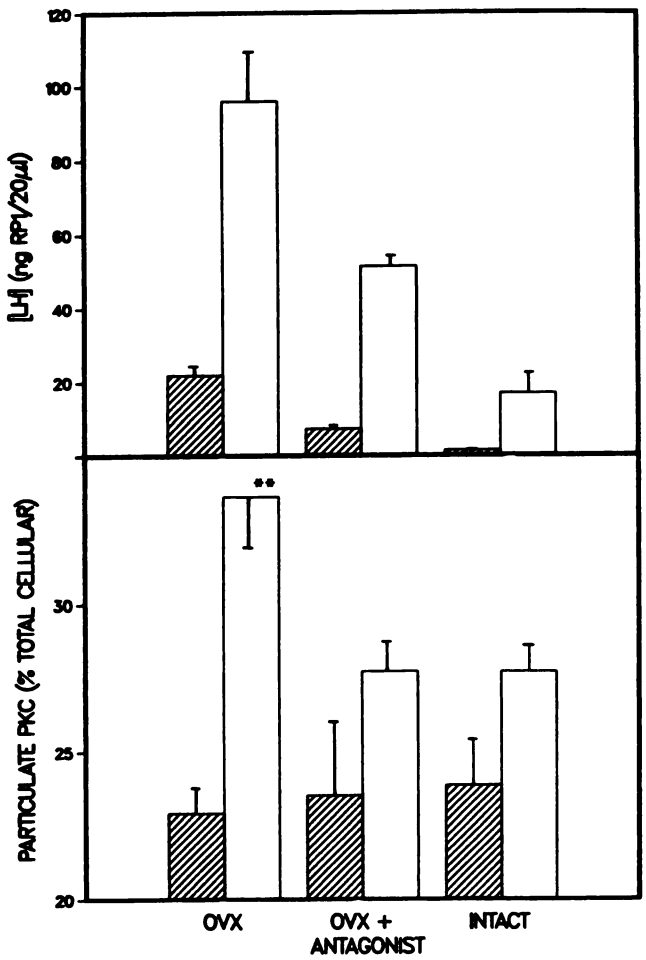

Fig. 1. GnRH-stimulated PKC redistribution is receptor mediated and gonadotrope specific. Groups of three or four rats were pretreated with either vehicle (0.2 ml of PBS/BSA, subcutaneously) or with the GnRH receptor antagonist D-pyroGlu[1]-D-Phe[2]-D-Trp[3]-Ser[4]-Tyr[5]-D-Trp[6]-Leu[7]-Arg[8]-Pro[9]-Gly[10]-amide (50 μg in vehicle) 30 min before administration of 2 μg of GnRH (subcutaneously in 0.1 ml of PBS/BSA, □) or vehicle alone (▨), and were sacrificed after a further 30 min. Anterior pituitaries were removed immediately and pooled for determination of particulate and cytosolic PKC as described. Samples of trunk blood were collected individually for determination of serum LH by RIA. The values shown are the mean ± SE of three to four determinations and are representative of those obtained in two similar experiments. **, $p < 0.01$ compared to appropriate control receiving no GnRH. *OVX*, ovariectomized.

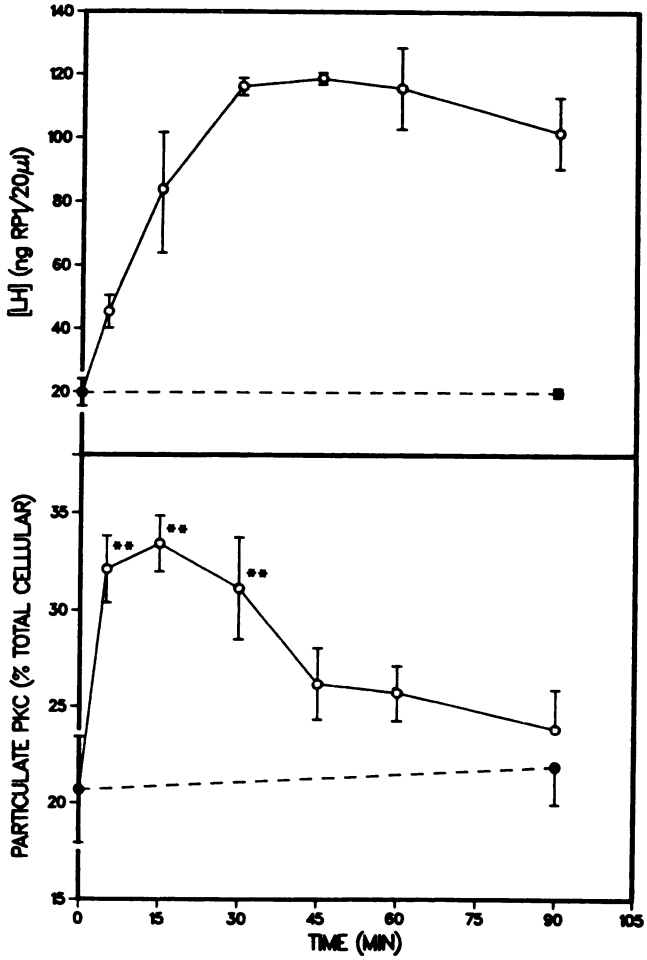

Fig. 2. Time course of GnRH-stimulated elevation of serum LH and particulate PKC. Groups of three rats received vehicle (●, 0.1 ml of PBS/BSA, subcutaneously) or GnRH (○, 2 μg in vehicle) at $t = 0$ and were sacrificed 0–90 min later as indicated. The values shown are the mean ± SE of three to four determinations and are representative of those obtained in three similar experiments. **, $p < 0.01$ compared to pooled control (vehicle treated) value of 21.3 ± 1.6% (mean ± SE, $n = 8$).

GnRH receptor antagonist (Fig. 1) indicates the effect to be both gonadotrope specific and GnRH receptor mediated.

In time course studies, administration of 2 μg of GnRH provoked a rapid increase in serum LH and in the proportion of PKC recovered in the particulate fraction (Fig. 2). Particulate PKC was elevated 5–30 min after administration of GnRH and did not significantly differ from control values thereafter. The effect of GnRH on PKC distribution was found to be dose dependent, particulate PKC being significantly elevated 30 min after administration of 1, 2, 4, and 8 μg of GnRH (Fig. 3).

Effect of Buserelin on serum LH and pituitary PKC distribution. Administration of 2 μg of Buserelin caused an increase in both serum LH and the proportion of cellular PKC recovered in the particulate fraction (Fig. 4). Particulate PKC was elevated 15 min after administration of Buserelin and had not returned to control values by 60 min. The effect of Buserelin on serum LH and on particulate PKC was found to be dose dependent, particulate PKC being significantly elevated 30 min after administration of 0.02, 0.2, and 2 μg of the peptide (Fig. 5).

Effect of D600 on Buserelin-stimulated redistribution of PKC. In order to test the possibility that hormone-stimulated redistribution of PKC occurred as a consequence of Ca^{2+} influx, the effect of Buserelin was reassessed in animals pretreated with the Ca^{2+} antagonist D600. In control groups receiving 0, 25, 250, or 2500 μg of D600 and no Buserelin, the calcium antagonist did not significantly effect total cellular PKC activity (1.34 ± 0.09, 1.46 ± 0.07, 1.47 ± 0.11, and 1.29 ± 0.16 pmol of ^{32}P incorporated/min/μg of protein, respectively), PKC distribution (particulate PKC activity was 25.6 ± 2.5, 22.2 ± 2.4, 21.2 ± 2.8, and 23.9 ± 2.5% of the total cellular activity, respectively), or serum LH. However, Buserelin-stimulated elevation of both serum LH and particulate PKC was inhibited by pretreatment with 2.5 mg of D600 (Fig. 6). The Buserelin-stimulated increase in serum LH was reduced by only 34% in the D600-pretreated animals in which no statistically significant redistribution of PKC was observed.

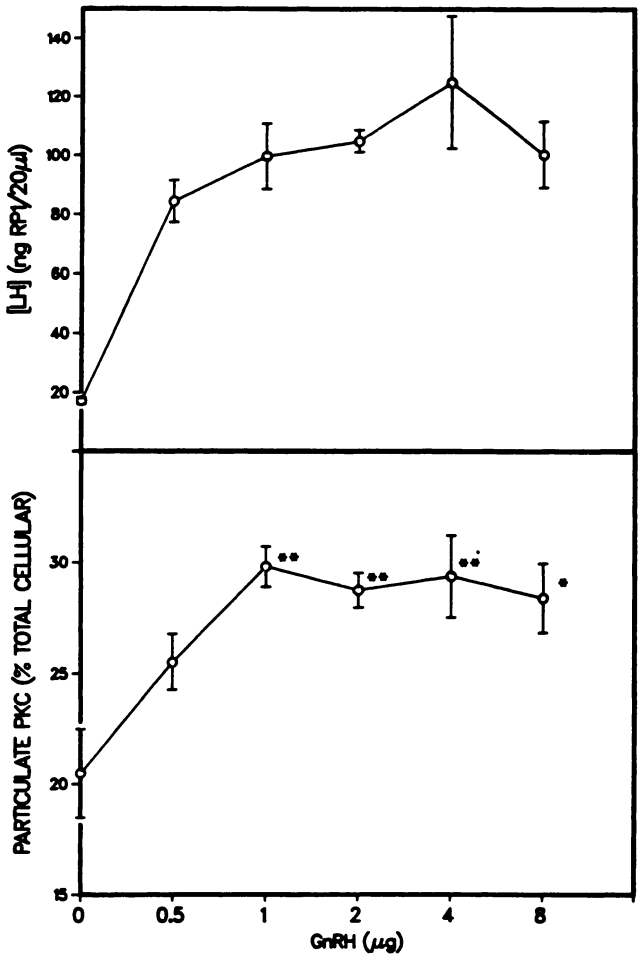

Fig. 3. Dose-response relationship for GnRH-stimulated elevation of serum LH and particulate PKC. Groups of three rats received 0–8 μg of GnRH (subcutaneously in 0.1 ml of PBS/BSA) 30 min before sacrifice. The values shown are the mean ± SE, of three to four determinations and are representative of those obtained in two similar experiments. *, $p < 0.05$, **, $p < 0.01$ compared to control group receiving vehicle alone.

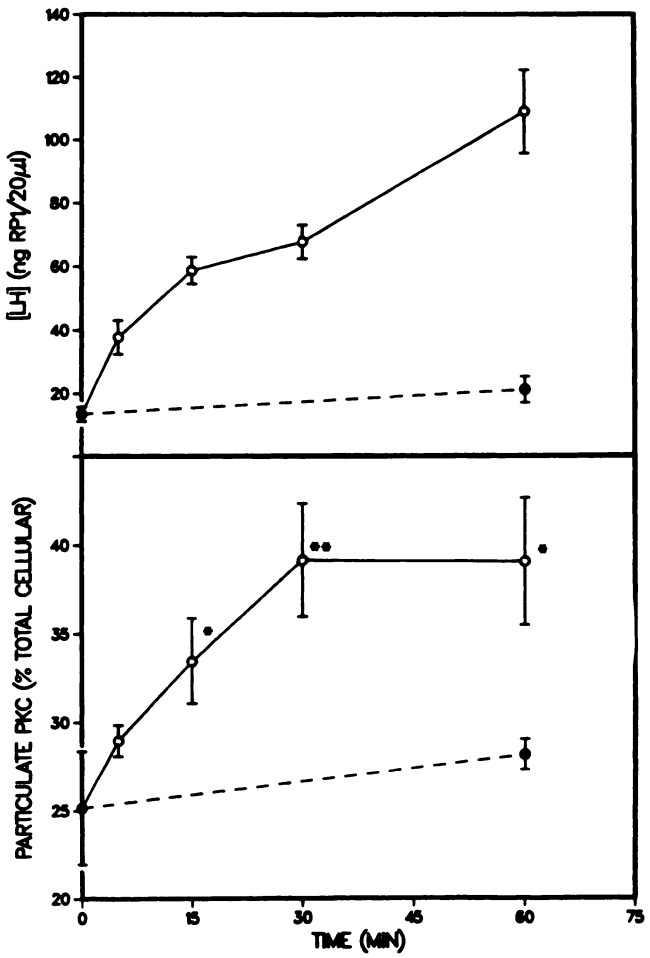

Fig. 4. Time course of Buserelin-stimulated elevation of serum LH and particulate PKC. Groups of three rats received 2 μg of Buserelin in 0.1 ml of PBS/BSA (○) or vehicle alone (●) at $t = 0$ and were killed 0–60 min later as indicated. The values shown are the mean ± SE, of three to four determinations and are representative of those obtained in two similar experiments. *, $p < 0.05$, **, $p < 0.01$ compared to pooled control (vehicle treated) value of 26.6 ± 1.6% (mean ± SE, $n = 8$).

Discussion

Although the precise mechanism of action of GnRH in the gonadotrope remains unclear, it is apparent that Ca^{2+} performs a second messenger function in this system (1, 2). Recent evidence suggests a role for metabolites of inositol phospholipids since GnRH stimulation of gonadotropes leads to the production of diacylglycerol by this route (21). Additionally, exogenous diacylglycerols have been shown to stimulate LH release from cultures of pituitary cells (23) and to synergistically enhance LH release provoked by the Ca^{2+}-selective ionophore A23187 (24). These studies first suggested a role for diacylglycerols as amplifiers of the Ca^{2+} signal. However, questions remain concerning the role of diacylglycerol production in LH release, as we have recently shown that GnRH-stimulated inositol phosphate formation can be markedly inhibited (by PMA or dioctanoylglycerol) without measurable reduction in LH release (35).

PKC activation and translocation remain potential mechanisms for the integration of the effects of hormone-stimulated Ca^{2+} mobilization and diacylglycerol production. We have, therefore, determined the effects of GnRH and Buserelin, *in vivo*, on the distribution of anterior pituitary PKC. In the present work we demonstrate that administration of GnRH and Buserelin to ovariectomized rats causes an increase in the proportion of cellular PKC recovered in the particulate fraction of anterior pituitary homogenates. This redistribution is inhibited in animals pretreated with a GnRH receptor antagonist, in intact (i.e., not ovariectomized) rats, and in rats pretreated with the Ca^{2+} channel blocker, D600.

The proportion of gonadotropes in the pituitary of intact rats of the age and sex used is approximately 15–20% (17). The proportion of gonadotrope tissue is increased 2–3 weeks after ovariectomy as evidenced by an increase in gonadotrope size (8), GnRH receptor numbers (36), and serum LH (Fig. 1). The demonstration that Buserelin-stimulated PKC redistribution is observed in ovariectomized rats, but not in intact rats, is therefore consistent with gonadotrope-specific PKC redistribution. Similarly, the antagonism of GnRH-stimulated PKC redistribution with the GnRH antagonist indicates the effect to be GnRH receptor mediated and, thus, gonadotrope specific. Importantly, the GnRH receptor antagonist alone had no effect on PKC distribution indicating that receptor occupancy alone is insufficient to provoke this response.

Hirota *et al.* (28) have reported particulate PKC to be ele-

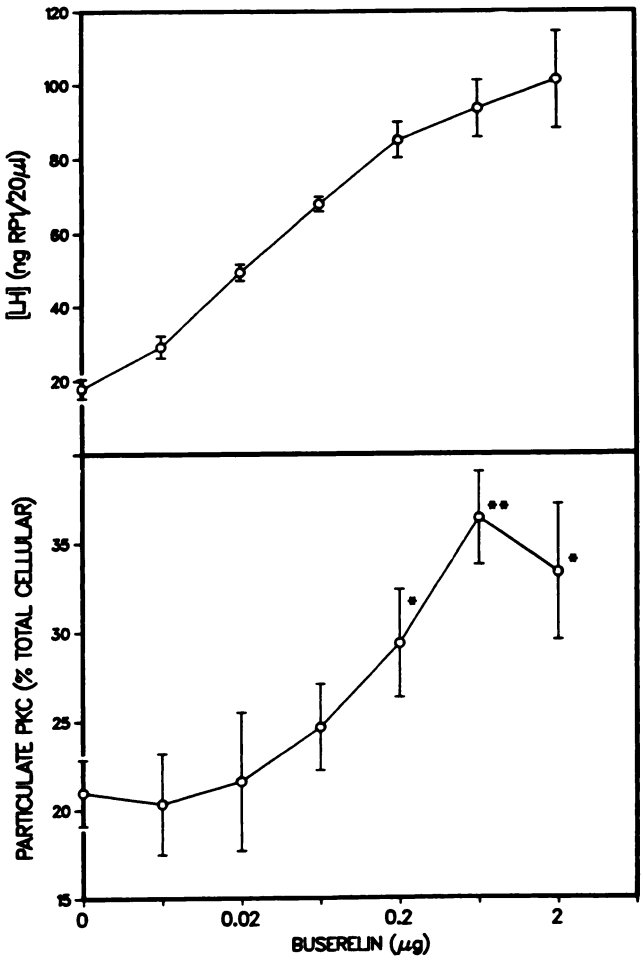

Fig. 5. Dose-response relationship for Buserelin-stimulated elevation of serum LH and particulate PKC. Groups of three rats received 0–2 μg of Buserelin (subcutaneously in 0.1 ml of PBS/BSA) 30 min before sacrifice. The values shown are the mean ± SE of three to four determinations and are representative of those obtained in two similar experiments. *, $p < 0.05$, **, $p < 0.01$ compared to control group receiving vehicle alone.

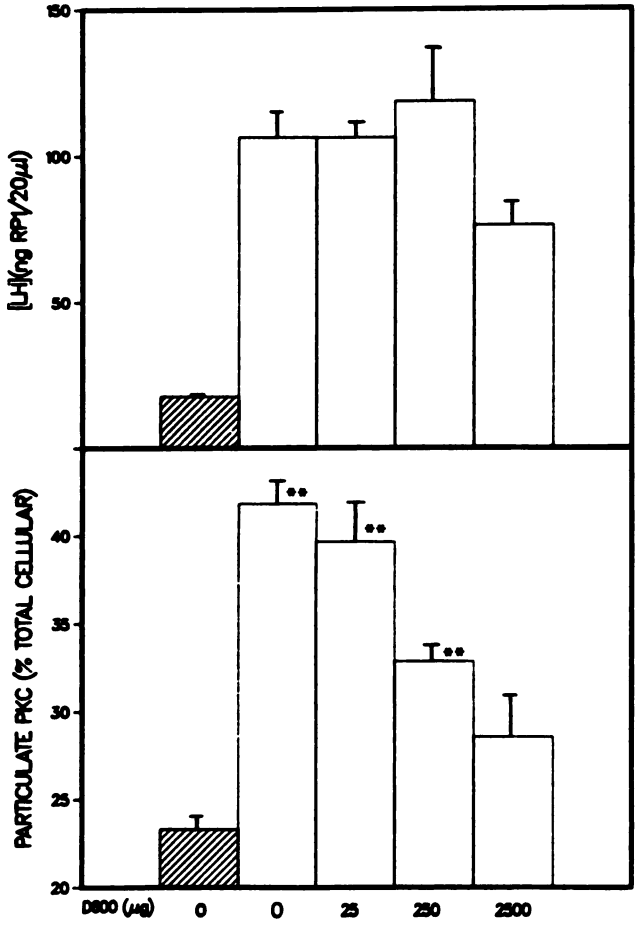

Fig. 6. Inhibition of Buserelin-stimulated elevation of serum LH and particulate PKC With D600. Groups of three rats received the indicated doses of D600 (intraperitoneally in 125 μl of Me_2SO) 30 min before administration of 1 μg of Buserelin (subcutaneously in 0.1 ml of PBS/BSA, ☐) or vehicle alone (▨), and were sacrificed after a further 30 min. As D600 treatment alone had no measurable effect on either particulate PKC or serum LH these four control groups have been combined in the "pooled control" group (▨). The values shown are the mean ± SE of three to four determinations, except for the pooled control where n = 15, and are representative of those obtained in three similar experiments. **, $p < 0.01$ compared to pooled control value.

vated in a time- and dose-dependent manner by addition of GnRH to gonadotrope-enriched static cultures of pituitary cells. Although this effect was apparently gonadotrope specific, it was not shown by the use of selective antagonists to be GnRH receptor mediated. Moreover, DEAE-cellulose purification of samples and correction for lipid-independent activity were not routinely used. Accordingly, it is difficult to assess the relative contributions of PKC and other, lipid-independent kinases to the observed response.

The effect of GnRH in PKC distribution *in vivo* is somewhat shorter in duration than that reported to occur in static cultures (particulate PKC elevated 5–60 min after administration of 10^{-7}M GnRH) (28). This difference is likely to reflect the rapid metabolism of GnRH *in vivo*. Indeed, in the present study, when the metabolically stable agonist Buserelin was used, particulate PKC was found to be significantly elevated as much as 60 min after administration (Fig. 4). Similarly, differences between the distribution kinetics of GnRH and the more hydrophobic analog, Buserelin, may be responsible for differences between the rates of onset of the effects of these peptides (Figs. 2 and 4).

Verapamil and its analogs constitute the most potent class of Ca^{2+} channel antagonists at inhibition of GnRH-stimulated LH release both *in vivo* and *in vitro* (37, 38). These compounds are considerably less potent at inhibition of GnRH-stimulated LH release (IC_{50} of D600 assessed in gonadotrope cell culture is 10^{-4}–10^{-5} M) (4) than at inhibition of cardiac contractility or excitation-contraction coupling in smooth muscle (IC_{50} typically 10^{-6}–10^{-9}M; for review see Ref. 39). The cardiovascular effects of these antagonists thus preclude the use of doses sufficient to abolish Buserelin-stimulated LH release *in vivo*. In spite of this limitation we were able to demonstrate inhibition of the Buserelin-stimulated elevation of both serum LH and particulate PKC by pretreatment with 2.5 mg of D600.

The mechanisms underlying PKC translocation are as yet not well known. Faeron and Tashjian (27) observed the redistribution of PKC activity from the cytosolic to the particulate fraction of GH_4C_1 cells within 1 min of addition of thyrotropin-releasing hormone. The authors noted that the rapidity of the response was consistent with the action of thyrotropin-releasing hormone on the hydrolysis of polyphosphoinositides and speculated that the production of diacylglycerol alone may be

sufficient for the activation of PKC and for the formation of a high affinity association with cell membranes.

Alternatively, several lines of evidence suggest that Ca^{2+} may play a role in regulating the distribution of PKC. It has been shown that elevation of the Ca^{2+} concentration of homogenization medium increases the proportion of cellular PKC activity recovered from the particulate fraction of rat cerebral cortex (40), GH_4C_1 cells (27), and rat pituitaries.[1] Purified PKC has been shown to associate with mixed micelles of Triton X-100, phosphatidylserine, and 1,2-dioleoylglycerol (41), and with inside-out vesicles prepared from erythrocytes (42), in a Ca^{2+}-dependent manner. Importantly, both effects were observed in the absence of added diacylglycerol or phorbol ester, and the latter effect occurred at physiologically relevant Ca^{2+} concentrations (100–500 nM). The binding of PKC to erythrocyte membrane vesicles was maximal at 500 nM Ca^{2+}, whereas enzyme activation occurs between 5 and 50 μM (in the absence of phorbol esters). Thus, it was suggested that, at intracellular Ca^{2+} levels, the binding reaction is likely to be more relevant and that such binding may be viewed as "priming" of the PKC system enabling its participation in transmembrane signaling.

A further possibility is that both diacylglycerol production and Ca^{2+} mobilization are necessary for hormone-stimulated redistribution of PKC. In support of this suggestion, Wolf *et al.* (42) have shown that phorbol esters synergistically enhance the Ca^{2+}-dependent binding of PKC to inside-out vesicles produced from human erythrocytes (42).

The inhibition of Buserelin-stimulated redistribution of PKC with the Ca^{2+} channel blocker D600 supports the view that redistribution of the enzyme occurs as a consequence of Ca^{2+} influx from the extracellular pool. The specificity of this effect is indicated by the observation that D600, at concentrations of up to 100 μM, exerts no direct effect on either basal or PMA-stimulated PKC activity and does not significantly influence the redistribution of PKC activity observed in pituitary homogenates on elevation of Ca^{2+} concentration.[2] It should also be noted that in control groups D600 pretreatment alone had no direct effect on the total cellular PKC activity or on the proportion of cellular PKC activity associated with the particulate fraction. Interestingly, D600 (2.5 mg) inhibited elevation of serum LH in response to Buserelin by only 34% in animals in which only a modest increase in particulate PKC (which did not attain statistical significance, $p > 0.2$) was observed. Moreover, pretreatment with 250 μg of D600 clearly inhibited the Buserelin-stimulated elevation of particulate PKC but had no such effect on serum LH. This degree of uncoupling of the two responses suggests that the proportion of PKC recovered from the particulate fraction in the absence of stimulated redistribution is sufficient to support considerable LH release on stimulation by endogenous diacylglycerol. In support of this possibility, neither acute GnRH-stimulated diacylglycerol production nor diacylglycerol-stimulated LH release is blocked by D600 (21, 24). Alternatively, PKC redistribution may prove to be unnecessary for acute hormone-stimulated LH release but could mediate other effects of the releasing hormone such as receptor regulation (43), target cell sensitivity (44), or gonadotropin biosynthesis (45). Interpretation of these data is, however, complicated by the possibility that a transient redistribution of PKC may have occurred prior to the death of these animals and that impaired blood circulation to the pituitary in D600-treated rats may exert nonspecific effects on pituitary responsiveness.

In conclusion, the data presented are consistent with the involvement of PKC in signal transduction in the gonadotrope at a locus distal to Ca^{2+} influx. Hormone-stimulated redistribution of PKC from an aqueous to a lipid-rich environment could affect the activity of the enzyme and may also play a role in determination of the cellular compartment in which protein phosphorylation by PKC occurs.

Note added in proof. Since submission of this manuscript we have shown that GnRH receptor occupancy and polyphosphoinositide turnover appear to be linked by a guanine nucleotide-binding protein (46).

Acknowledgments

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