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Stimulation of progesterone and prostaglandin E_2 production by lipoxygenase metabolites of arachidonic acid

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The role of several lipoxygenase metabolites of arachidonic acid in the action of luteinizing homone-releasing hormone (LHRH) on ovarian hormone production was investigated. Like LHRH, treatment of rat granulosa cells with 5-HETE, 5-HPETE, 12-HETE, 15-HETE or 15-HPETE stimulated progesterone (P) and prostaglandin E_2 (PGE_2) production. 12-HEPE was most potent and stimulated P and PGE_2 equally well. By contrast, 5-HETE stimulated P better than PGE_2, while 15-HETE was a potent stimulator of PGE_2 but not of P. Stimulation of P and PGE_2 by LHRH or 12-*O*-tetradecanoylphorbol 13-acetate (TPA) was further augmented by several HETEs and HPETEs. Like protein kinase C, arachidonic acid metabolites appear to mediate the multiple actions of LHRH in the ovary.

Progesterone; Prostaglandin E_2; Arachidonic acid; Hormone action; Lipoxygenase

1. INTRODUCTION

In the rat ovary, LHRH stimulates arachidonic acid (AA) liberation from membrane phospholipids [1,2]. Treatment with melittin, a phospholipase A_2 activator, as well as administration of exogenous AA enhances hormone production in isolated ovarian cells [3,4]. The action of LHRH or AA is blocked by nordihydroguaiaretic acid but not by indomethacin [3], suggesting that the lipoxygenase metabolites of AA may be involved in the action of LHRH on ovarian steroidogenesis. In this study, we further examine the direct effects of several lipoxygenase metabolites of AA, including hydroxyeicosatetraenoic acids (HETEs) and hydroperoxyeicosatetraenoic acids (HPETEs), on ovarian steroid and prostaglandin production.

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2. MATERIALS AND METHODS

Immature Sprague-Dawley female rats purchased from Charles River Canada, Inc., were injected subcutaneously with 12 IU pregnant mare's serum gonadotropin (PMSG, Sigma). After 48 h, the rats were killed by cervical dislocation on the morning of day 25 of age. Granulosa cells were harvested by puncturing the ovarian follicles with a fine hypodermic needle under the dissecting microscope as described [5]. The cells were suspended in MEM (modified) with Eagle's salts and supplemented with glutamine, antibiotics, and nonessential amino acids. Aliquots of the cell suspension (2×10^5/ml) were added to 24-well culture plates (Falcon) and incubated at 37°C under an atmosphere of 5% CO_2 in air. The concentration of progesterone (P) in the culture medium was determined by a specific RIA with an antiserum kindly provided by Dr D.T. Armstrong of the University of Western Ontario [6]. The intra-assay coefficient of variations (C.V.) was 5.0% and inter-assay C.V., 5.9%. The prostaglandin E_2 concentrations in the culture medium were determined by specific RIA with an antiserum kindly provided by Dr T.G. Kennedy of the University of Western Ontario [7]. The intra-assay C.V. of PGE_2 was 6.7% and the inter-assay C.V. was 9.6%. The results are presented as means ± SE of quadruplicate determinations. Statistical significance of the data was determined by analysis of variance. A P value of <0.05 was considered significant. For all figures, similar or identical results were obtained in two or three separate experiments.

LHRH and TPA were purchased from Sigma and the pregnant mare's serum gonadotropin was a gift from the NIDDK

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and the National Hormone and Pituitary Program (University of Maryland School of Medicine). HETEs and HPETEs were purchased from Biomol Labs (Philadelphia, PA).

3. RESULTS

3.1. *Effects of HETEs and HPETEs on P production*

Rat granulosa cells were incubated for 5 h in the absence or presence of increasing concentrations of 5-HETE, 5-HPETE, 12-HETE, 15-HETE or 15-HPETE (10^{-7}–10^{-5} M). At 10^{-6} M, all treatments resulted in a slight but significant increase in P formation. At 10^{-5} M, all compounds (except 15-HPETE) further stimulated P production. The following order of potency was observed: 12-HETE > 5-HETE > 5-HPETE = 15-HETE.

3.2. *Synergistic effects of HETE and HPETE on P production*

15-HETE and 15-HPETE, either alone or in combination, were added to granulosa cells for 5 h (fig.2). At 10^{-6} M, 15-HETE or 15-HPETE caused a slight but significant stimulation of P production, by ~36 and 31%, respectively. Interestingly, in the combined presence of 15-HETE and 15-HPETE, P accumulation was markedly increased (by 140%) when compared with P levels in the control incubations.

3.3. *Effects of HETEs on PGE_2 production*

Granulosa cells were treated with 5-, 12- or 15-HETE and the effects on P as well as PGE_2 production were examined. As seen in fig.3 (upper panel), at 5×10^{-6} M, 12-HETE was most potent and caused a 4.1-fold increase in P formation. 5-HETE and 15-HETE caused a 3.5- and 2.4-fold increase in P accumulation, respectively, vs control incubations. Interestingly, these HETEs also stimulated PGE_2 production in the same ex-

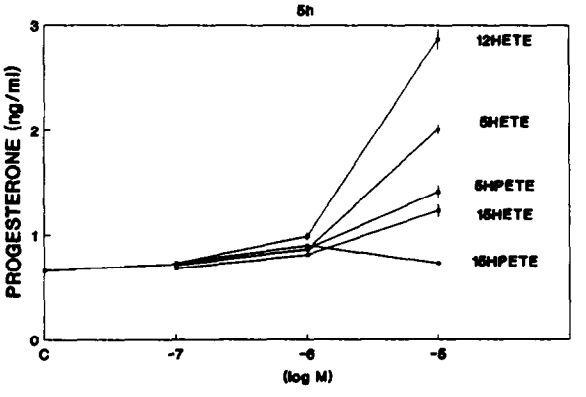

Fig.1. Effects of HETEs and HPETEs on P production. C, controls (untreated cells).

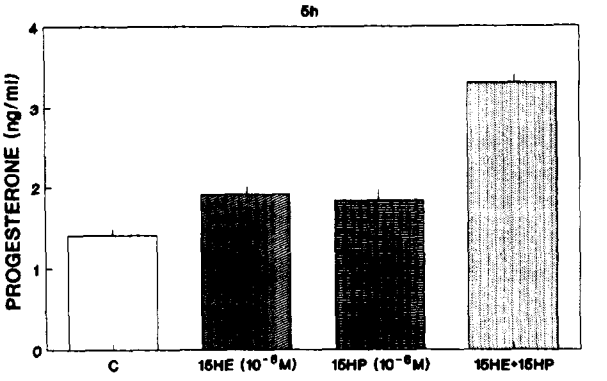

Fig.2. Synergistic effects of 15-HETE (15HE) and 15-HPETE (15HP) on P production.

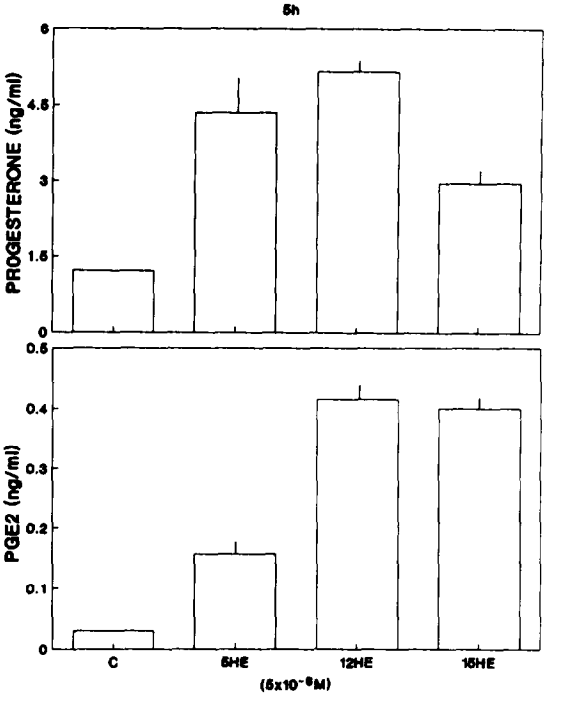

Fig.3. Effects of HETEs on P (upper panel) and PGE_2 (lower panel) production. C, controls; 5HE, 5-HETE; 12HE, 12-HETE; 15HE, 15-HETE.

periments (lower panel). Unlike their action on P production, the effect of 15-HETE was as potent as 12-HETE on PGE_2 formation. 15-HETE or 12-HETE caused an approx. 15-fold increase in PGE_2 accumulation in the culture medium. By contrast, 5-HETE was considerably less potent when compared with 12- or 15-HETE, but still resulted in significant increase of PGE_2 formation when compared with the control incubations.

3.4. *Interactions of HETEs or HPETEs with LHRH*

The effects of HETEs and HPETEs on the stimulation of P production by LHRH were further investigated (fig.4, upper panel). At the minimum effective dose (i.e. 10^{-6} M), the AA metabolites stimulated basal P production slightly. A more effective stimulation of P was observed with 10^{-6} M LHRH. Concomitant treatment with LHRH and the various AA metabolites caused further increases in P accumulation, by 20–93%, when compared with the effect of LHRH alone.

PGE_2 production in the same experiments was also determined (fig.4, lower panel). 5-HETE and 5-HPETE, at the minimum effective dose which stimulated P, altered neither basal nor LHRH-induced PGE_2 formation. On the other hand, 12-HETE, 15-HETE or 15-HPETE significantly increased PGE_2 levels when compared with the control incubations. Furthermore, 12-HETE, 15-HETE and 15-HPETE augmented the stimulatory effect of LHRH on PGE_2 production by 2-, 2.9- and 2.5-fold, respectively, vs LHRH treatment alone.

3.5. *Interactions of HETEs or HPETEs with TPA*

The addition of the protein kinase C activator (TPA), at 10^{-7} M, to granulosa cells caused a marked increase in P production (fig.5, upper panel). All HETEs and HPETEs tested significant-

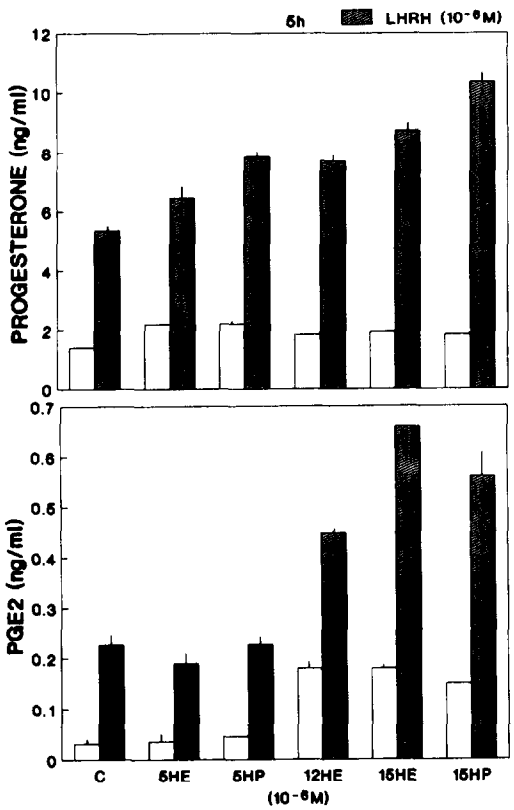

Fig.4. Interactions of HETEs or HPETEs with LHRH on P (upper panel) and PGE_2 (lower panel) production. Same abbreviations as in fig.3.

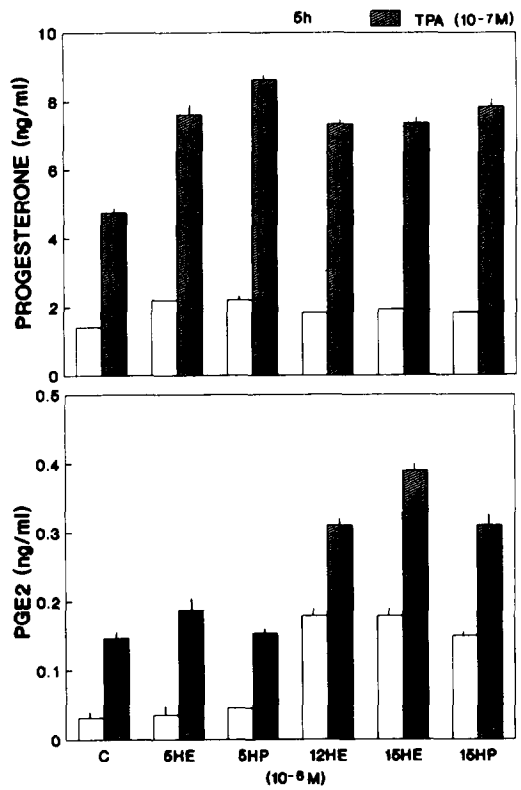

Fig.5. Interactions of HETEs or HPETEs with TPA on P (upper panel) and PGE_2 (lower panel) production. Same abbreviations as in fig.3.

ly augmented the stimulatory effect of TPA, by 55–83%, when compared with P levels induced by TPA alone.

In the same experiments, TPA alone caused a 4.9-fold increase in PGE_2 (lower panel). While 5-HETE or 5-HPETE did not significantly affect PGE_2 production induced by TPA, concomitant treatment with 12-HETE, 15-HETE or 15-HPETE further enhanced TPA-stimulated PGE_2 accumulation.

4. DISCUSSION

We have recently proposed that lipoxygenase metabolites of AA may be involved in the actions of LHRH [3]. This hypothesis is further investigated in the present study by using several HETEs and HPETEs. The results indicate that at least some lipoxygenase metabolites of AA are capable of enhancing the formation of P by rat granulosa cells in a dose-dependent manner. The stimulatory effect of 12-HETE on P production appears to be more potent than that of 5-HETE, 5-HPETE, 15-HETE or 15-HPETE. Interestingly, the concomitant presence of 15-HETE and 15-HPETE can further amplify the stimulatory effect of either compound alone. In addition to P, formation of PGE_2 is also stimulated by several of the AA metabolites. At 5×10^{-6} M, 12-HETE and 15-HETE are more potent than 5-HETE in this regard.

Previous studies have demonstrated that LHRH induces AA liberation from granulosa cells [1,2]. AA or its metabolites stimulate basal P production as well as potentiate P formation induced by LHRH or TPA [3,4]. We hereby show that 5-HETE, 5-HPETE, 12-HETE, 15-HETE and 15-HPETE increase basal P production and further augment the LHRH-induced stimulation of formation. Also, at 10^{-6} M, 12-HETE, 15-HETE and 15-HPETE stimulate basal PGE_2 formation and potentiate the stimulation of PGE_2 formation induced by LHRH. Since very similar results are observed with TPA, the facilitatory effects of HETEs and HPETEs on LHRH-induced P and PGE_2 production may be due to the interaction of these AA metabolites with LHRH-activated protein kinase C. The present findings that HETEs and HPETEs potentiate the action of LHRH or TPA on P and PGE_2 formation correlate well with our earlier demonstration that AA potentiates the effect of LHRH and TPA on P production [3]. To our knowledge, this is the first report that HETEs and HPETEs stimulate the ovarian production of PGE_2 and P in the same experiments.

There is increasing evidence to support the notion that lipoxygenase metabolites of AA are potent mediators of hormone production in different endocrine tissues. For example, in the pituitary, lipoxygenase products of AA metabolism have been shown to stimulate prolactin [8] and LH release [9,10]. 5-HETE shows an insulinotropic effect in pancreatic islets in a concentration-dependent manner [11]. In bovine corpus luteum, 5-HETE reduces the biosynthesis of P and 6-keto-$PGF_{1\alpha}$, while the synthesis of $PGF_{2\alpha}$ is unaffected [12]. The 5-lipoxygenase pathway is of special interest because 5-HPETE and 5-HETE can be rapidly converted to leukotrienes, which are presumably the most active of the lipoxygenase metabolites of AA [13,14]. It has been reported that leukotrienes are effective stimulators of LH release from dispersed rat anterior pituitary cells [9,10,15]. In view of the present demonstration of stimulatory effects of 5-HETE and 5-HPETE on P and PGE_2 production, the role of leukotrienes on ovarian cell function warrants further investigation.

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