Regulation of Gonadotropin mRNA Levels in Cultured Rat Pituitary Cells by Gonadotropin-Releasing Hormone (GnRH): Role for Ca^{2+} and Protein Kinase C†

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ABSTRACT: Incubation of cultured rat pituitary cells with gonadotropin-releasing hormone (GnRH, 1 nM) resulted in a rapid elevation of gonadotropin subunit steady-state mRNA levels(α, 2.2-fold, LHβ, 2.1-fold, and FSHβ 2.2-fold increases at 30 min). Addition of actinomycin D abolished the stimulatory effect of GnRH upon α and LHβ and reduced the effect upon FSHβ mRNA levels. The effect of GnRH is biphasic, where the early phase is being observed at 30–60 min, while the late phase is noticed between 12–24 h. A significant decrease in FSHβ mRNA levels was found after 6 h of incubation when using a stable GnRH analog. The unique profile of the time response enabled us to attempt to dissect the signal transduction cascade involved in the neurohormone action. Addition of the protein kinase C (PKC) activator, 12-*O*-tetradecanoylphorbol 13-acetate (TPA), or the Ca^{2+} ionophore, ionomycin, mimicked the profile of GnRH-induced α and LHβ mRNA elevation. The two phases of FSHβ mRNA elevation induced by GnRH could be mimicked by TPA, while the decrease at 6 h was mimicked by ionomycin. The rapid stimulatory effect of GnRH on gonadotropin subunit mRNA levels was abolished by the PKC inhibitors, staurosporine and GF 109203X. Similarly, the rapid stimulatory effect of GnRH on α and LHβ, but not FSHβ, was abolished in Ca^{2+}-free medium. While additivity in LH release is obtained upon the combined addition of TPA and ionomycin for 30 min of incubation, LHβ and FSHβ gene expression is inhibited. Since GnRH is known to elevate Ca^{2+} and activate PKC, we conclude that whereas simultaneous signaling elicits exocytosis, divergence of signaling by Ca^{2+} and PKC is most likely responsible for gonadotropin gene expression.

Gonadotropin-releasing hormone (GnRH[1]) is the first key hormone of the reproductive system; nevertheless, its mechanism of action upon gonadotropin secretion, and in particular gonadotropin synthesis, is still not known [see Naor (1990) for a review]. The gonadotropin luteinizing hormone (LH) and follicle-stimulating hormone (FSH), as well as the thyroid-stimulating hormone (TSH), are members of the pituitary glycoprotein hormone family sharing a common α-subunit and a specific β-subunit [for reviews, see Pierce and Parsons (1980) and Gharib et al. 1990]. Binding of GnRH to its receptor, which is found exclusively on pituitary gonadotrophs (Naor & Childs, 1986) and has been cloned recently (Kaiser et al., 1992; Kakar et al., 1992; Reinhart et al., 1992; Tsutsumi et al., 1992), triggers a cascade of molecular events, which includes the following: activation of a G protein (Perrin et al., 1989; Limor et al., 1989); accelerated phosphoinositide turnover (Naor et al., 1986; Morgan et al., 1987); release of Ca^{2+} from internal stores as well as Ca^{2+} influx (Chang et al., 1986; Limor et al., 1987; Naor et al., 1988; Leong, 1991; Tse et al., 1993; Vanecek & Klein, 1993); activation of phospholipase D (PLD; Netiv et al., 1991); stimulation of protein kinase C (PKC; Naor et al., 1985a; Hirota et al., 1985); release of arachidonate; and formation of active lipoxygenase products (Naor & Catt, 1981; Naor et al., 1985b; Kiesel et al., 1991; Dan-Cohen et al., 1992). While the molecular events involved in gonadotropin release are well-documented [see Naor (1990) for a review], little is known about the second messengers involved in gonadotropin biosynthesis [see Gharib et al. (1990), Mercer (1990), and Counis and Jutisz (1991) for reviews]. Using *in vitro* approaches, some investigators found increased α but not LHβ mRNA levels after GnRH challenge (Hubert et al., 1988; Weiss et al., 1990). While some investigators found an increase in LHβ mRNA levels by GnRH and proposed that PKC was involved in this response (Andrews et al., 1988), others did not find elevation of steady-state levels of LHβ mRNA (Salton et al., 1988). Still others reported that GnRH elevates both α and LHβ mRNA levels (Starzec et al., 1989b; Attardi et al., 1989) and proposed that both PKC and protein kinase A (PKA) participate in GnRH action (Starzec et al., 1989a,b).

Less is known about the regulation of FSHβ gene expression by GnRH *in vitro*, and a decrease in FSHβ mRNA levels after 6–12 h of static incubation with GnRH was reported (Attardi et al., 1989; Weiss et al., 1990). Thus, the effect of GnRH on gonadotropin gene expression is controversial and might be partially resolved by differences obtained either in the *in vitro* system (e.g., static culture vs pulsatile administration of GnRH to perifused cells) or in animal models tested (e.g., intact vs castrated vs castrated and testosterone/estrogen-replaced rats) (Weiss et al., 1990; Haisenleder et al., 1993). Also, most of the studies on GnRH-regulated gonadotropin gene expression *in vitro* have utilized a long incubation protocol (5–72 h). Since we had recently reported a rapid stimulation of α-subunit mRNA levels by GnRH in the gonadotroph-like cell line αT3-1 (Ben-Menahem et al., 1992), we decided to investigate GnRH actions in cultured rat pituitary cells and the potential second messengers involved in GnRH regulation of gonadotropin gene expression.

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[1] Abbreviations: GnRH, gonadotropin-releasing hormone; GnRHa, [D-Trp^6]GnRH analog; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; LH, luteinizing hormone; FSH, follicle-stimulating hormone; PRL, prolactin; GADPH, glyceraldehyde-3-phosphate dehydrogenase; BSA, bovine serum albumin; dCTP, deoxycytidine 5′-triphosphate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PKC, protein kinase C; PKA, protein kinase A; DMSO, dimethyl sulfoxide.

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EXPERIMENTAL PROCEDURES

GnRH was purchased from Peninsula Labs (San Carlos, CA). The GnRH analog, [D-Trp^6]GnRH (GnRHa), was a gift from Dr. R. Millar (Cape Town, South Africa). The calcium ionophore, ionomycin, was purchased from Boehringer (Mannheim, Germany). Bovine serum albumin (BSA), 12-*O*-tetradecanoylphorbol 13-acetate (TPA), trypsin, actinomycin D, soybean trypsin inhibitor, and DNAse were from Sigma (St. Louis, MO). Staurosporine was from Kyowa Co. (Tokyo, Japan). Bisindolylmaleimide (GF 109203X) was purchased from Calbiochem (Laufelfingen, Switzerland). α-subunit and LHβ cDNA probes were kindly provided by Dr. W. W. Chin (Boston, MA). Rat FSHβ as well as prolactin (PRL) cDNAs was kindly provided by Dr. R. A. Maurer (Iowa City, IA) and by Dr. J. A. Martial (Liege, Belgium). All media, sera, and antibiotics for cell culture were purchased from Biological Industries (Kibbutz Beit Ha-Emek, Israel). [α-^{32}P]dCTP was purchased from Rotem Industries (Beersheba, Israel).

Methods

Preparation and Stimulation of Pituitary Cell Cultures. Pituitary glands from 25-day-old Wistar-derived male rats were used for cell preparation, as previously described (Naor et al., 1988). Cells were grown in 5 mL of medium 199 supplemented with 6% charcoal-stripped horse serum (HS), penicillin (100 IU/mL), and streptomycin (0.1 mg/mL). After 3–4 days of culture, pituitary cells (5×10^6 per 60-mm dish, Sterilin, Hounslow, England) were washed three times with fresh medium 199, and stimulants were added in 5 mL of medium at the indicated concentrations for the given length of time. HEPES (10 mM) was used to buffer cells treated for up to 1 h, while for longer periods the medium contained 0.1% BSA and antibiotics.

LH Release. The medium from the culture described above was collected and stored frozen for the LH-RIA. The kit for LH-RIA was kindly provided by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), the Center for Population Research of the National Institute of Child Health and Human Development (NICHD), and the Agricultural Research Service of the U.S. Department of Agriculture, as well as the University of Maryland School of Medicine. Results are expressed in terms of the RP-3 preparation of the NIDDK of the NIH (Dan-Cohen et al., 1992).

RNA Isolation and Analysis. Total RNA was extracted from cells in 5 M guanidinium thiocyanate containing 8% 2-mercaptoethanol and isolated by the LiCl method as described by Cathala et al. (1983). RNA samples (4 μg) were slot-blotted onto GeneScreen using a slot-blot manifold (Schleicher and Schull, Dassal, Germany), and the samples loaded in each lane were separated into two. Following baking and prehybridization, the membranes were hybridized overnight with the specific cDNA insert labeled to high specific activity using a random primer labeling kit (Boehringer, Mannheim, Germany). One of the two halves of each sample derived from the slot-blot analysis was hybridized with gonadotropin subunit cDNA, and the second corresponding half was hybridized with either prolactin (PRL) or the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNAs, which were used as an internal control. Thereafter, filters were washed at high stringency, and autoradiography was followed by densitometric scanning of autoradiograms.

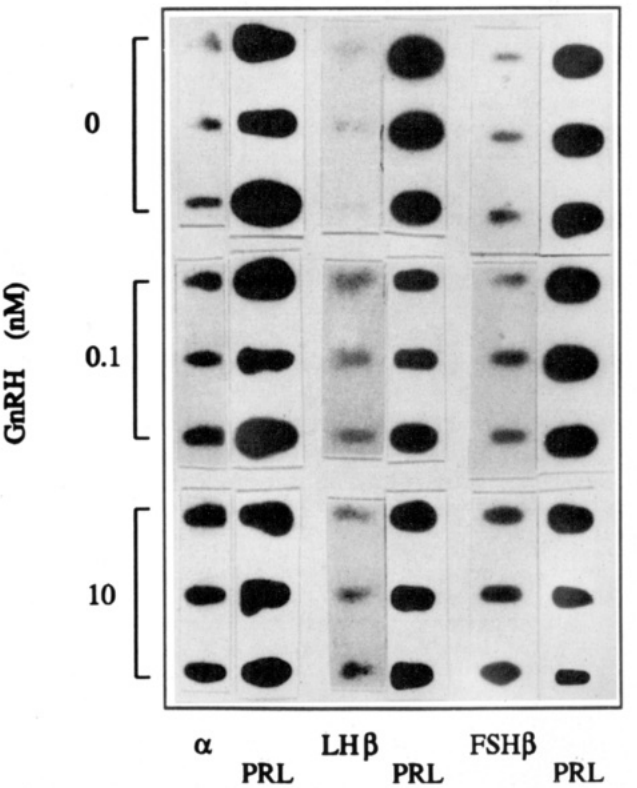

FIGURE 1: Effect of GnRH on gonadotropin subunit mRNA levels in cultured rat pituitary cells. Cells in triplicate were treated with or without GnRH (0.1 and 10 nM) for 30 min of incubation. An autoradiograph of slot-blot hybridization with purified labeled cDNA inserts to common α, LHβ, and FSHβ is shown. A prolactin (PRL) probe serves as the marker for RNA loading.

Statistical Analysis. The hybridization signals for gonadotropin subunit mRNAs in each group were normalized to the hybridization signals for the lactotroph hormone prolactin (PRL) mRNA (when cultured cells were stimulated with GnRH) or for the housekeeping gene GAPDH mRNA (when ionomycin or TPA were used). An arbitrary unit of 1 represents the control vehicle treated cultures. Statistical comparisons between control and treatment groups were performed using the Student's *t*-test: $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

RESULTS

Effect of GnRH on Gonadotropin mRNA Levels in Cultured Pituitary Cells. Gonadotropin mRNA levels were determined following treatment of cultured rat pituitary cells with GnRH using slot-blot analysis. As shown in Figure 1, GnRH (0.1 and 10 nM) increased α, LHβ, and FSHβ subunit mRNA levels. Prolactin mRNA, which was not changed by the GnRH treatment, served as a control for mRNA loading. The effect of GnRH on gonadotropin mRNA levels was dose-related (Figure 2). A significant increase was detected at 0.1 nM for α and LHβ and at 1 nM for FSHβ mRNA levels after 30 min of incubation. Pretreatment of the cultured cells with the transcriptional inhibitor actinomycin D abolished the stimulatory effect of GnRH upon α and LHβ mRNA levels (Figure 3). The basal FSHβ mRNA level was slightly increased by actinomyin D, and the GnRH effect was reduced but not abolished. A time response study of the effect of GnRH (1 nM) on α, LHβ, and FSHβ mRNA levels is shown in Figure 4. A very rapid first phase of increase in mRNA levels was detected for LHβ and FSHβ at 30 min and at 60 min for α-subunit. The rapid increase was followed by a gradual

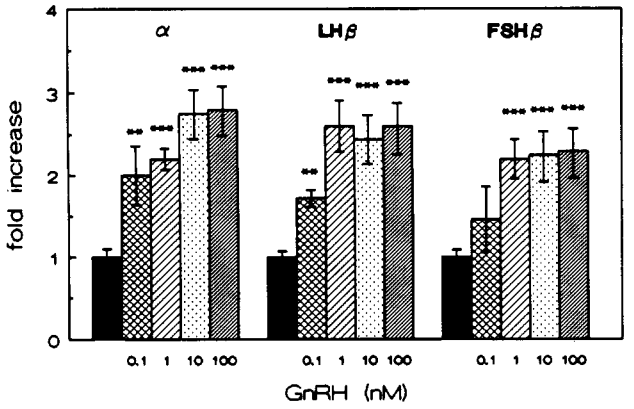

FIGURE 2: Effect of dose response of GnRH upon gonadotropin subunit mRNA levels. Cells were treated with the indicated concentrations of GnRH for 30 min. Slot-blot analysis was performed with purified labeled cDNA inserts to common α, LHβ, and FSHβ. An arbitrary unit of 1 represents the control value obtained by densitometry as described in Methods. Results are expressed as mean ± SEM (n = 4–15). $^{**}p < 0.01$; $^{***}p < 0.001$.

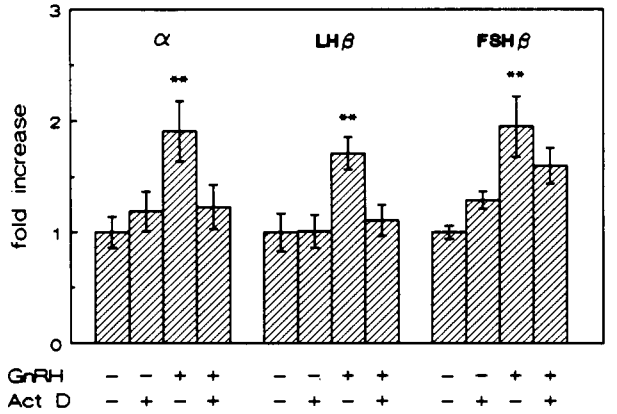

FIGURE 3: Effect of actinomycin D on GnRH-induced gonadotropin subunit mRNA elevation. Cells were pretreated with actinomycin D (2.5 μg/mL) for 60 min followed by GnRH (1 nM) for 30 min. Slot-blot analysis was performed as described above. Results are expressed as mean ± SEM (n = 4–8). $^{**}p < 0.01$.

decrease to the basal level, followed by a secondary rise between 12 and 24 h (Figure 4).

Interestingly, between the two phases of mRNA elevation by GnRH, we could demonstrate a specific decrease in FSHβ but not α or LHβ mRNA levels at 6 h of incubation, which was best seen when we used a stable GnRH analog (GnRHa) (Figure 5). Indeed, addition of GnRHa (0.1 nM) to cultured rat pituitary cells for 6 h resulted in the elevation of α- and LHβ-subunit mRNA levels, while FSHβ mRNA levels decreased by 45% at this time point (Figure 5).

Effect of TPA on Gonadotropin mRNA Levels in Cultured Pituitary Cells: Role of PKC. We then initiated studies to elucidate the nature of the second messengers involved in GnRH action. Addition of the known PKC activator TPA to the cultured cells resulted in biphasic stimulation of gonadotropin subunit mRNA levels (Figure 6). A relatively high concentration of TPA (100 ng/mL) was more effective than a lower dose (1 ng/mL) in the biphasic elevation of α- and LHβ-subunit mRNA levels.

The opposite effect was observed with FSHβ mRNA levels. Whereas the low dose of TPA was active, the higher dose of TPA was less effective on both phases of GnRH action (30 min and 24 h). Hence, different PKC subtypes with variable responsiveness to TPA might be involved in GnRH elevation of gonadotropin subunit mRNA accumulation. Nevertheless,

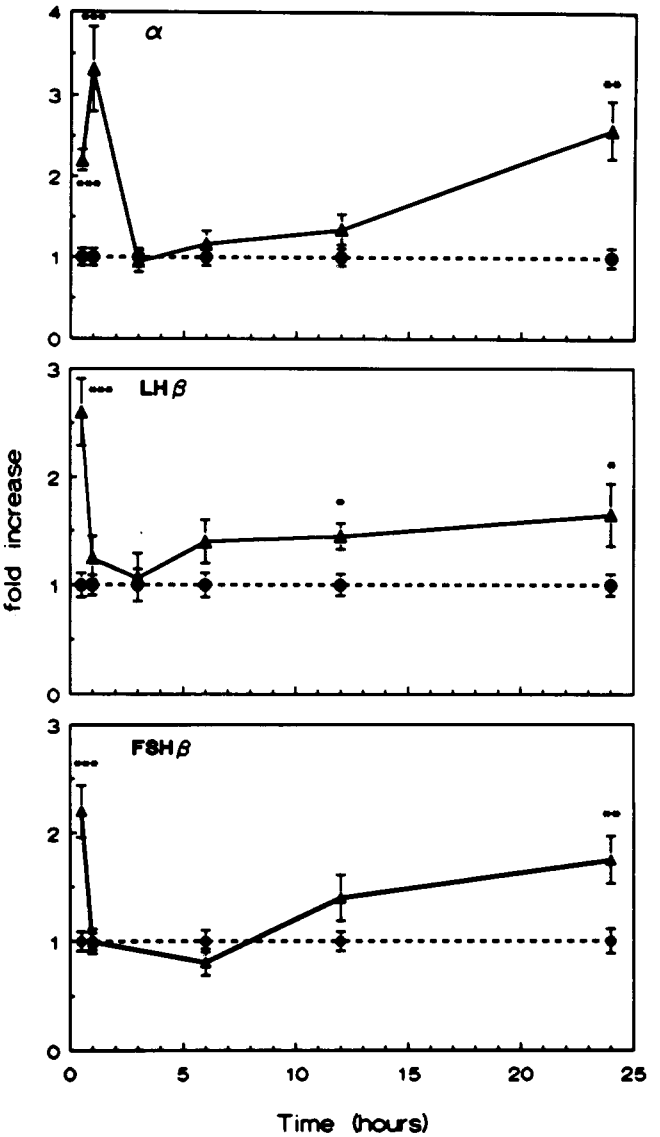

FIGURE 4: Effect of time response of GnRH upon gonadotropin subunit mRNA levels. Cells were treated with GnRH (1 nM; triangles) for the indicated time. Slot-blot analysis was performed as described above. Results are mean ± SEM (n = 6–15). $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

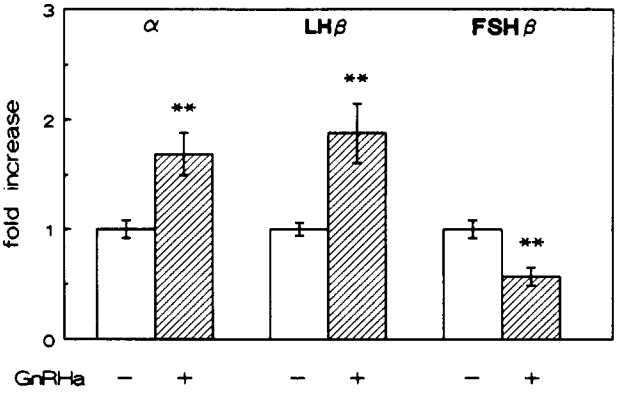

FIGURE 5: Effect of [D-Trp^6]GnRH analog (GnRHa) on gonadotropin subunit mRNA levels. Cells were treated with (striped bars) or without (empty bars) the GnRH analog (0.1 nM) for 6 h. Slot-blot analysis was performed as described above. Results are mean ± SEM (n = 7–11). $^{**}p < 0.01$.

the time response shown here suggests that PKC is involved in the biphasic elevation of gonadotropin subunit (α, LHβ, and FSHβ) mRNA levels.

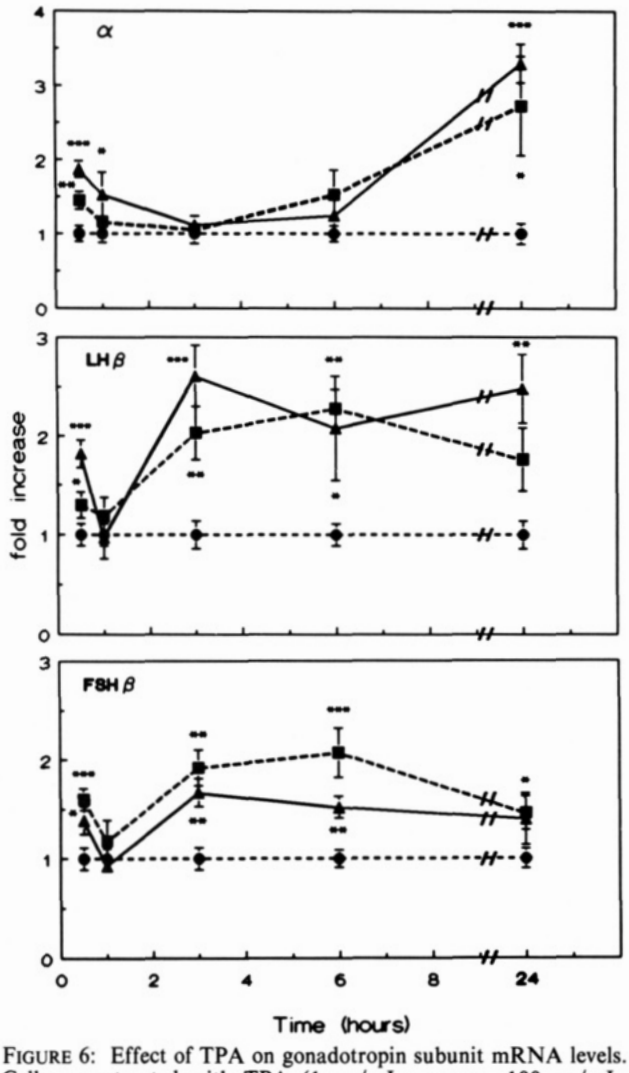

FIGURE 6: Effect of TPA on gonadotropin subunit mRNA levels. Cells were treated with TPA (1 ng/mL, squares; 100 ng/mL, triangles) or with vehicle alone (0.01% DMSO, circles) for the time indicated. Other details were as described above. Results are mean ± SEM (n = 6–15). *p < 0.05; **p < 0.01; ***p < 0.001.

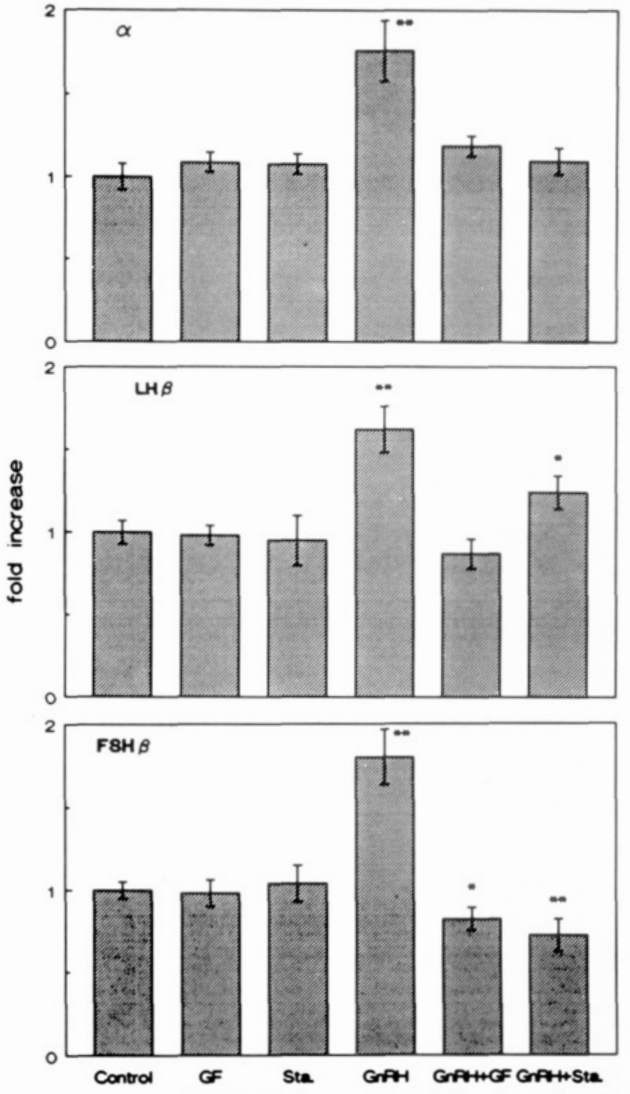

FIGURE 7: Effect of PKC inhibition on GnRH-induced gonadotropin subunit mRNA levels. Cells were pretreated with staurosporine (Sta, 10 nM, 10 min) or with GF 109203X (GF, 2.5 μM, 20 min). Staurosporine-treated cells were then washed several times to remove the drug. GnRH (1 nM) was then added for an additional 30 min, and slot-blot analysis was performed as described above. Results are mean ± SEM (n = 8–14). *p < 0.05; **p < 0.01 vs control. mRNA levels of cells treated with drugs + GnRH were significantly lower than those with GnRH alone for all three subunits.

Further support for the role of PKC in GnRH action is demonstrated by the use of PKC inhibitors. As shown in Figure 7, the stimulatory effect of GnRH upon gonadotropin subunit mRNA elevation (30 min) was abolished or markedly inhibited by the PKC inhibitors staurosporine and GF 109203X. The drug concentrations utilized here were based upon effective doses in cell systems (Toullec et al., 1991; Mischak et al., 1993).

Effect of Ca^{2+} Ionophore on Gonadotropin mRNA Levels in Cultured Pituitary Cells: Role of Ca^{2+}. We also examined the potential role of Ca^{2+} in GnRH action (Figure 8). Addition of the Ca^{2+} ionophore, ionomycin, to cultured pituitary cells resulted in a rapid and significant increase in α-subunit and LHβ mRNA levels (Figure 8). Whereas the effect of ionomycin on α-subunit mRNA levels persisted for several hours, the effect on LHβ rapidly declined to basal levels (1 h) and reappeared at 12 h of incubation. Interestingly, ionomycin (1 μM) decreased FSHβ mRNA levels markedly at 1–12 h of incubation, followed by an increase at 24 h. Hence, Ca^{2+} mobilization and influx differentially regulate gonadotropin subunit mRNA levels. Further support for a differential role for Ca^{2+} in GnRH-induced gonadotropin gene expression was demonstrated by the removal of Ca^{2+} (Figure 9). Indeed, incubation of cultured rat pituitary cells with GnRH for 30 min in Ca^{2+}-free medium abolished the stimulation of α-subunit and LHβ mRNA levels, while the elevation of FSHβ mRNA levels was only slightly reduced (Figure 9). Thus, Ca^{2+} plays an important role in the differential effects of GnRH upon gonadotropin subunit gene expression.

Combined Effect of TPA and Ionomycin upon Gonadotropin mRNA Levels and LH Release. Since GnRH elevates $[Ca^{2+}]_i$ and stimulates PKC activity [see Naor (1990) and Stojilkovic and Catt (1992) for reviews], we also treated pituitary cells with TPA and ionomycin together. As shown in Figure 10, the combination of TPA and ionomycin resulted in a surprising decrease in responsiveness for GnRH-induced LHβ and FSHβ mRNA and with no additivity in α mRNA levels. This was in direct contrast to the combined effect of TPA and ionomycin upon LH release from cultured pituitary cells, which was additive at the time point (30 min) examined here (Figure 11).

DISCUSSION

Although there has been a relatively large number of reports on the mechanism of action of GnRH upon gonadotropin

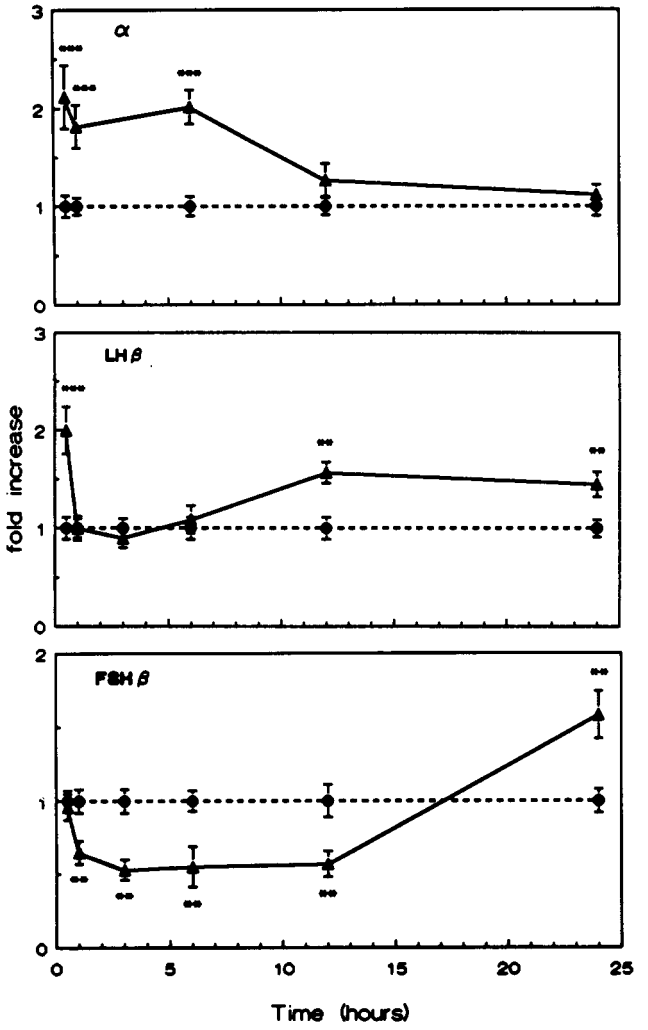

FIGURE 8: Effect of ionomycin on gonadotropin subunit mRNA levels. Cells were treated with ionomycin (1 μM, triangles) or with vehicle alone (0.01% DMSO, circles) for the times indicated. Other details are described above. Results are mean ± SEM (n = 8–15). $^{**}p < 0.01$; $^{***}p < 0.001$.

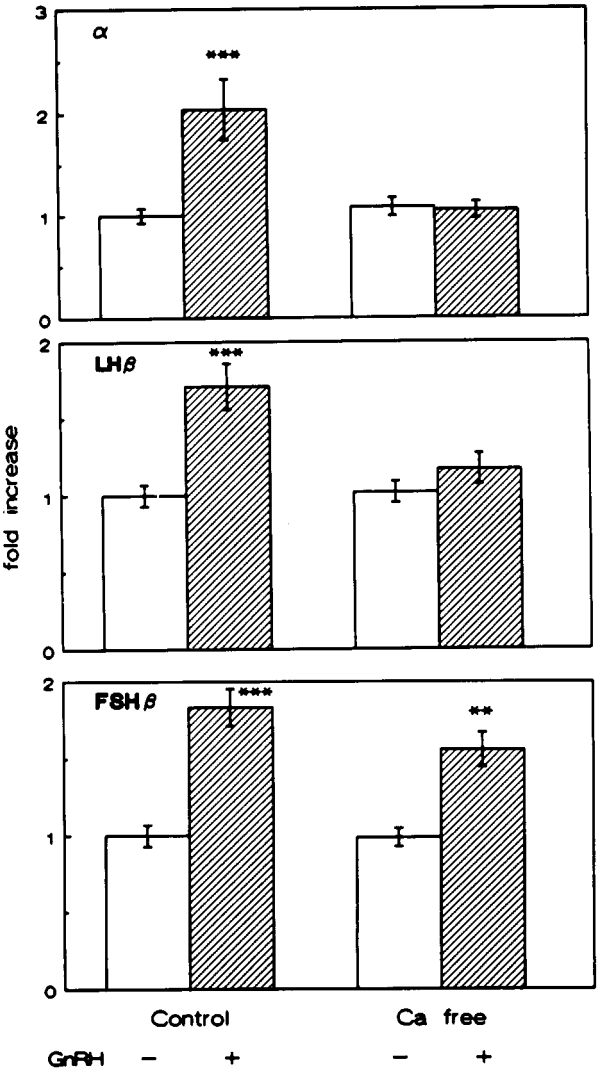

FIGURE 9: Effect of Ca^{2+} removal upon GnRH-induced gonadotropin subunit mRNA levels. Cells were treated with (striped bars) or without (empty bars) GnRH (1 nM) in regular DMEM medium (control) or in Ca^{2+}-free DMEM/EGTA (250 μM) for 30 min. Slot-blot analysis was performed as described above. Results are expressed as mean ± SEM (n = 6–14). $^{**}p < 0.01$; $^{***}p < 0.001$.

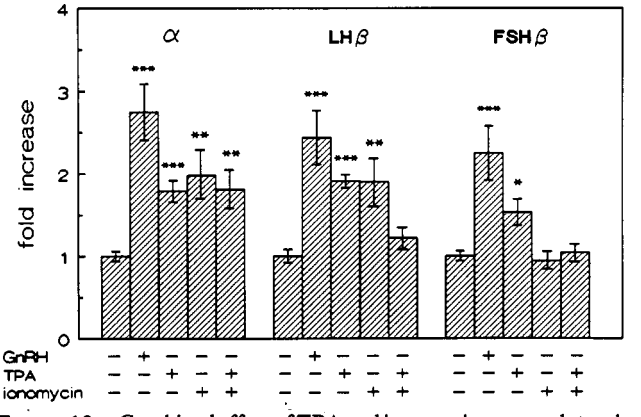

FIGURE 10: . Combined effect of TPA and ionomycin on gonadotropin subunit mRNA levels. Cells were treated with GnRH (10 nM), TPA (100 ng/mL), and ionomycin (1 μM) or with TPA + ionomycin for 30 min. Slot-blot analysis was performed as described above. Results are mean ± SEM (n = 9–16). $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

secretion [Naor (1990) and Stojilkovic and Catt (1992) for reviews], very little is known about the regulation of gonadotropin biosynthesis by GnRH, particularly in terms of signal transduction. Controversy also exists in studies on GnRH-induced gonadotropin gene expression *in vitro*. We therefore initially characterized the effect of GnRH on the steady-state levels of common α, LHβ, and FSHβ mRNA levels. The effect was biphasic, with rapid elevation at 30 min and a second rise between 12 and 24 h of incubation. The rapid response at 30 min is unique for the induction of trophic hormone mRNAs, but more common for the induction of immediate early genes such as the protooncogenes c-fos and c-jun, which might participate by means of the AP-1 complex or by binding to other transcription factors in mediating GnRH action on gonadotropin synthesis.

The time course described here differs from those of other reports, which found stimulation of α alone, LHβ alone, or both between 6 and 24 h of incubation, corresponding to our second phase of GnRH action (Hubert et al., 1988; Andrews et al., 1988; Attardi et al., 1989; Starzec et al., 1989a; Weiss et al., 1990). Nevertheless, the incubation of pituitary fragments with GnRH (1 nM) resulted in a rapid (1 h) and significant elevation of α and LHβ, but not FSHβ, gene transcription (Shupnik, 1990). Our results with actinomycin D are in agreement with those of Shupnik (1990) and Salton et al. (1988), since we noticed that the transcriptional inhibitor abolished the effect of GnRH upon α and LHβ mRNA levels and only reduced the effect upon FSHβ mRNA levels. It is

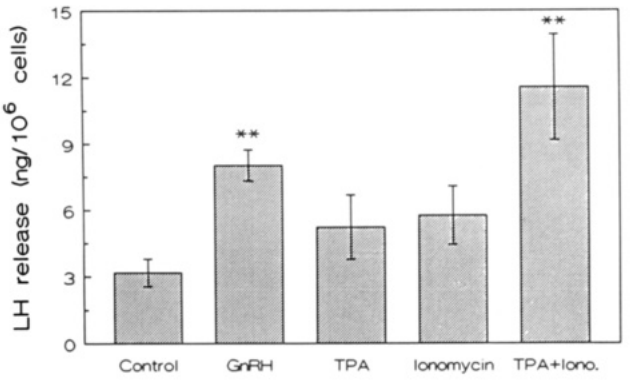

FIGURE 11: . Combined effect of TPA and ionomycin on LH release. Cells were treated with GnRH (10 nM), TPA (100 ng/mL), and ionomycin (1 μM) or with TPA + ionomycin for 30 min. LH released to the medium was measured by RIA. Results are mean ± SEM (n = 6–8). $^{**}p < 0.01$.

therefore possible that regulation of FSHβ mRNA levels by GnRH is both transcriptional and posttranscriptional in nature.

Since GnRH is secreted from the hypothalamus in a pulsatile manner every 60–120 min (Belchetz et al., 1978; Sarkar & Fink, 1980; Fink et al., 1984), the first rapid phase observed in our study might represent the physiological response to the neurohormone pulses. Furthermore, our results might also explain the molecular basis for the "priming" phenomenon (Pickering & Fink, 1979), in which secondary exposure of gonadotrophs to GnRH elicits a larger exocytotic response in terms of LH and FSH release as compared to the priming challenge.

Since Ca^{2+} and PKC are the main candidates to serve as second messengers in GnRH-induced gonadotropin secretion [Naor (1990) and Stojilkovic and Catt (1992) for reviews], we initiated this study to elucidate the role of Ca^{2+} and PKC in GnRH-induced gonadotropin gene expression. The biphasic nature of GnRH-induced gonadotropin gene expression and the surprising decrease in FSHβ mRNA levels at 6 h enabled us to attempt to dissect the signaling mechanism involved by pharmacological reconstitution. Indeed, addition of the PKC ligand TPA resulted in the elevation of gonadotropin mRNA levels with good agreement with the time response of GnRH-induced gonadotropin mRNA elevation. On the other hand, the rapid elevation by GnRH of α-subunit and LHβ mRNA levels (30 min), as well as the second phase of elevation of LHβ and FSHβ (24 h), could be mimicked by the use of the Ca^{2+} ionophore, ionomycin.

The effect of GnRH on FSHβ mRNA levels was more complex and also involved a reduction in mRNA levels at 6 h of incubation. Interestingly, ionomycin elevated α and LHβ mRNA levels, but decreased FSHβ mRNA levels at 1–12 h of incubation. Thus, PKC might mediate GnRH elevation of FSHβ mRNA levels at 30 min and 24 h, while Ca^{2+} might be involved in reduction of FSHβ mRNA levels at 6 h and in the secondary rise at 24 h. Indeed, a switch from negative to positive hormonal or second messenger regulation of transcription factors has been described (Foulkes et al., 1992). This unique effect of ionomycin on the reduction of FSHβ mRNA levels is surprising in the light of the known stimulatory effect of Ca^{2+} ionophores on FSH release (Krey et al., 1993).

It is commonly thought that in secretory cells exocytosis precedes gene expression and might also mediate the process [see Turgeon and Waring (1992) for a review]. The effect of ionomycin described here demonstrates dissociation between exotyosis and gene expression. Thus, different mechanisms might mediate GnRH action on gonadotropin release vs synthesis. Another dissociation between exocytosis and gene expression was evident when we stimulated cells with both ionomycin and TPA. While additivity between Ca^{2+} ionophore and TPA in terms of LH release was observed here, the combination was inhibitory to LHβ and FSHβ gene expression. Since GnRH activates both arms of the phosphoinositide cycle, namely, Ca^{2+} elevation and PKC activation, we propose that while simultaneous activation by GnRH of Ca^{2+} and PKC elicits exocytosis, divergence of signaling is responsible for differential gonadotropin gene expression. Divergence of signaling by Ca^{2+} and PKC might be achieved by different time response, by different Ca^{2+} pools and/or PKC subspecies (Nishizuka, 1992), or by different downstream elements such as AP-1, AP-2, or CREB. It is known that cAMP can activate α and LHβ mRNA accumulation [reviewed in Counis and Jutisz (1991)]. However, since GnRH does not activate the cAMP cascade [Naor et al., 1975; see Naor (1990) for a review], it is possible that elevated Ca^{2+} utilizes the cAMP response element binding protein (CREB) for gene expression, as shown for other systems (Sheng et al., 1990; Dash et al., 1991). Alternatively, since both cAMP and PKC can converge at the transcription factor AP-2 (Imagawa et al., 1987; Hyman et al., 1989), it is possible that the observed effects of forskolin and cholera toxin on gonadotropin gene expression (Starzec et al., 1989a,b) were mediated by AP-2, which is also the target for PKC.

It is interesting to note that whereas a dose of 100 ng/mL TPA was more effective than a lower dose of 1 ng/mL in the elevation of α and LHβ mRNA levels, the opposite was observed in the elevation of FSHβ mRNA levels where the lower dose was more effective. We therefore suggest that different PKC isozymes with different affinities for phorbol esters (Nishizuka, 1988, 1992; Tsutsumi et al., 1993) might be involved in mediating GnRH action. Furthermore, Ca^{2+}-independent forms of PKC subspecies such as new PKCs and atypical PKCs (Nishizuka, 1992), some of which are also present in the pituitary (Naor et al., 1993), could mediate the effect of GnRH on FSHβ gene expression in a Ca^{2+}-independent fashion. Inhibition of GnRH action upon gonadotropin mRNA elevation by the PKC inhibitors staurosporine and GF 109203X, as well as by incubating the cells in Ca^{2+}-free medium, lends further support to the role of different PKC subspecies and Ca^{2+} in the neurohormone action.

The results presented here indicate that the gonadotropin subunits are differentially regulated by Ca^{2+} and PKC. Furthermore, dissociation exists between exotysosis and gene expression. Although the gonadotropins (LH and FSH) are produced in monohormonal or multihormonal gonadotrophs [see Naor and Childs (1986) for a review], different profiles and physiological changes of serum LH and FSH levels are observed. We therefore propose that differential regulation of gonadotropin gene expression by GnRH via Ca^{2+} and PKC might contribute to the different profiles of LH and FSH, which are responsible for normal reproductive function.

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