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PROSTAGLANDIN STIMULATION OF OVARIAN ORNITHINE DECARBOXYLASE IN VITRO*

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Summary: Prostaglandins E_1 and E_2 caused a 5-10 fold stimulation of ornithine decarboxylase activity in granulosa cells isolated from porcine ovarian follicles. The minimally effective concentration of prostaglandin E_2 was 10 ng/ml and the plateau of activity was reached at 500 ng/ml. Prostaglandin $F_{2\alpha}$ was ineffective. 1-Methyl,3-isobutyl-xanthine, a phosphodiesterase inhibitor, potentiated the effect of both submaximal and maximal effective doses of prostaglandin E_2, suggesting that the effect of prostaglandin E_2 is mediated by cAMP. The effect of prostaglandin E_2 was similar to that of luteinizing hormone and a cAMP analogue, 8-Bromo-cAMP.

INTRODUCTION

The activity of ovarian ornithine decarboxylase (EC 4.1.1.17) has been repeatedly demonstrated to increase dramatically following administration of luteinizing hormone in vivo (1-7). We have recently shown that luteinizing hormone as well as follicle stimulating hormone can stimulate ornithine decarboxylase in porcine ovarian granulosa cells under defined conditions in vitro (8). Our most recent studies suggest that this effect of gonadotropins is mediated by cAMP (9).

Prostaglandin E_2 has also been shown to stimulate ovarian ornithine decarboxylase activity when administered to rats in vivo (10). In the current study we have further characterized prostaglandin effects on ovarian ornithine decarboxylase by demonstrating a direct effect on a particular ovarian cell type (the porcine granulosa cell) in vitro. In addition, we have compared the effects of several prostaglandins, related these effects to those produced by luteinizing hormone and by 8-Bromo-cAMP and demonstrated the enhancement of the prostaglandin response by inhibition of cAMP phosphodiesterase.

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MATERIALS AND METHODS

Materials. Ovine luteinizing hormone (NIH-LH-S19)** was obtained from the Hormone Distribution Office, National Institute of Arthritis, Metabolism and Digestive Diseases. Prostaglandins were obtained from the Upjohn Company. 8-Bromo-cAMP was purchased from the Sigma Chemical Company and 1-methyl,3-isobutyl-xanthine (MIX) from the Aldrich Chemical Company. DL-(1-^{14}C) ornithine monohydrochloride (specific activity: 45 mCi/mmole) was purchased from New England Nuclear.

Tissue Preparation. Porcine ovaries were collected at a local slaughter house and the granulosa cells isolated from small follicles (11). Approximately 10^8 cells/flask were incubated for 4 h in 10 ml serum-free Medium 199 as previously described (8). Luteinizing hormone (200 ng/ml), prostaglandins, 8-Bromo-cAMP (0.5 mM), and MIX were added at the beginning of incubation in respective experiments. Prostaglandins were dissolved in ethanol and the entire dose was added in a 10 µl volume per flask. MIX was dissolved in dimethylsulfoxide and the entire dose was added in a 25 µl volume per flask (final concentration of MIX was 0.25 mM). Controls received appropriate volume of ethanol, dimethylsulfoxide or both.

Ornithine Decarboxylase Assay. Following incubation granulosa cells were separated from the incubation medium, washed, homogenized and the cytosol was prepared for the assay of ornithine decarboxylase activity as previously described (8,12). The results are expressed as pmol/mg protein per 30 min. Each sample was assayed in duplicate.

Total protein in the cytosol was determined by the method of Lowry et al. (13). The precision of the culture technique and ornithine decarboxylase assay has been characterized previously (8). In each experiment shown in the Results section, granulosa cells were from a single pool of cells isolated on a particular day from the 100-200 ovaries. Each experiment was repeated at least once with a different batch of ovaries and a similar pattern of response was obtained.

RESULTS

The Effect of Prostaglandins on Ovarian Ornithine Decarboxylase Activity. Preliminary studies on the time course of prostaglandin stimulation of ornithine decarboxylase revealed the earliest stimulation at 2 h and a maximum between the 4th and 6th h, similar to that of gonadotropins (8). All subsequent experiments were performed 4 h after addition of stimulating agents. As shown in Fig. 1, prostaglandin E_1 and prostaglandin E_2 caused a 5-6 fold stimulation of ornithine decarboxylase activity at 100 ng/ml and a 9-10 fold stimulation at 1000 ng/ml. Prostaglandin E_2 was a more potent stimulator at a lower dose ($p < 0.01$) but about equal to prostaglandin E_1 at a higher dose used. Prostglandin $F_{2\alpha}$ was without effect. Prostaglandin E_2 was then

** Ovine luteinizing hormone (NIH-LH-S19) contains less than 0.05 NIH-FSH-S1 units/mg.

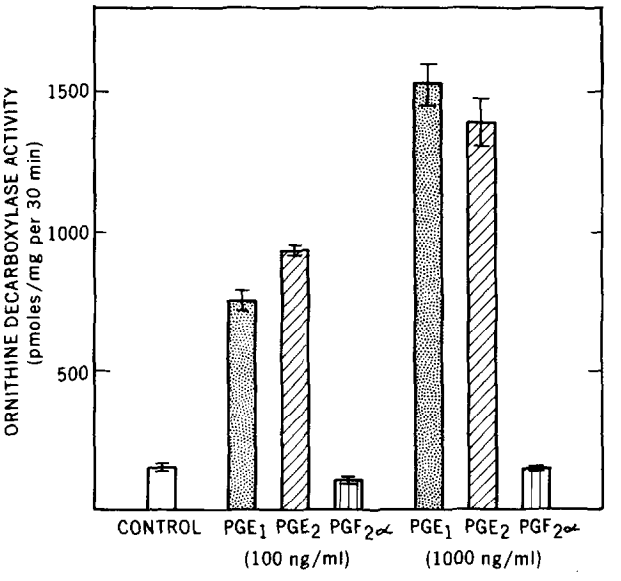

Figure 1. Effect of prostaglandins (PG) E_1, E_2 and $F_{2\alpha}$ on ornithine decarboxylase activity of porcine granulosa cells. Results are means ± SEM of determinations on 3 replicate cultures.

selected for all subsequent experiments.

Figure 2 shows the dose-respone curve of prostaglandin E_2. The minimally effective dose was 10 ng/ml and the maximum effective concentration was 500 ng/ml.

Effect of Phosphodiesterase Inhibitor on Prostaglandin-Stimulated Ornithine Decarboxylase. Addition of MIX, a phosphodiesterase inhibitor to both submaximal (50 ng/ml) and maximal effective dose of prostaglandin E_2 (1000 ng/ml), potentiated the effect of prostaglandin E_2 on ornithine decarboxylase (Fig. 3). This is consistent with the mediation of prostaglandin effect by cAMP. MIX alone was without effect, suggestive of low basal (unstimulated) cAMP production of these cells. This is in agreement with our previous findings (9).

Comparison of Effects of Prostaglandin E_2 Luteinzing Hormone and 8-Bromo-cAMP on Ovarian Ornithine Decarboxylase. At maximally effective doses, prostaglandin E_2 caused slightly greater stimulation of the enzyme activity than the saturating dose of luteinizing hormone in the experiment illustrated

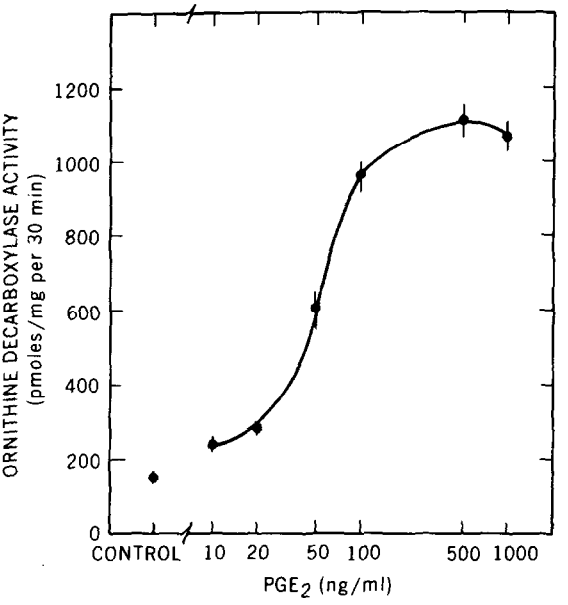

Figure 2. Effect of various concentrations of prostaglandin (PG) E_2 on ornithine decarboxylase activity of porcine granulosa cells. Each point is a mean and range of determinations on 2 replicate cultures.

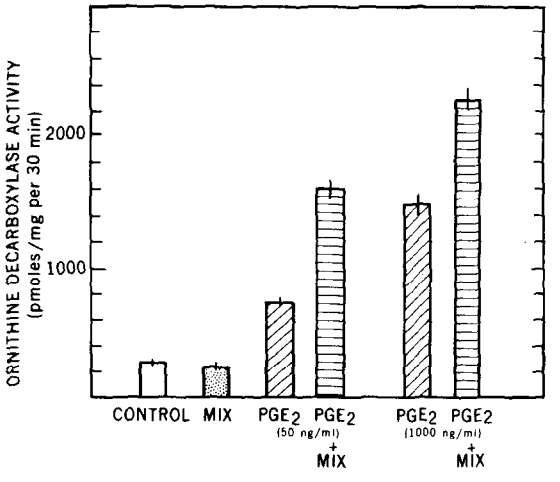

Figure 3. Effect of submaximally (50 ng/ml) and maximally (1000 ng/ml) effective concentrations of prostaglandin (PG) E_2 with and without MIX (0.25 mM) on ornithine decarboxylase activity of porcine granulosa cells. Results are means and range of determinations on 2 replicate cultures.

in Fig. 4. However, when the experiment was repeated with 3 additional

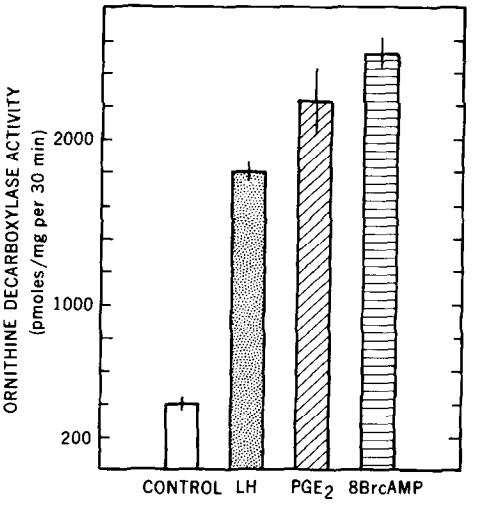

Figure 4. Effect of luteinizing hormone (LH), prostaglandin (PG) E_2 (1000 ng/ml) and 8-Bromo-cAMP (0.5 mM) on ornithine decarboxylase activity of porcine granulosa cells. Results are means and range of determinations on 2 replicate cultures.

batches of granulosa cells, the difference between prostaglandin E_2 and luteinizing hormone was not significant (paired t-test). Prostaglandin E_2 and 8-Bromo-cAMP were similar in their stimulation of the enzyme activity.

DISCUSSION

To our knowledge, this is the first demonstration of *in vitro* stimulation of ovarian ornithine decarboxylase by prostaglandins. The effect is shown to be dose-dependent and restricted to prostaglandins of the E series. The effect of saturating concentration of prostaglandin E_2 and luteinizing hormone were similar, which is in good agreement with the observation of Lamprecht *et al.* (10) in rat ovary *in vivo*. The minimally effective dose of prostaglandin E_2 (10 ng/ml) which augmented ornithine decarboxylase activity in our preparation was recently shown to be effective in stimulation of cAMP accumulation in isolated rat granulosa cells (14). Our observation that MIX, a potent cAMP phosphodiesterase inhibitor, potentiated the effect of both submaximal and maximal effective concentrations of prostaglandin E_2, is consistent with cAMP mediation of this effect. We have found similar potentiation of gonadotropin effect on ornithine decarboxylase activity by MIX (9).

Ornithine decarboxylase can thus be viewed along with progesterone secretion and morphological luteinization as an aspect of granulosa cell function which can be stimulated by either gonadotropins or prostaglandins with cAMP as the probable mediator. The physiological coordination of these events is only partially understood. Luteinizing hormone appears to stimulate accumulation of prostaglandins in the ovary; this effect is evidently a consequence of luteinizing hormone stimulation of adenylyl cyclase rather than a prerequisite for cAMP generation (10,15). The stimulation of ornithine decarboxylase activity seems to follow these early steps in hormone action. As the rate-limiting enzyme in polyamine synthesis, however, ornithine decarboxylase may be involved in the regulation of more distal aspects of differentiated function in the ovary. Such a view is supported by the close association of the stimulation of ornithine decarboxylase activity and steroidogenesis in the ovary and the ability to block progesterone secretion with inhibitors of polyamines (16).

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