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Mechanism of the rapid antigonadotropic action of prostaglandins in cultured luteal cells

(prostaglandin $F_{2\alpha}$/luteolysis/gonadotropin receptor/adenylate cyclase)

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ABSTRACT **A reproducible method for dissociation and culture of rat luteal cells is described. The concentration of LH required to produce half-maximal stimulation of progesterone secretion was 50 ng/ml. The effects of prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on basal and luteinizing hormone (LH)-stimulated progesterone production were examined. Both prostaglandins stimulated basal progesterone production but PGE_2 was about twice as active, showing a 2-fold maximal stimulation at 0.75 μM. When either prostaglandin was incubated simultaneously with LH, a dose-dependent inhibition of progesterone secretion occurred; $PGF_{2\alpha}$ was 4 times more active than PGE_2, showing 50% inhibition at a concentration of 40 × nM. Thus, both prostaglandins are more active as antagonists than as agonists of LH with respect to progesterone secretion. $PGF_{2\alpha}$ also inhibited LH-stimulated adenylate cyclase activity and cyclic AMP accumulation. The block in progesterone secretion was reversed by addition of dibutyryl cyclic AMP (1 mM) but not by theophylline (5 mM) alone. These data and the finding that $PGF_{2\alpha}$ did not affect the specific binding activity of the LH receptor in intact luteal cells indicate that the rapid action of prostaglandins in luteal cells is due to a block of LH-dependent production of cyclic AMP which results in a decrease in progesterone secretion.**

Regression of the corpus luteum signals the termination of the estrus and menstrual cycles and is characterized by a rapid decline in progesterone production. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), a putative physiological luteolysin, has been shown to reduce luteal function *in vivo* in several species (1), and the reduction of circulating progesterone occurs as early as 30 min in the rat (2). On the other hand, *in vitro* studies with luteal tissue do not reveal any consistent inhibitory effect of $PGF_{2\alpha}$ on progesterone production. Behrman *et al.* (3) reported that, in cultured explants of hamster luteal tissue, $PGF_{2\alpha}$ produced little effect on basal progesterone production but, when coincubated with luteinizing hormone (LH), a clear block of LH-stimulated progesterone production occurred. This observation was confirmed by others in studies on cultured granulosa cells (4).

The antigonadotropic effect of $PGF_{2\alpha}$ may be due to an induced loss of receptors for LH in the luteal cell. Hichens *et al.* (5) demonstrated such an action of $PGF_{2\alpha}$ but, in later studies, Grinwich *et al.* (6) found that the loss of LH receptors occurred several hours after the decrease in circulating progesterone. Thus, the loss of LH receptors may explain the long-term antigonadotropic action of $PGF_{2\alpha}$ *in vivo*, but the acute action of $PGF_{2\alpha}$ appears to be independent of the number of LH binding sites in luteal tissue. The possibility exists that $PGF_{2\alpha}$ may interfere with binding activity of the LH receptor in the corpus luteum, and it was shown (2) that $PGF_{2\alpha}$ caused a rapid and marked inhibition of accumulation of ^{125}I-labeled human chorionic gonadotropin (hCG) by corpora lutea *in vivo* coincident with a decrease in circulating progesterone within 30 min. However, interpretation of these data was confounded by possible effects of $PGF_{2\alpha}$ on blood flow because $PGF_{2\alpha}$ was found to produce a similar effect on luteal accumulation of ^{125}I-labeled prolactin (7).

To further examine the interaction between $PGF_{2\alpha}$ and LH on luteal progesterone production, it was deemed important to study the early and direct actions of these agents on luteal cells *in vitro*. The present studies describe the effect of PGE_2 and $PGF_{2\alpha}$ on LH-dependent progesterone secretion in conjunction with studies on the effect of $PGF_{2\alpha}$ on binding of gonadotropin to isolated luteal cells in culture. In this same model the effect of simultaneous incubation of luteal cells with LH, dibutyryl cyclic AMP [$(Bt)_2$-cAMP], and theophylline in the presence and absence of $PGF_{2\alpha}$, in addition to the effect of $PGF_{2\alpha}$ and LH on adenylate cyclase and cAMP accumulation, was examined to provide information on the possible site of action of prostaglandins.

MATERIALS AND METHODS

Animals. Immature (26-day-old) rats (CD strain, Charles River Laboratories, Wilmington, MA) were given a subcutaneous injection of 50 international units (IU) of pregnant mare serum (Gestyl, Organon) followed, 64 hr later, by a second subcutaneous injection of 25 IU of hCG (A.P.L., Ayerst).

Dispersion of Luteal Cells. Ovaries were removed 7 days after hCG injection, and the cells were dispersed in Ca^{2+}-free medium (medium 1) (no. 138, GIBCO, Grand Island, NY) containing 2000 IU of collagenase (Worthington, Freehold, NJ) and 3000 IU of deoxyribonuclease (Worthington) per g of tissue for 1 hr at 37° under 95% O_2/5% CO_2. The contents of the flask were filtered through nylon mesh (Nyten, Tetko Inc.) and centrifuged (5 min, 100 × *g*); the supernatant fraction was discarded and the pellet was washed three times with fresh medium 1. The final cell concentration (10^6 cells per ml) was made up in minimal essential medium with 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonate buffer and Earles's salts (medium 2) (no. 236, GIBCO). Cell numbers were determined with a hemocytometer, and cell viability was tested by the trypan blue dye test (8). Luteal cells comprised more than 60% of the total cells based on their size and granular lipid inclusions as seen in the light microscope.

Incubation of Luteal Cells. Incubations were carried out

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Abbreviations: PG, prostaglandin; LH, luteinizing hormone; hCG, human chorionic gonadotropin; cAMP, cyclic AMP; $(Bt)_2$-cAMP, $N^6O^{2'}$-dibutyryl adenosine 3':5'-cyclic monophosphoric acid; IU, international units.

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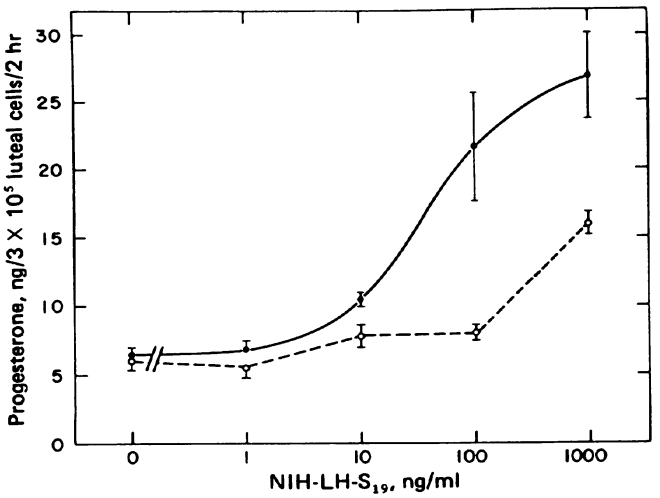

FIG. 1. Dose-response of isolated luteal cells to LH and effect of $PGF_{2\alpha}$ (1.1 μM) on LH-dependent progesterone secretion. Points are mean ± SEM of duplicate determinations. ●, LH; ○, LH + $PGF_{2\alpha}$. Statistical analyses: stimulation by 1 ng of LH, not significant; stimulation by 10 ng of LH vs. no LH, $P < 0.05$; stimulation by 100 and 1000 ng of LH vs. no LH, $P < 0.001$; inhibition by $PGF_{2\alpha}$, $P < 0.025$.

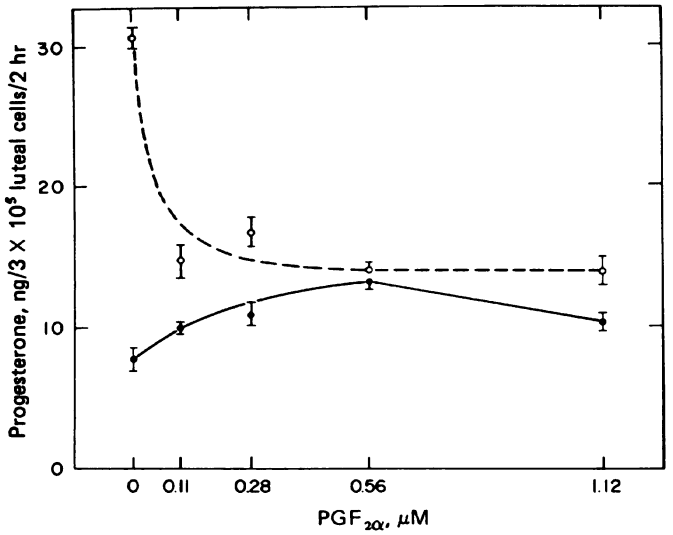

FIG. 2. Dose-response of isolated luteal cells to $PGF_{2\alpha}$ in the absence (●) and presence (○) of LH (50 ng/ml). Same experimental conditions as in Fig. 1. Statistical analysis: inhibition of LH by $PGF_{2\alpha}$, $P < 0.001$; stimulation of basal progesterone secretion by $PGF_{2\alpha}$, $P < 0.05$.

in 25-cm^2 Falcon flasks (Dickinson Co, Oxnard, CA). Each flask contained about 0.5–2 × 10^6 cells in 2 ml of medium 2. The dispersed cells were preincubated for 1 hr at 37° under 95% O_2/5% CO_2 and the medium was decanted before addition of the different hormone preparations in fresh medium 2; the cells were incubated in hormones for 1–2 hr. At the end of the last incubation, cell viability was checked again (8) and, in all studies, was found to be >90%.

Progesterone Radioimmunoassay. Progesterone production by isolated luteal cells was determined by radioimmunoassay (9). In a limited study it was found that the progesterone content of the medium reflected levels seen when tissue and medium were assayed for progesterone.

Extraction and Radioimmunoassay of cAMP. At the end of the incubation period described above, 150 μl of medium was removed for progesterone assay. Cells were lysed, and intracellular cAMP was combined with that of the medium. Contaminating nucleotides were removed by passage over neutral alumina as described in method C of Salomon *et al.* (10). Total cAMP content was determined by radioimmunoassay (RIA kit, Schwarz/Mann, Orangeburg, NY). The assay was sensitive to 0.025 pmol with minimal crossreactivity to other nucleotides (ATP, 0.0001%; AMP, 0.0001%; and cGMP, 0.01%).

Assay of Adenylate Cyclase Activity. Adenylate cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] activity was determined as described by Kuehl *et al.* (11) based on labeling of intracellular ATP by incubation of the cells with [^{14}C]adenine (40–60 mCi/mmol; New England Nuclear, Boston, MA).

Cell isolation was as described above; however, during enzymatic dispersion and preincubation, [^{14}C]adenine was added to the medium (4–10 μCi). In all studies, flasks contained 5–8 × 10^6 cells. After incubation in the various hormonal preparations, cells were lysed, intracellular contents were combined with medium, and [^{14}C]cAMP was isolated according to procedure C of Salomon *et al.* (10). Loss of [^{14}C]cAMP due to extraction was corrected based on recovery of [^{3}H]cAMP.

Binding Assays. Iodination of hCG was carried out in the presence of lactoperoxidase as described (5). Luteal cells (6 × 10^5 cells per tube) were incubated in 12 × 75 mm glass culture tubes for 1 hr at 37° in the presence of 10,000 dpm of ^{125}I-labeled hCG (specific activity, 30,000 dpm/ng). Nonspecific binding was determined by measuring binding in the presence of 200 IU of hCG. The tubes were centrifuged at 1000 × *g*, the supernatant fractions were discarded, and the pellets were washed twice with 1 ml of medium 2. Bound radioactivity was determined in the washed luteal cell pellets.

Hormones and Reagents. $PGF_{2\alpha}$ and PGE_2 were kindly supplied by John Pike, The Upjohn Co. (Kalamazoo, MI). Purified ovine LH (NIH-LH-S19) was a gift from the National Institute of Arthritis, Metabolism, and Digestive Disease; purified hCG was a gift of Martin Hichens, Merck Laboratories (West Point, PA). $(Bt)_2$ cAMP and theophylline were purchased from Sigma (St. Louis, MO).

RESULTS

Effect of LH and $PGF_{2\alpha}$ on Progesterone Secretion. Progesterone secretion by luteal cells in culture in response to various concentrations of LH are shown in Fig. 1. No increase in progesterone secretion was seen at an LH concentration of 1 ng/ml, but a significant increase was observed at 10 ng/ml. Maximal stimulation of progesterone secretion by LH (1 μg/ml) was about 5-fold greater than that seen in the absence of LH. In subsequent studies (data not shown), LH at 1 μg/ml was found to produce maximal stimulation of progesterone secretion.

Also shown in Fig. 1 is the effect of $PGF_{2\alpha}$ (1.1 μM) on progesterone secretion in the presence of increasing concentrations of LH. With LH at 10 and 100 ng/ml, $PGF_{2\alpha}$ completely inhibited the stimulation of progesterone secretion. With LH at 1 μg/ml, stimulation of progesterone secretion was not completely inhibited by $PGF_{2\alpha}$. These data indicate that increasing concentrations of LH may override the inhibition of progesterone secretion produced by $PGF_{2\alpha}$ which is consistent with data from acute *in vivo* studies (12).

The dose response effect of $PGF_{2\alpha}$ on progesterone secretion by luteal cells is shown in Fig. 2. Basal progesterone production was significantly elevated by $PGF_{2\alpha}$ in a dose-dependent manner and maximal stimulation by $PGF_{2\alpha}$ was seen at 0.56 μM. At a $PGF_{2\alpha}$ concentration of 1.1 μM, the degree of stimulation of progesterone secretion was minimal; the basis of this decrease is not clear. A toxic effect is not excluded, although

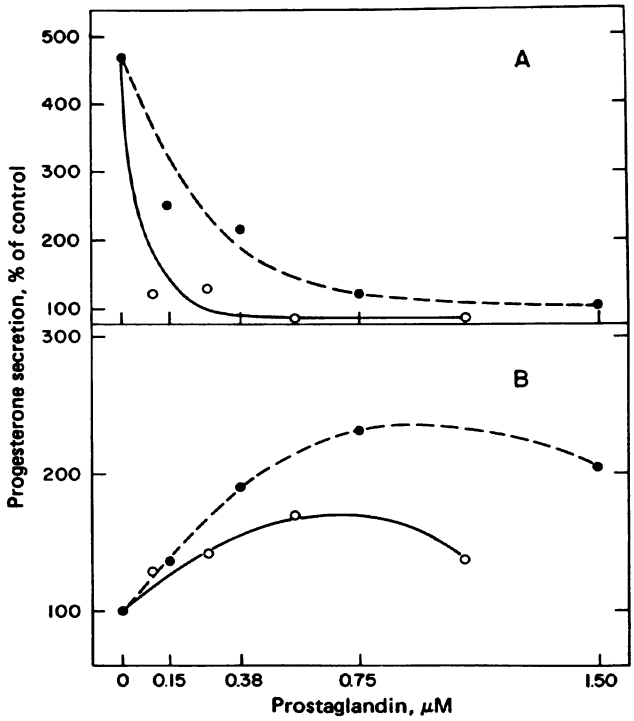

FIG. 3. (*A*) Dose-response of isolated luteal cells to $PGF_{2\alpha}$ (○) and PGE_2 (●) in the presence of LH at 50 ng/ml, the concentration that gave half-maximal stimulation of progesterone secretion. The concentrations of $PGF_{2\alpha}$ and PGE_2 producing 50% inhibition of LH action were 40 nM and 0.16 μM, respectively. (*B*) Dose-response of isolated luteal cells to $PGF_{2\alpha}$ (○) and PGE_2 (●) in absence of LH.

the trypan blue test did not show any dose-dependent effect of $PGF_{2\alpha}$ on the number of dead cells.

The effect of various concentrations of $PGF_{2\alpha}$ on progesterone secretion produced by the concentration of LH (50 ng/ml) that caused half-maximal stimulation is shown in Fig. 2. A highly significant block of LH-dependent progesterone secretion was exhibited at all concentrations of $PGF_{2\alpha}$ and was maximal at the lowest concentration (0.11 μM) tested; 50% inhibition occurred at about 40 nM $PGF_{2\alpha}$. The estimated concentration of $PGF_{2\alpha}$ necessary to block LH-dependent progesterone secretion was about one-fifth that necessary to stimulate progesterone secretion maximally (Fig. 3).

Effect of PGE_2 on Progesterone Secretion. PGE_2 has been shown (13) to increase progesterone secretion by luteal tissue, and in the present studies a marked dose-dependent effect of PGE_2 on basal progesterone secretion was demonstrated (Fig. 4). The maximal stimulation of progesterone secretion (about 2.5-fold) was exhibited at about 0.75 μM PGE_2. Similar to that seen with $PGF_{2\alpha}$, a slight decrease from the maximal stimulation of progesterone was apparent at the highest concentration of PGE_2 (1.5 μM); again, no association with cell viability was apparent from the trypan blue test.

As demonstrated with $PGF_{2\alpha}$, PGE_2 also attenuated the stimulation of progesterone secretion produced by LH, although this response was not as marked as with $PGF_{2\alpha}$. Maximal inhibition of LH action was seen at about 1.5 μM PGE_2. The concentration of PGE_2 estimated to decrease LH stimulation of progesterone secretion by 50% was 0.16 μM (Fig. 3). On the basis of these calculations, $PGF_{2\alpha}$ was about 4 times more active than PGE_2 in antagonizing the action of LH, but both were clearly antagonists of LH.

Effect of $PGF_{2\alpha}$ on ^{125}I-Labeled hCG Binding. Because binding of gonadotropin to its receptor is generally believed to

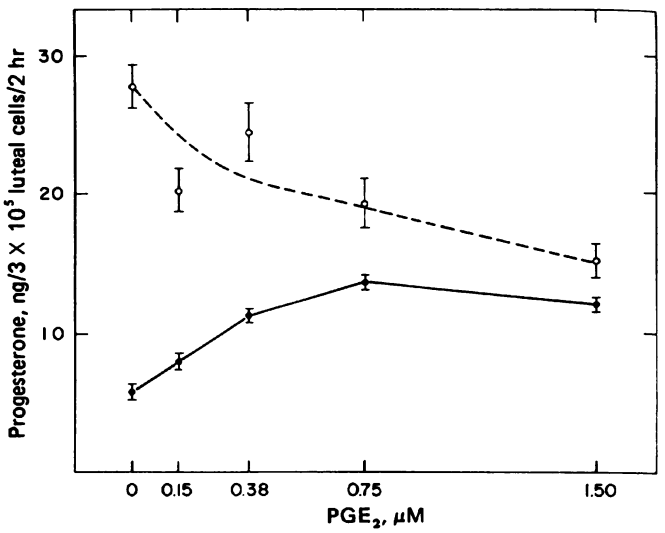

FIG. 4. Dose-response of isolated luteal cells to PGE_2 in the absence (●) and presence (○) of LH (50 ng/ml). Same experimental conditions as in Fig. 1. Statistical analysis: inhibition of LH by PGE_2, $P < 0.05$; stimulation of basal progesterone secretion by PGE_2, $P < 0.005$.

be the first step in the sequence of reactions leading to progesterone synthesis, a decrease in binding induced by $PGF_{2\alpha}$ would explain the antagonism of LH stimulation of progesterone secretion seen in the present studies. However, in binding studies, $PGF_{2\alpha}$ at 1.1 μM did not affect specific binding of ^{125}I-labeled hCG to luteal cell receptors. Specific binding of ^{125}I-labeled hCG was 21 ± 1.5% in control cells and 22.5 ± 1% in $PGF_{2\alpha}$-treated cells. In this same study, $PGF_{2\alpha}$ blocked hCG and LH stimulation of progesterone production (data not shown).

Effect of $PGF_{2\alpha}$ on $(Bt)_2$-cAMP-Dependent Progesterone Secretion. Because the action of LH is suggested to be mediated by cAMP (14), we studied the effect of exogenous $(Bt)_2$-cAMP on $PGF_{2\alpha}$ inhibition of LH-dependent progesterone secretion. $PGF_{2\alpha}$ at a concentration of 1.4 μM did not block the effect of $(Bt)_2$-cAMP on stimulation of progesterone secretion, although it completely abrogated the stimulatory effect of LH (Fig. 5). Moreover, when luteal cells were incubated with $(Bt)_2$-cAMP

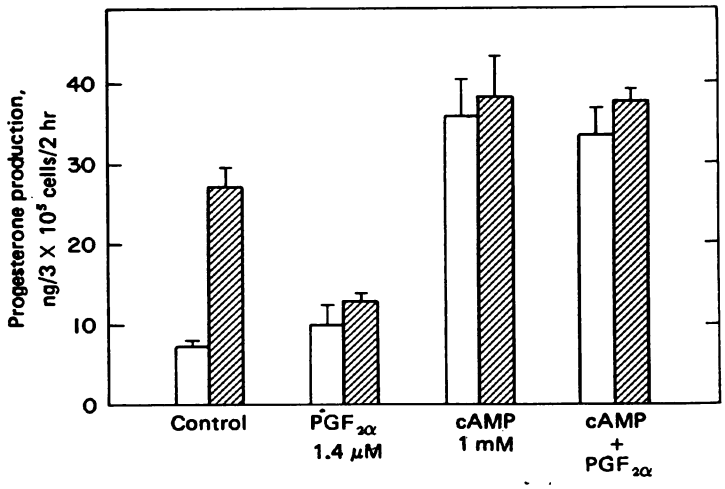

FIG. 5. Effect of addition of $(Bt)_2$-cAMP (designated cAMP) on the $PGF_{2\alpha}$-induced block of LH-dependent progesterone secretion. Each bar represents the mean ± SEM of four replicates. Open bars: no LH; hatched bars: LH (50 ng/ml). Statistical analysis: $(Bt)_2$-cAMP LH group vs. no LH, $P < 0.001$; LH group vs. LH + $PGF_{2\alpha}$, $P < 0.025$; $(Bt)_2$-cAMP group vs. no LH, $P < 0.001$; $(Bt)_2$-cAMP vs. $(Bt)_2$-cAMP + $PGF_{2\alpha}$, not significant.

Table 1. Effect of $PGF_{2\alpha}$ on theophylline-induced progesterone production

Addition	Progesterone, ng/ml: Without theophylline	With theophylline
None	3.9 ± 0.1	6.6 ± 1.2
LH	10.2 ± 1.3	9.4 ± 0.1
$PGF_{2\alpha}$	4.3 ± 0.1	5.9 ± 0.3
LH + $PGF_{2\alpha}$	4.8 ± 0.2	7.5 ± 0.5

Cells (3×10^5/flask) were incubated for 2 hr. Values are given as means (±SEM) of duplicate values. The concentrations used were: theophylline, 5 mM; $PGF_{2\alpha}$, 0.56 μM; and LH, 50 ng/ml. For theophylline-treated samples compared to control, $P < 0.025$; $PGF_{2\alpha}$ reduced progesterone secretion in presence of LH ($P < 0.01$) and in presence of LH + theophylline ($P < 0.05$).

and LH, no effect of $PGF_{2\alpha}$ was seen. From these studies it appears that the $PGF_{2\alpha}$-induced block of LH-dependent progesterone secretion may be due to inhibition of cAMP accumulation in the luteal cell.

Effect of Theophylline on the Antigonadotropic Action of $PGF_{2\alpha}$. Theophylline was added to medium 2 at concentrations of 0, 1, 2.5, 5, and 10 mM in the presence and absence of $PGF_{2\alpha}$ (0.56 μM). Under these conditions, basal progesterone secretion was increased significantly and was maximal at 1 mM theophylline. $PGF_{2\alpha}$ had no effect on the progesterone response of the cells to theophylline. In a subsequent experiment, the effect of 5 mM theophylline on progesterone secretion in the presence and absence of LH and/or $PGF_{2\alpha}$ was studied (Table 1). The level of progesterone secretion produced by LH was not significantly different in the presence or absence of theophylline. Coincubation of luteal cells with LH, $PGF_{2\alpha}$, and theophylline resulted in a level of progesterone secretion that was significantly lower than that seen with theophylline and LH. From this and the earlier study, it was concluded that $PGF_{2\alpha}$ had no effect on the stimulation of progesterone secretion seen with theophylline alone, and theophylline did not prevent inhibition of LH-dependent progesterone secretion by $PGF_{2\alpha}$. From these studies it was concluded that the antigonadotropic action of $PGF_{2\alpha}$ appears not to be due to stimulation of cAMP degradation.

Effect of LH and $PGF_{2\alpha}$ on Adenylate Cyclase Activity and Accumulation of cAMP. LH (100 ng/ml) produced a highly significant increase ($P < 0.001$) in both adenylate cyclase activity and accumulation of cAMP in cultured luteal cells (Table 2), whereas $PGF_{2\alpha}$ (1.1 μM) had little effect. However, when $PGF_{2\alpha}$ was coincubated with LH, the magnitude of LH stimulation of adenylate cyclase and cAMP accumulation was reduced by about 50% ($P < 0.05$ and $P < 0.001$, respectively). $PGF_{2\alpha}$ at this concentration in the presence of theophylline did not completely block the action of LH because a significant increase ($P < 0.001$) in adenylate cyclase and cAMP accumulation was still seen. Progesterone secretion was significantly increased ($P < 0.001$) by LH, slightly but not significantly increased by $PGF_{2\alpha}$, and significantly reduced ($P < 0.001$) when LH and $PGF_{2\alpha}$ were coincubated when compared to the level seen with LH alone. On the basis of these data, it appears that $PGF_{2\alpha}$ inhibited LH-dependent adenylate cyclase activity, resulting in a decrease in LH-dependent cAMP accumulation and a subsequent decrease in progesterone secretion.

DISCUSSION

The present studies demonstrate that dissociated rat luteal cells in culture respond in a dose-dependent manner to LH by increasing progesterone secretion. Basal progesterone secretion in the absence of LH was always observed, but it is not known if this level of progesterone secretion was independent of LH because gonadotropin, bound to the cells *in situ*, may have been carried over in culture. A preincubation of the cells in medium containing no hormone or other treatments was routinely carried out to allow viable cells to attach to the culture flask and recover from enzymatic treatment. Nonviable cells and cell fragments were always seen in the medium and were removed after preincubation; progesterone content was about 3 times as high after preincubation as that seen with subsequent incubation in identical media.

Table 2. Effect of LH and $PGF_{2\alpha}$ on adenylate cyclase activity, cAMP accumulation, and progesterone secretion in luteal cells cultured in the presence of theophylline

Addition	Adenylate cyclase activity, cpm/flask	cAMP accumulation, pmol/10^6 cells	Progesterone secretion, ng/ml
None	448 ± 35	10.7 ± 0.6	22.4 ± 0.5
LH	1497 ± 194	33.5 ± 3.3	34.2 ± 0.7
$PGF_{2\alpha}$	435 ± 59	11.1 ± 1.7	23.5 ± 0.6
LH + $PGF_{2\alpha}$	790 ± 43	21.2 ± 1.1	26.7 ± 0.7

Cells were incubated with theophylline (5 mM) for 1 hr in the presence of LH (100 ng/ml) and/or $PGF_{2\alpha}$ (1.1 μM). Values are given as the mean ± SEM. Statistical analysis: adenylate cyclase activity—control vs. LH, $P < 0.001$; control vs. LH + $PGF_{2\alpha}$, $P < 0.025$; LH vs. LH + $PGF_{2\alpha}$, $P < 0.05$; cAMP accumulation—control vs. LH, $P < 0.001$; control vs. LH + $PGF_{2\alpha}$, $P < 0.001$; LH vs. LH + $PGF_{2\alpha}$, $P < 0.001$; progesterone secretion—control vs. LH, $P < 0.001$; control vs. LH + $PGF_{2\alpha}$, $P < 0.001$; LH vs. LH + $PGF_{2\alpha}$, $P < 0.001$.

Both PGE_2 and $PGF_{2\alpha}$ stimulated progesterone secretion in a dose-dependent manner with maximal stimulation observed at about 1 μM, although PGE_2 was twice as effective as $PGF_{2\alpha}$ in this regard. A similar response of $PGF_{2\alpha}$ has been shown (12) in hypophysectomized rats under conditions in which LH was presumably absent. Kuehl *et al.* (11) reported that both PGE_2 and $PGF_{2\alpha}$ stimulated adenylate cyclase activity in mouse ovarian tissue *in vitro*. Presumably, this action of the prostaglandins was the basis for the increased progesterone secretion seen in the present experiments.

When progesterone secretion was elevated by incubation of luteal cells in medium containing LH, both PGE_2 and $PGF_{2\alpha}$ produced a dose-dependent inhibition of progesterone secretion. The concentration of prostaglandin necessary to produce inhibition of progesterone secretion in the presence of LH was considerably less than that required to stimulate basal progesterone secretion in the absence of LH. In these studies $PGF_{2\alpha}$ was about 4 times as potent an antagonist as PGE_2. *In vivo*, PGE_2 is about $1/10$ as active as $PGF_{2\alpha}$ in causing a decrease in circulating progesterone (3). The concentration of $PGF_{2\alpha}$ (40 nM) required to inhibit LH-dependent progesterone secretion in the present study is compatible with the concentration of $PGF_{2\alpha}$ ($K_d = 10^{-7}$M) necessary to occupy specific receptors in corpora lutea (15). Thus, it can be argued that inhibition of hormone action may be the actual response to prostaglandins in luteal cells rather than the mediation of LH action in mouse ovarian tissue suggested by Kuehl *et al.* (11). *In vivo*, the ovary is constantly exposed to LH and, in the absence of this gonadotropin, luteal progesterone production rapidly wanes (16). Thus, it is suggested that direct antagonism of LH action in the luteal cell may be the basis for the rapid action of prostaglandins in decreasing luteal progesterone production.

The antigonadotropic action of prostaglandin could occur at several sites: inhibition of LH binding to its receptor, inhi-

bition of adenylate cyclase activity induced by LH, or increase in cAMP degradation. The present studies show that addition of $(Bt)_2$-cAMP to the culture medium completely reversed the $PGF_{2\alpha}$-induced block of LH-dependent progesterone secretion. This observation provides evidence that the action of $PGF_{2\alpha}$ was not due to cell death *per se* and indicates that cAMP-dependent processes in steroidogenesis were not affected by $PGF_{2\alpha}$. In an earlier study (2) it was shown that $PGF_{2\alpha}$ *in vivo* caused a rapid block of gonadotropin accumulation in corpora lutea, and such an effect would explain the antigonadotropic action of $PGF_{2\alpha}$. However, the present studies demonstrate that a decrease in specific binding of gonadotropin did not occur in luteal cells incubated with $PGF_{2\alpha}$, although progesterone secretion induced by gonadotropin was blocked. Thus, it is concluded that the site of the early action of prostaglandin in blocking LH response was not due to an effect on LH binding to its receptor.

The reversal by exogenous $(Bt)_2$-cAMP of the $PGF_{2\alpha}$-induced block of LH-dependent progesterone secretion lends support to the conclusion that $PGF_{2\alpha}$ may inhibit intracellular cAMP accumulation produced by LH. This observation is consistent with the report of Lahav *et al.* (17) showing that $PGF_{2\alpha}$ blocked accumulation of cAMP in slices of rat ovarian tissue incubated with LH, but no data were shown that $PGF_{2\alpha}$ blocked progesterone production or was active in the presence of a phosphodiesterase inhibitor. The block by $PGF_{2\alpha}$ of LH-dependent progesterone secretion may possibly occur by a decrease in adenylate cyclase activity or an increase in phosphodiesterase activity because either response would lead to a decrease in intracellular levels of cAMP. The present experiments show that $PGF_{2\alpha}$ did not block the ability of theophylline to increase progesterone production, and theophylline at supermaximal concentrations did not prevent expression of the antigonadotropic action of $PGF_{2\alpha}$, although the magnitude of the inhibition by $PGF_{2\alpha}$ was attenuated in the presence of theophylline, possibly due to a higher basal level of cAMP.

Direct assay of the effect of $PGF_{2\alpha}$ on LH-dependent adenylate cyclase activity and cAMP accumulation in cultured luteal cells showed that $PGF_{2\alpha}$ inhibited this LH-dependent response in the presence of theophylline. These data, in addition to almost identical effects on progesterone secretion, point to this action of $PGF_{2\alpha}$ as the probably site of the rapid antigonadotropic action of prostaglandin. Failure of $PGF_{2\alpha}$ to completely block the action of LH in the presence of theophylline may have been due to a shift in the dose-response curve of $PGF_{2\alpha}$ due to theophylline treatment or to other recognized actions of theophylline such as changes in intracellular calcium distribution. Nonetheless, the highly significant and reproducible inhibition by $PGF_{2\alpha}$ of the LH-induced increase in adenylate cyclase activity and cAMP accumulation lend credence to the conclusion that this may be the site of the early action of $PGF_{2\alpha}$ in blocking LH-dependent progesterone secretion.

The present data are consistent with earlier reports showing a similar response of $PGF_{2\alpha}$ in cultured explants of hamster corpora lutea (3) and in cultures of porcine, bovine, and human luteinized granulosa cells exposed to $PGF_{2\alpha}$ for several hours (18). Grinwich *et al.* (19) have shown that $PGF_{2\alpha}$ *in vivo* blocked LH-dependent progesterone synthesis and cAMP accumulation in luteal tissue several hours after $PGF_{2\alpha}$ treatment but, during this interval, a marked loss of LH receptors occurred. However, progesterone secretion is reduced within 30 min of exposure to $PGF_{2\alpha}$ and remains low (2, 6). From the present data it is concluded that the rapid effect of $PGF_{2\alpha}$ on progesterone secretion, which occurs in minutes, is due to inhibition of LH-dependent cAMP accumulation and not to inhibition of LH binding to its receptors. Later effects of $PGF_{2\alpha}$ may be due to loss of LH receptors.

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