Arachidonic Acid and Lipoxygenase Products Stimulate Gonadotropin α-Subunit mRNA Levels in Pituitary αT3-1 Cell Line: Role in Gonadotropin Releasing Hormone Action†

David Ben-Menahem,‡ Zurit Shraga-Levine,‡ Rona Limor,§ and Zvi Naor*,‡

Department of Biochemistry, The George S. Wise Faculty of Life Sciences, and Timsit Institute of Reproductive Endocrinology, Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv 69978, Israel

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ABSTRACT: The role of arachidonic acid (AA) and its lipoxygenase metabolites in gonadotropin releasing hormone (GnRH) induced α-subunit gene expression was investigated in the transformed gonadotroph cell line αT3-1. The stable analog [D-Trp^6]GnRH (GnRHa) stimulated [^{3}H]AA release from prelabeled cells after a lag of 1–2 min. Addition of AA stimulated α-subunit mRNA levels in a dose-dependent manner, a significant effect being detected at 5 μM AA. Among various lipoxygenase metabolites of AA, only the 5-lipoxygenase products 5-hydroxyeicosatetraenoic acid (5-HETE) and leukotriene C_4 (LTC_4) stimulated α-subunit mRNA levels. However, while 5-HETE and LTC_4 (0.1 nM each) were active already after 30 min of incubation, similar to GnRHa, AA (20 μM) stimulated α-mRNA levels after 1 h of incubation. Addition of the phospholipase A_2 inhibitor 4-bromophenacyl bromide (BPB) or the selective 5-lipoxygenase inhibitor L-656,224 inhibited GnRHa elevation of α-subunit mRNA by 65%, while the cyclooxygenase inhibitor indomethacin had no effect. Addition of AA (20 μM) or LTC_4 (0.1 nM) to normal cultured rat pituitary cells mimicked the rapid (30 min) stimulatory effect of GnRH (1 nM) upon α-subunit, LHβ, and FSHβ mRNA levels, while 5-HETE (0.1 nM) stimulated only FSHβ mRNA levels at this time point. Thus AA and selected 5-lipoxygenase products, in particular LTC_4, participate in GnRHa-induced α-subunit mRNA elevation.

Gonadotropin releasing hormone (GnRH)[1] regulates the synthesis and release of the gonadotropins luteinizing hormone (LH) and follicle stimulating hormone (FSH). LH and FSH together with thyroid stimulating hormone (TSH) are members of the pituitary glycoprotein hormone family, sharing a common α-subunit and a specific β-subunit [see Pierce and Parsons (1980) and Gharib et al. (1990) for reviews].

The signaling events involved in GnRH-induced gonadotropin release include interaction with a G-protein, enhanced phosphoinositide turnover, Ca^{2+} mobilization and influx, activation of phospholipase D, translocation and activation of protein kinase C (PKC), release of arachidonic acid (AA), and formation of bioactive lipoxygenase products [see Naor (1990) and Naor et al. (1993) for reviews].

Much less is known about GnRH-induced gonadotropin synthesis, in particular in terms of signaling [see Gharib et al. (1990) for a review]. Some researchers suggested the involvement of protein kinase A (PKA) and protein kinase C (PKC) in mediating GnRH-induced gonadotropin gene expression (Counis & Jutisz, 1991). Others have reported involvement of PKC in GnRH regulation of α-subunit (Horn et al., 1991; Ben-Menahem et al., 1992; Schoderbek et al., 1993; Kay et al., 1994) or LHβ mRNA elevation (Andrews et al., 1988). We have reported differential roles for Ca^{2+} and PKC in mediating GnRH elevation of gonadotropin subunits mRNA (Ben-Menahem et al., 1994).

The recent availability of a transformed gonadotroph cell line (αT3-1) (Windle et al., 1990), which was derived by targeted tumorigenesis in transgenic mice and is capable of producing the common α-subunit, has enabled studies on α-gene expression in homogeneous cell populations (Horn et al., 1991; Ben-Menahem et al., 1992; Schoderbek et al., 1993; Kay et al., 1994). Previous studies have suggested a potential role for AA and its lipoxygenase products in GnRH-induced gonadotropin secretion (Naor & Catt, 1981; Naor et al., 1985; Hulting et al., 1985), and more recent studies have demonstrated the formation of bioactive lipoxygenase products of AA by GnRH in cultured rat pituitary cells (Kiesel et al., 1991; Dan-Cohen et al., 1992). We now investigate the effect of AA and several of its lipoxygenase metabolites on α-subunit mRNA levels in αT3-1 cells.

EXPERIMENTAL PROCEDURES

Materials

The GnRH analog [D-Trp^6]GnRH (GnRHa) was a gift from Dr. R. Millar (Cape Town, South Africa). Bovine serum albumin (BSA), trypsin, soybean trypsin inhibitor, DNase, indomethacin, and 4-bromophenacyl bromide (BPB) were from Sigma (St. Louis, MO). [^{3}H]Arachidonic acid was from New England Nuclear (240 Ci/nmol, 8880 GBq/mmol; Boston). Hydroxy fatty acids (15-, 12-, and 5-HETE), peptidoleukotrienes (LTC_4, LTD_4, LTE_4), and the lipoxygenase inhibitor 7-chloro-2-[(4-methoxyphenyl)methyl]-3-methyl-5-propyl-4-benzofuranol (L-656,224) were the kind

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* To whom correspondence should be addressed. FAX: 972-3-6415053.

‡ Department of Biochemistry, The George S. Wise Faculty.

§ Timsit Institute of Reproductive Endocrinology, Sackler Faculty of Medicine.

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[1] Abbreviations: GnRH, gonadotropin releasing hormone; LH, luteinizing hormone; FSH, follicle stimulating hormone; LTB_4, LTC_4, LTD_4, and LTE_4, leukotriene B_4, C_4, D_4, and E_4, respectively; HETE, hydroxyeicosatetraenoic acid; AA, arachidonic acid; PKC, protein kinase C; DG, diacylglycerol; L-656,224, 7-chloro-2-[(4-methoxyphenyl)-methyl]-3-methyl-5-propyl-4-benzofuranol; BPB, 4-bromophenacyl bromide; PLA_2, phospholipase A_2.

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gift of Dr. Ford-Hutchinson of Merck Frosst Canada Inc. (Point Duval, PQ). The rat α-subunit and LHβ cDNA probes were kindly provided by Dr. W. Chin (Boston, MA). Rat FSHβ cDNA was kindly provided by Dr. R. A. Maurer (Iowa City, IA). 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

αT3-1 Cell Culture. αT3-1 cells were subcultured into 60-mm tissue culture dishes (Sterilin, Hounslow, U.K.). Cells were grown in Dulbecco's modified Eagle's medium (DMEM) (5 mL) supplemented with 5% fetal calf serum (FCS), 5% horse serum (HS), penicillin (100 units/mL), and streptomycin (0.1 mg/mL). At 70–80% confluency the cells were washed, and stimulants were added in 5 mL of DMEM at the indicated concentration for the given length of time. When the stimulation period was up to 1 h, 10 mM HEPES was added to the medium. For stimulation periods longer than 6 h the medium contained 0.1% BSA and antibiotics.

Preparation and Stimulation of Pituitary Cell Cultures. Cells were prepared from pituitary glands from 25-day-old Wistar-derived male rats as previously described (Dan-Cohen et al., 1992). Pituitary cells were grown in medium 199 (5 mL), 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), were washed and stimulants were added in medium 199 as indicated above.

Determination of Arachidonic Acid Release. αT3-1 cells (10^6 cells/well/2 mL) were prelabeled with [^{3}H]arachidonic acid (0.2 μCi/well) for 24 h. Cells were then washed several times with DMEM to remove the unincorporated [^{3}H]-arachidonic acid. The labeled cells were then incubated with GnRHa for various times in DMEM containing 1 mg/mL BSA. Thereafter the medium was removed and centrifuged, and the radioactivity was determined.

RNA Isolation and Analysis. Total RNA was extracted from cells in 5 M guanidium 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, and the samples loaded in each lane were separated into two. One of the halves of each sample derived from the slot-blot analysis was hybridized with relevant gonadotropin subunit cDNA, and the second, corresponding half was hybridized with the cDNA for the housekeeping protein glyceraldehyde 3-phosphate dehydrogenase (GAPDH), which was used as an internal control. Following hybridization, filters were washed at high stringency and autoradiographed at −70 °C. Steady-state levels of mRNAs were quantitated with densitometric scanning of autoradiograms. The data were corrected for variability in loading by calculation as a ratio to GAPDH.

Statistical Analysis. The hybridization signals for gonadotropin subunit mRNA in each group were normalized to the hybridization signals for GAPDH. An arbitrary unit of 1 represents the control values. Statistical comparisons between control and treatment groups were performed using Student's t test: $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

RESULTS

Initially we studied the effect of the stable analog [D-Trp^6]-GnRH (GnRHa) on [^{3}H]arachidonic acid ([^{3}H]AA) release from prelabeled αT3-1 cells (Figure 1A). [^{3}H]AA release by

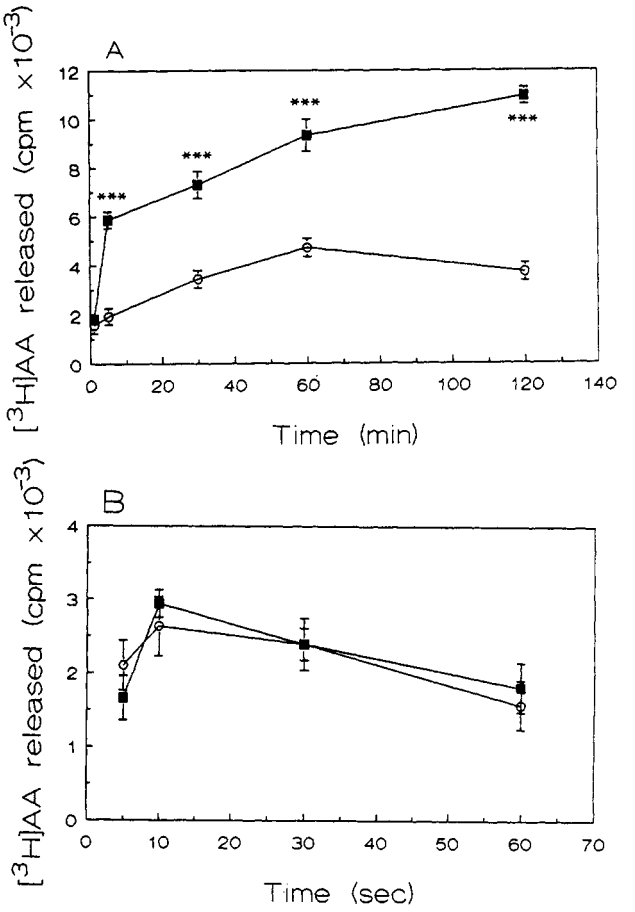

FIGURE 1: Effect of [D-Trp^6]GnRH analog on arachidonic acid release from αT3-1 cells. The cells (10^6/well) were prelabeled with [^{3}H]arachidonic acid (0.2 μCi/well) for 24 h. After several washes, the cells were incubated with (■) or without (○) GnRHa (10 nM) for the indicated times (A, minutes; B, seconds) in DMEM containing 1 mg/mL BSA. At the end of the incubation, medium was removed and radioactivity was determined. Results are means ± SEM (n = 3) from a representative experiment. $^{***}p < 0.001$.

GnRHa (10 nM) revealed a lag period of at least 1 min (Figure 1B), while significant release was detected after 5 min of incubation and at later time points as shown in Figure 1A. Stimulation of [^{3}H]AA release by GnRHa was dose-related with the maximal effect being obtained at 10 nM analog (Figure 2).

Since arachidonate and LTC_4 were reported to be involved in GnRH-induced gonadotropin secretion (Kiesel et al., 1991; Dan-Cohen et al., 1992), we examined here the effect of the fatty acid and its lipoxygenase metabolites on α-subunit mRNA levels. Stimulation of α-mRNA levels by AA is dose-related, and the effect is significantly detected at 5 μM AA (Figure 3). Various lipoxygenase metabolites of AA were examined for their potential to elevate α-mRNA levels, and only 5-HETE and LTC_4 (0.1 nM each) were found to be active (Figure 4). The stimulatory effect of LTC_4 and 5-HETE on α-mRNA levels is dose-related, and maximal response was observed at 0.1 nM of both metabolites (Figure 5 and Table 1). Interestingly this range of LTC_4 concentration can be achieved in cultured pituitary cells stimulated by GnRH (Dan-Cohen et al., 1992).

We then proceeded to investigate the effect of AA, LTC_4, and 5-HETE on α-mRNA levels at time points at which GnRHa has been reported to elevate α-mRNA levels (Ben-Menahem et al., 1992) (Figure 6). Although GnRHa elevates α-mRNA levels already after 30 min of incubation, AA was found to be active only after 1 h of incubation (Figure 6). On

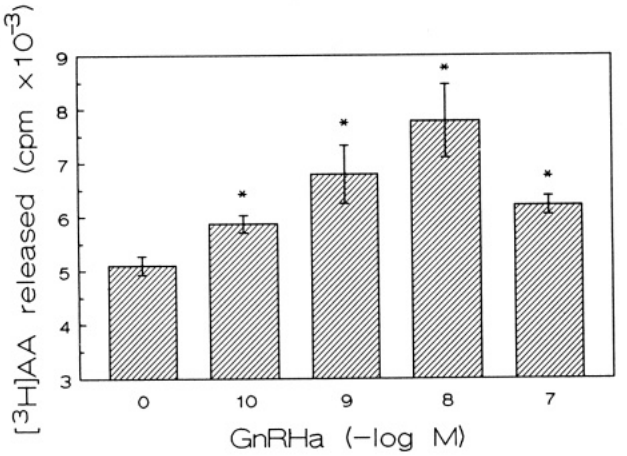

FIGURE 2: Dose–response study of the effect of [D-Trp⁶]GnRH analog on arachidonic acid release in αT3-1 cells. Prelabeled cells (as in Figure 1) were incubated with increasing concentrations of GnRHa for 2 h, and [³H]AA release into the medium was determined. Results are means ±SEM (n = 3) of a representative experiment. $^{*}p < 0.05$.

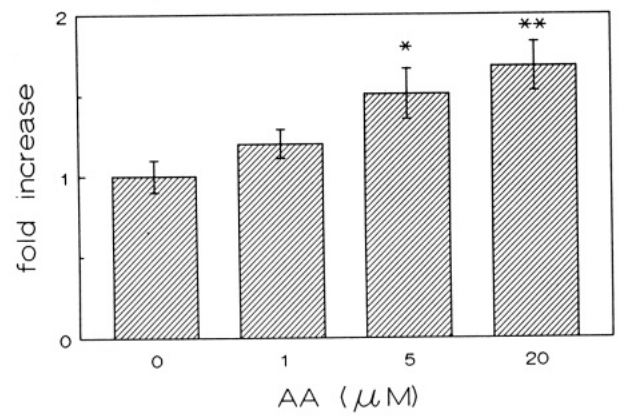

FIGURE 3: Effect of arachidonic acid (AA) on α-mRNA levels in αT3-1 cells. Cells were treated with increasing concentrations of AA for 1 h, and α-mRNA levels were determined as described in Methods. Results are expressed as means ± SEM (n = 8–13). $^{*}p<0.05$; $^{**}p < 0.01$.

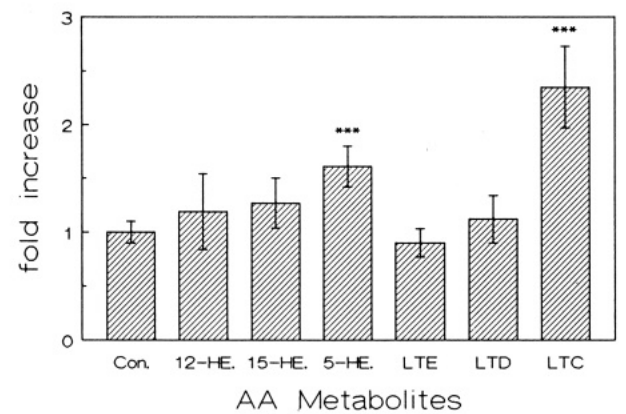

FIGURE 4: Effect of lipoxygenase metabolites of arachidonic acid on α-mRNA levels in αT3-1 cells. Cells were treated with 12-HETE (1 nM), 15-HETE (0.1 nM), 5-HETE (0.1 nM), LTE_4 (1 nM), LTD_4 (1 nM), or LTC_4 (0.1 nM) for 1 h, and α-mRNA levels were determined as described in Methods. Results are means ± SEM (n = 4–9). $^{***}p < 0.001$.

the other hand, the active metabolites LTC_4 and 5-HETE elevated α-mRNA levels also at the early time point of 30 min. The late effect of GnRHa on α-mRNA levels at 24 h was smaller in magnitude and was mimicked by AA, LTC_4, and 5-HETE.

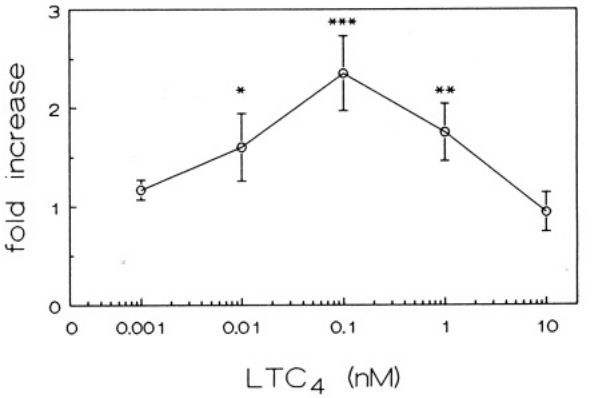

FIGURE 5: Dose–response of leukotriene C_4 (LTC_4) effect on α-mRNA levels in αT3-1 cells. Cells were treated with increasing concentrations of LTC_4 for 1 h, and α-mRNA was determined as described in Methods. Results are means ± SEM (n = 4–7). $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Table 1: Dose–Response of 5-HETE Effect on α-mRNA Levels in αT3-1 Cells[a]

5-HETE (M)	α-mRNA (fold increase)
control	1 ± 0.15
10^{-12}	1.21 ± 0.32
10^{-11}	1.68 ± 0.32[b]
10^{-10}	1.63 ± 0.16[b]
10^{-9}	1.43 ± 0.17[b]

[a] Cells were treated with increasing concentrations of 5-HETE for 1 h, and α-mRNA was determined as described in Methods. Results are mean ± SEM (n = 4–8). [b] $p < 0.05$.

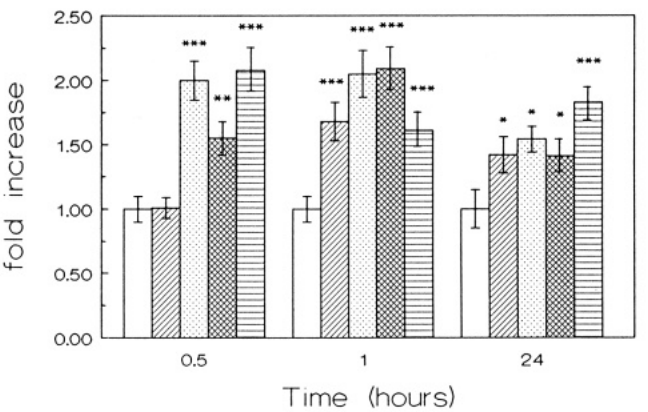

FIGURE 6: Effect of [D-Trp⁶]GnRH analog, arachidonate, LTC_4, and 5-HETE on α-mRNA levels in αT3-1 cells. Cells were untreated (open bars) or were treated with arachdionate (20 μM, hatched bars), GnRHa (10 nM, stippled bars), LTC_4 (0.1 nM, crosshatched bars), or 5-HETE (0.1 nM, lined bars) for the indicated times. α-mRNA levels were determined as described in Methods. Results are means ± SEM (n = 8–13). $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

In order to investigate whether AA and its metabolites participate in GnRH-induced α-mRNA elevation, we pretreated the cells with the phospholipase A_2 inhibitor 4-bromophenacyl bromide (BPB), with the selective 5-lipoxygenase inhibitor L-656,224, and with the cyclooxygenase inhibitor indomethacin in the presence of GnRHa (Figure 7). Elevation of α-mRNA levels by GnRHa was inhibited by BPB and by L-656,224 by 65%, while indomethacin had no effect (Figure 7).

To rule out the possibility that the effects observed here are restricted to the αT3-1 cell line, we examined the effect of AA, LTC_4, and 5-HETE on gonadotropin subunits mRNA levels in normal cultured rat pituitary cells (Figure 8). AA (20 μM) and LTC_4 (0.1 nM) mimicked the rapid stimulation (30 min) of α-subunit, LHβ, and FSHβ mRNA elevation

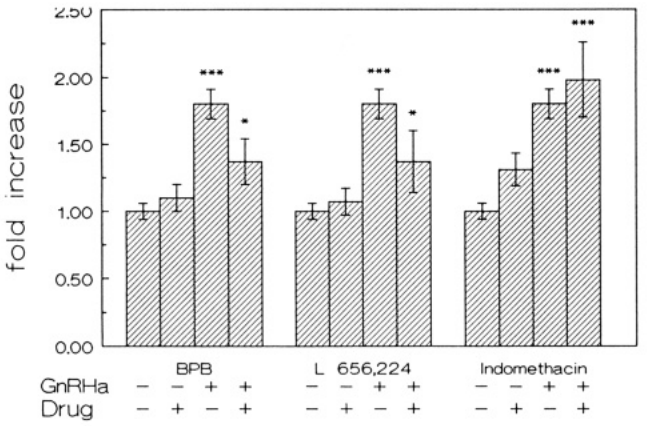

FIGURE 7: Effect of inhibitors of arachidonate metabolism on [D-Trp^6]-GnRH-induced α-mRNA elevation. Cells were pretreated with BPB (10 μM), L-656,224 (3 μM), or indomethacin (2 μM) for 10 min, followed by GnRHa (0.1 nM) for 60 min. α-mRNA levels were determined as described in Methods. Results are means ± SEM (n = 5–9). $^*p < 0.05$; $^{***}p < 0.001$.

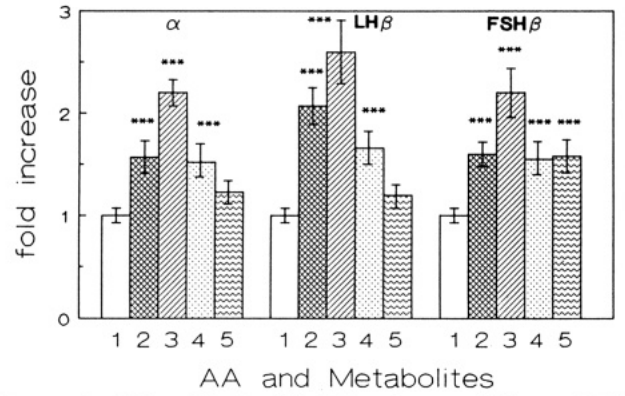

FIGURE 8: Effect of [D-Trp^6]GnRH analog, arachidonate, LTC_4, and 5-HETE on gonadotropin subunit mRNA levels in cultured rat pituitary cells. The cultured cells were washed to remove serum and were either untreated (1) or treated with (2) AA (20 μM), (3) GnRHa (1 nM), (4) LTC_4 (0.1 nM), or (5) 5-HETE (0.1 nM) for 30 min. mRNA levels were determined as described in Methods. Results are means ± SEM (n = 11–22). $^{***}p < 0.001$.

induced by GnRH (1 nM), while 5-HETE (0.1 nM) stimulated only FSHβ mRNA levels at this time point.

DISCUSSION

We have recently described the effect of GnRHa on α-mRNA levels in the transfected gonadotroph cell line αT3-1 (Ben-Menahem et al., 1992). The effect is biphasic with a rapid phase observed at 30–60 min of incubation and a secondary rise at 24 h. We suggested that sequential activation by Ca^{2+} and protein kinase C (PKC) is involved in the first phase of α-subunit mRNA elevation by GnRHa, while simultaneous activation by Ca^{2+} and PKC might be involved in mediation of the second phase (D. Ben-Menahem et al., submitted). In another study we and others have demonstrated the formation of lipoxygenase products of AA in GnRH-stimulated pituitary cells and suggested that lipoxygenase metabolites such as LTC_4 are involved in GnRH-induced gonadotropin secretion (Naor & Catt, 1981; Naor et al., 1985; Hulting et al., 1985; Kiesel et al., 1991; Dan-Cohen et al., 1992). Although AA is considered as a potential second messenger [see Naor (1990) for a review] very little is known about the role of AA in gene expression. We therefore examined here the effect of AA and some of its metabolites on α-subunit mRNA levels.

Release of AA by GnRHa revealed a lag of at least 1 min, similar to activation of phospholipase D by GnRHa in αT3-1 cells (Netiv et al., 1992). On the other hand, activation of phospholipase C by GnRH in αT3-1 cells is rapid and can be detected after 30 s (Horn et al., 1991). Sequential activation of phospholipases by GnRH will generate various second messengers such as Ca^{2+}, early and late diacylglycerol (DG), and phosphatidic and arachidonic acids. One common site of action for the above messenger molecules is the activation of PKC subspecies (Nishizuka, 1992). It is therefore possible that early DG and Ca^{2+} activate Ca^{2+}-dependent PKC subtypes which participate directly or indirectly in phospholipase D and phospholipase A_2 activation (Nishizuka, 1992; Asaoka et al., 1992). The released AA and late DG might then participate in activation of Ca^{2+}-independent as well as Ca^{2+}-dependent PKC subtypes (Naor et al., 1988; Nishizuka, 1992; Asaoka et al., 1992), resulting in gonadotropin α-subunit gene expression. Alternatively, AA and its active lipoxygenase metabolites might elevate α-subunit mRNA levels by direct activation of a novel transcription factor, similar to the action of other second messengers. Still another possibility is that AA does not act directly but is being converted to LTC_4. The newly formed leukotriene exits the cell and binds to neighboring cells or the same cell to specific receptors which are coupled to phospholipase C (Anderson et al., 1986). In this case an autocrine/paracrine loop is formed, converting LTC_4 to a first messenger capable of perpetuating the signal transduction evoked by the primary ligand (Dan-Cohen et al., 1992). The observation that LTC_4 and 5-HETE but not AA mimicked the rapid (30 min) effect of GnRHa on α-subunit mRNA elevation, as well as the similar inhibition of the GnRHa response by phospholipase A_2 and 5-lipoxygenase inhibitors, supports the notion that the active species mediating GnRH actions is LTC_4 or 5-HETE.

To rule out the possibility that stimulation of α-subunit mRNA levels by AA and its lipoxygenase products is due to the transformed nature of αT3-1 cells, we confirmed the data in normal cultured rat pituitary cells, which unlike αT3-1 cells express also the other subunits of the gonadotropin hormones, namely, LHβ and FSHβ. We chose the 30-min time point since we have recently shown that this is the earliest time point at which we could demonstrate the stimulatory effect of GnRH upon gonadotropin subunit gene expression (Ben-Menahem & Naor, 1994). Still, some differences were observed between the two cell systems. While AA elevated α-subunit mRNA levels in αT3-1 cells only after 1 h of stimulation, in cultured pituitary cells AA exerted its stimulatory effect already after 30 min, similar to GnRH. Also, while 5-HETE stimulated α-subunit mRNA levels in αT3-1 cells, it showed no effect on α-subunit mRNA levels in cultured pituitary cells at this early time point. Stimulation of LHβ mRNA levels by AA and LTC_4 and stimulation of FSHβ mRNA levels by AA, LTC_4, and 5-HETE in cultured pituitary cells suggest that AA and selected lipoxygenase products might be involved in regulation of gonadotropin gene expression by GnRH.

Removal of Ca^{2+} and inhibition of PKC were sufficient to block the GnRHa response upon α-subunit mRNA elevation (D. Ben-Menahem et al., submitted). Also, stimulation of AA release by GnRH reveals a lag of at least 1 min (Figure 1), while stimulation of phosphoinositide turnover and Ca^{2+} mobilization have been demonstrated in the first 30 s after exposure of αT3-1 cells to GnRH (Horn et al., 1991). We therefore suggest that AA release and formation of the lipoxygenase active metabolites are downstream of Ca^{2+}

mobilization and PKC activation. Nevertheless, since GnRH-induced α-mRNA elevation is abolished by a GnRH antagonist (Ben-Menahem et al., 1992) or by Ca^{2+} removal and inhibiton of PKC but is only inhibited (65%) by inhibitors of AA metabolism (Figure 7), we suggest that a fraction of the GnRHa response (35%) is mediated by Ca^{2+} and PKC independent of the AA pathway.

Very little is known about the involvement of AA and its lipoxygenase metabolites in gene expression. AA and leukotriene B_4 were reported to be involved in TPA induction of tumor necrosis factor gene expression (Horiguchi et al., 1989). Also, AA metabolites may function in activation of latent transcription factors by interferon α (Hannigan & Williams, 1991). We demonstrate here a role for AA and its 5-lipoxygenase products in GnRH-induced α-subunit gene expression. The results shed further light on GnRH actions, the first key hormone of the reproductive cycle, and on molecular events downstream of Ca^{2+} mobilization and PKC activation in cellular signaling.

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REFERENCES

Anderson, T., Schlegel, W., Monod, A., Krause, K. H., Stendahl, O., & Lew, D. P. (1986) *Biochem. J. 240*, 333–340.

Andrews, W. V., Maurer, R. A., & Conn, P. M. (1988) *J. Biol. Chem. 263*, 13755–13761.

Asaoka, Y., Nakamura, S.-I., Yoshida, K., & Nishizuka, Y. (1992) *Trends Biochem. Sci. 17*, 414–417.

Ben-Menahem, D., & Naor, Z. (1994) *Biochemistry 33*, 3698–3704.

Ben-Menahem, D., Shraga, Z., Lewy, H., Limor, R., Hammel, I., Stein, R., & Naor, Z. (1992) *Biochemistry 31*, 12893–12898.

Cathala, G., Savouret, J.-F., Bernadita, M., West, B. L., Karin, M., Martial, J. A., & Baxter, J. D. (1983) *DNA 2*, 329–335.

Counis, R., & Jutisz, M. (1991) *Trends Endocrinol. Metab. 2*, 181–187.

Dan-Cohen, H., Sofer, Y., Schwartzmann, M. L., Natarajan, R. D., Nadler, J. L., & Naor, Z. (1992) *Biochemistry 31*, 5442–5448.

Gharib, S. D., Wierman, M. E., Shupnik, M. A., & Chin, W. W. (1990) *Endocr. Rev. 11*, 177–199.

Hannigan, G. E., & Willims, B. R. (1991) *Science 251*, 204–207.

Horiguchi, J., Spriggs, D., Imamura, K., Stone, R., Luebbers, R., & Kufe, D. (1989) *Mol. Cell. Biol. 9*, 252–258.

Horn, F., Bilezikjiaan, L. M., Perrin, M. H., Bosma, M. M., Windle, J. J., Huber, K. S., Blount, A. L., Hille, B., Vale, W., & Mellon, P. L. (1991) *Mol. Endocrinol. 5*, 347–355.

Hulting, A. L., Lindgren, J. A., Hokfelt, T., Eneroth, P., Werner, S., Patrono, C., & Samuelsson, B. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 3834–3838.

Kay, T. W. H., Chedrese, P. J., & Jameson, J. L. (1994) *Endocrinology 134*, 568–573.

Kiesel, L., Przylipiak, A. F., Habenicht, A. J., Przylipiak, M. S., & Runnebaum, B. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 8801–8805.

Naor, Z. (1990) *Endocr. Rev. 11*, 326–353.

Naor, Z. (1991) *Mol. Cell. Endocrinol. 80*, C181–C186.

Naor, Z., & Catt, K. J. (1981) *J. Biol. Chem. 256*, 2226–2229.

Naor, Z., Kiesel, L., Vanderhoek, J. Y., & Catt, K. J. (1985) *J. Steroid Biochem. 23*, 711–717.

Naor, Z., Shearman, M. S., Kishimoto, A., & Nishizuka, Y. (1988) *Mol. Endocrinol. 2*, 1043–1048.

Naor, Z., Shraga-Levine, Z., Marantz, Y., Reiss, N., & Ben-Menahem, D. (1993) in *Molecular and Clinical Advances in Pituitary Disorders* (Melmed, S., Ed.) pp 281–286, Endocrine Research and Education Inc., Los Angeles.

Nishizuka, Y. (1992) *Science 258*, 607–614.

Pierce, J. G., & Parsons, J. F. (1980) *Annu. Rev. Biochem. 50*, 465–495.

Schoderbek, W. E., Roberson, M. S., & Maurer, R. A. (1993) *J. Biol. Chem. 286*, 3903–3910.

Windle, J. J., Weiner, R. I., & Mellon, P. L. (1990) *Mol. Endocrinol. 4*, 597–603.