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Effects of Prostaglandin $F_{2\alpha}$ on Agonist-Induced Progesterone Production in Cultured Bovine Luteal Cells

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ABSTRACT

The present study examines the effects of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on basal and agonist-stimulated progesterone (P_4) production utilizing long-term, serum-free cultures of bovine luteal cells. During the first 24 h of culture, $PGF_{2\alpha}$ had no significant effect on P_4 production, and was unable to inhibit either luteinizing hormone (LH)- or dibutyryl cAMP (dbcAMP)-stimulated increases in P_4. Treatment with $PGF_{2\alpha}$ on Day 1 produced a moderate, nonsignificant ($P>0.05$) inhibition of cholera toxin (CT)- and forskolin (FKN)-stimulated P_4 synthesis. Beyond Day 1 of culture (Days 3–11), $PGF_{2\alpha}$ continued to have no significant effect on basal P_4 production, but suppressed all stimulatory effects of LH, dbcAMP, CT and FKN. Treatment with indomethacin inhibited prostaglandin synthesis by the cultured cells and also elevated levels of P_4 from Days 3 to 11 of culture. Concurrent treatment with $PGF_{2\alpha}$ suppressed the steroidogenic effect of indomethacin.

From these studies it was concluded that in cultured bovine luteal cells, $PGF_{2\alpha}$ does not affect basal P_4 production, but is able to inhibit agonist-stimulated P_4 production at a site beyond the accumulation of cAMP. This inhibitory effect is not apparent during the first 24 h of culture, but appears after Day 1 and persists throughout the remaining 10 days of the culture period.

INTRODUCTION

While prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is luteolytic in many species, the manner in which it brings about regression of the corpus luteum (CL) is unknown. Although $PGF_{2\alpha}$ consistently induces luteal regression in cows (Inskeep, 1973), progesterone (P_4) is increased above control levels by $PGF_{2\alpha}$ in 1- to 4-h incubations of bovine luteal tissue slices (Speroff and Ramwell, 1970; Hansel et al., 1973) or dispersed luteal cells (Hixon and Hansel, 1979). In contrast, O'Grady et al. (1972) and Hall and Robinson (1979) reported that $PGF_{2\alpha}$ inhibited basal P_4 production by incubated luteal tissue from the rabbit and rat, respectively. Other workers found that $PGF_{2\alpha}$ had no effect on basal levels of P_4, but was able to suppress luteinizing hormone (LH)-stimulated P_4 in tissue slices or dispersed luteal cells from the rat and the ewe (Evrard et al., 1978; Wright et al., 1980; Fletcher and Niswender, 1982). However, these in vitro incubations have been short term (acute) in nature, and might not reflect the chronic effects of $PGF_{2\alpha}$ on the CL.

Thomas et al. (1978) demonstrated that while $PGF_{2\alpha}$ inhibited LH-stimulated steroidogenesis in cultured rat luteal cells, the addition of dibutyryl cAMP (dbcAMP) was able to overcome the effects of the prostaglandin. These authors concluded that the mechanism of action of $PGF_{2\alpha}$ was to block the LH-dependent formation of cAMP. In contrast, Jordan (1981) showed that luteal cells from $PGF_{2\alpha}$-treated rats were less responsive to both LH and dbcAMP in vitro, and concluded that the major site of actin of $PGF_{2\alpha}$ was at a point distal to the accumulation of cAMP.

Cholera toxin (CT) and forskolin (FKN) are both agents which activate adenylate cyclase. CT stimulates the enzyme through interaction with the guanine nucleotide regulatory subunit (Cassel and Pfeuffer, 1978; Gill and Meren, 1978). FKN may stimulate the catalytic subunit directly (Seamon and Daly, 1981), or may require an additional component for cyclase activation (Stengel et al., 1982; Brooker et al., 1983). Both compounds are used in the present study to increase P_4 production without involving the LH receptor.

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Due to paradoxical effects of $PGF_{2\alpha}$ in vivo and in vitro, and the variability in the response to the prostaglandin in vitro, the mechanism of action of $PGF_{2\alpha}$ has thus far remained elusive. In the present study, the effects of $PGF_{2\alpha}$ on basal P_4 production, as well as on steroidogenesis stimulated by LH, CT, FKN, dbcAMP and indomethacin were investigated. Serum-free cultures of bovine luteal cells were employed to examine the long-term, direct effects of $PGF_{2\alpha}$ in a controlled environment.

MATERIALS AND METHODS

Hormones and Materials

The sources of treatments were: $PGF_{2\alpha}$-Tris salt and $N^6,O^{2'}$-dibutyryladensine 3′:5′-cyclic monophosphate (dbcAMP), Sigma (St. Louis, MO); cholera toxin (CT) and forskolin (FKN), Calbiochem (San Diego, CA); indomethacin, Merck, Sharp & Dohme (Rahway, NJ); and LH, NIAMDD-bLH-4. Ham's F12 culture medium and antibiotics were obtained from Grand Island Biological Co. (Grand Island, NY). Insulin, transferrin and selenium were obtained from Collaborative Research (Lexington, MA). The collagenase used was Worthington, Type 1, (Freehold, NJ) and the N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (Hepes) was purchased from Calbiochem. Cells were cultured in 25-cm^2 polystyrene tissue culture flasks, Corning Glass Works (Corning, NY).

Experimental Procedures

Corpora lutea were removed per vaginum from regularly cycling., nonlactating dairy cows of various breeds on Days 8–12 of the estrous cycle and placed into Ham's F12 culture medium containing 24 mM Hepes (pH 7.35) at 4°C for transport to the laboratory. Luteal tissue was dissociated with collagenase (2000 U/g tissue) as previously described (Pate and Condon, 1982).

Tissue culture flasks were pretreated for 1 h at 37°C with Ham's F12 containing 10% calf serum to promote attachment of the cells. The serum-containing medium then was removed and the flasks were rinsed with a double volume of Ham's F12 without serum. The dissociated luteal cells then were added to these flasks (1×10^6 cells/flask) in a final volume of 4 ml of Ham's F12-Hepes culture medium containing 100 U/ml penicillin, 100 μg/ml streptomycin and 2.5 μg/ml fungizone. The medium was supplemented with 5 μg/ml insulin, 5 μg/ml transferrin, and 5 ng/ml selenium.

Cultures were incubated at 37°C. Spent culture medium was collected and replaced with fresh medium after 24 h (Day 1) and subsequently changed every 48 h for a total of 11 days. Culture medium was frozen and stored at −20°C until the time of assay. All treatments were added at the beginning of the culture period and replaced with each medium change, at the concentrations indicated in the figure legends. Each treatment was performed in duplicate for each CL. Cells were quantitated throughout the culture period using an ocular micrometer square. Each flask was counted in 5 locations, a minimum of 3 times during the culture period. Steroidogenic activity was then normalized per 5×10^5 cells attached to the culture flask.

Progesterone in the spent culture medium was quantitated by radioimmunoassay of unextracted samples with antiprogesterone-11-bovine serum albumin (BSA) serum (GDN-337) donated by Dr. Gordon Niswender. This antiserum does not cross-react significantly with any abundant steroid in our samples (Gibori et al., 1977). [1,2-^{3}H]Progesterone was obtained from New England Nuclear (Boston, MA). The intraassay coefficient of variation was 9.2%, the interassay coefficient of variation was 14.7%. The limit of sensitivity was 0.1 ng/ml. All standards and samples were assayed in duplicate. Quantitation of 6-keto-$PGF_{1\alpha}$, the stable metabolite of prostacyclin (PGI_2), by radioimmunoassay was performed by the method of Silvia et al. (1984). The intraassay coefficient of variation was 11.7%, and the interassay coefficient of variation was 16.5%. Radioimmunoassay of $PGF_{2\alpha}$ was by the method of Pexton et al. (1975) as modified by Ottobre et al. (1984).

Statistical Analysis

Differences among treatments were determined by analysis of variance and comparisons made using the Student-Newman-Keuls multiple range test.

RESULTS

When luteal cells were cultured in the presence of either LH (10 ng/ml), CT (1 nM) or FKN (1 μM) for 24 h, P_4 production increased ($P<0.05$) above control levels (Fig. 1, Day 1 of culture). Prostaglandin $F_{2\alpha}$, at a level of 10 ng/ml, had no significant effect on P_4 levels. During this first 24-h period, $PGF_{2\alpha}$ did not influence the LH-stimulated increases in P_4. Values for CT or FKN in combination with $PGF_{2\alpha}$ did not differ significantly from either the agonist-stimulated values or control values. However, when the luteal cells were exposed to these treatments for an additional 48 h (to Day 3 of culture), the effect of $PGF_{2\alpha}$ was quite different. Neither LH nor $PGF_{2\alpha}$ had any effect on P_4 on Day 3, while CT and FKN greatly increased P_4 production (Fig. 2). In contrast to Day 1, when $PGF_{2\alpha}$ was combined with either CT or FKN for another 48 h, the stimulatory effects of CT and FKN were completely suppressed. This inhibitory action of $PGF_{2\alpha}$ remained throughout the rest of the culture period. Figure 3 depicts P_4 production on Day 9 of culture. As on Day 1, LH, CT and FKN elevated P_4 above control levels, and although $PGF_{2\alpha}$ had no effect on basal steroidogenesis, it was able to completely inhibit the stimulatory effects of LH, CT or FKN.

To further examine the possible mechanism of action of $PGF_{2\alpha}$, the effect of prostaglandin

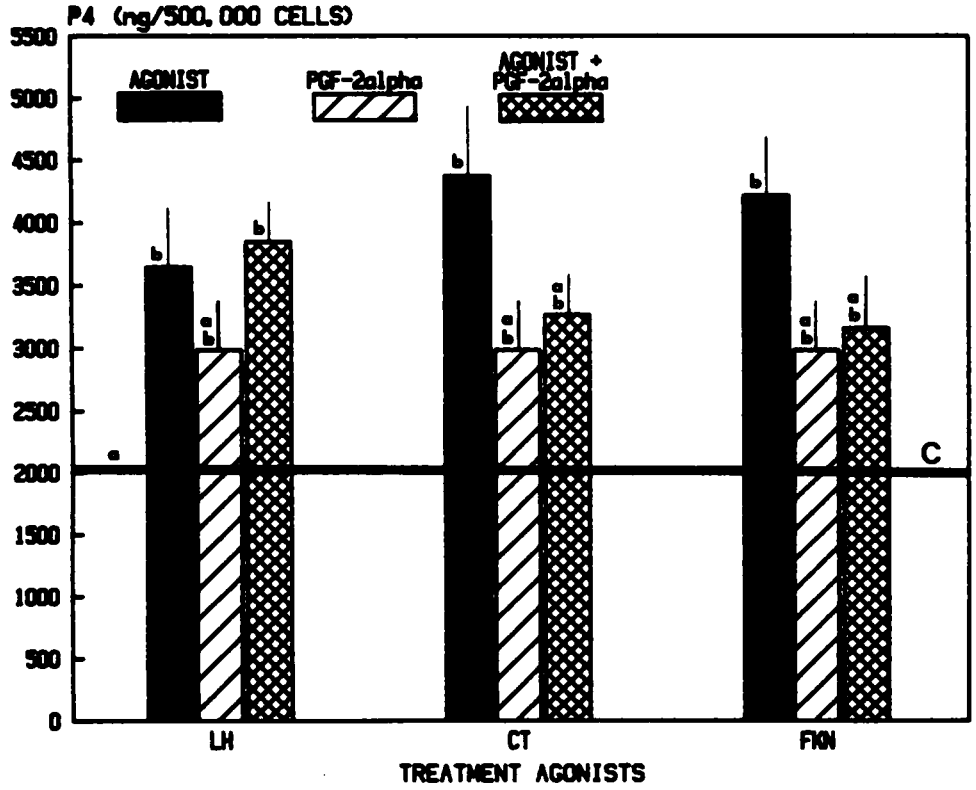

FIG. 1. Progesterone (P_4) production by luteal cells on Day 1 of culture. Cells were treated with either LH (10 ng/ml), cholera toxin (*CT*, 1 nM) or forskolin (*FKN*, 1 μM), alone and in combination with $PGF_{2\alpha}$ (10 ng/ml). *C*=Control (*solid line*). Bars within a group with different *superscript letters* are significantly different ($P<0.05$, LH and CT; $P<0.01$, FKN). Mean ± SEM, n=5.

on dbcAMP-stimulated P_4 was evaluated. The results of this experiment are depicted in Figs. 4 and 5, which represent Days 1 and 9 of culture, respectively. Dibutyryl cAMP (100 μM) increased P_4 on both Day 1 and Day 9, while $PGF_{2\alpha}$ did not alter basal steroidogenesis. On Day 1, $PGF_{2\alpha}$ had no effect on dbcAMP-stimulated P_4, but on all days after Day 1 (data shown for Day 9 only), the increase in P_4 evoked by dbcAMP was abolished by the simultaneous presence of $PGF_{2\alpha}$.

Since luteal cells are capable of prostaglandin synthesis, the effects of indomethacin (an inhibitor of prostaglandin synthesis) both alone and combined with $PGF_{2\alpha}$ were examined. The synthesis of 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ by cultured luteal cells on Days 1, 5 and 11 is shown in Table 1. On all 3 days LH was without effect on 6-keto-$PGF_{1\alpha}$ production. The addition of indomethacin, either alone or in combination with LH, completely inhibited synthesis of 6-keto-$PGF_{1\alpha}$ on Days 1 and 5. A similar trend was seen on Day 11, however the levels of 6-keto-$PGF_{1\alpha}$ were much lower and these differences were not significant. Indomethacin also inhibited synthesis of $PGF_{2\alpha}$ by the cultured cells.

While indomethacin blocks luteal prostaglandin synthesis, it was also capable of promoting luteal progesterone formation. Unlike the other steroidogenic stimulants examined thus far, indomethacin did not produce a significant increase in P_4 on Day 1 of culture (Fig. 6). Therefore, it was not possible to evaluate an inhibitory effect of $PGF_{2\alpha}$ in the presence of indomethacin on Day 1. However, upon further exposure to indomethacin, the cultured cells responded with large increases in P_4 synthesis. As shown for Day 3 and Day 9 of culture (Figs. 7 and 8, respectively), P_4 was

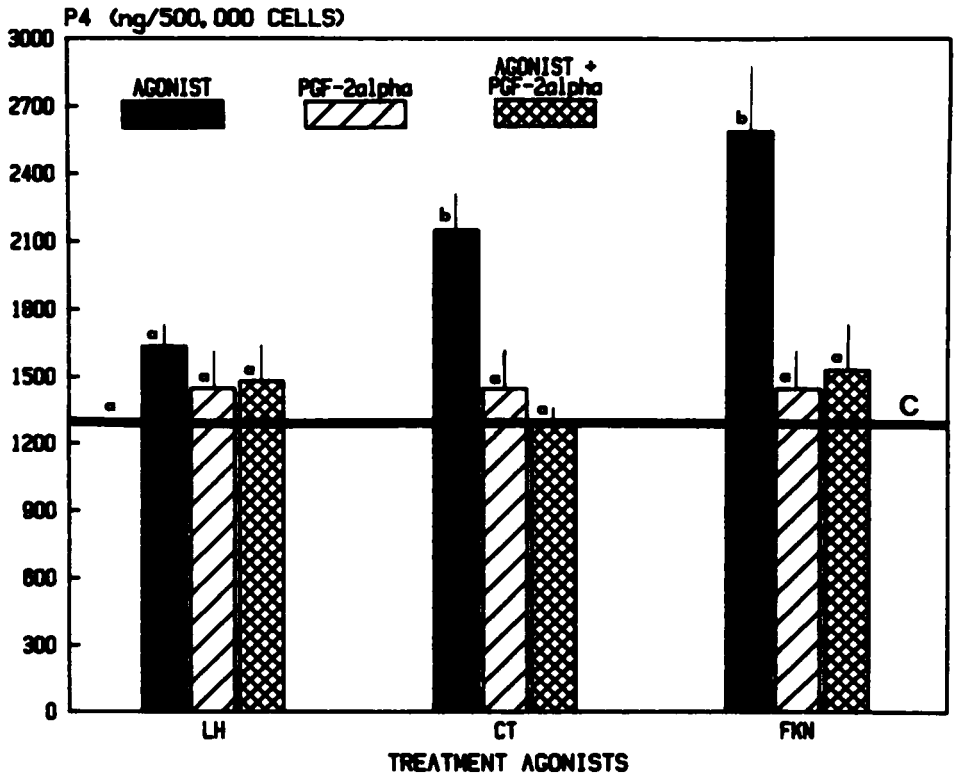

FIG 2. P_4 production on Day 3 of culture. Treatments were as described in Fig. 1. Bars within a group with different *superscript letters* are significantly different ($P<0.01$, FKN; $P<0.001$, CT). Mean ± SEM, n=5.

elevated in the presence of indomethacin. However, simultaneous treatment with $PGF_{2\alpha}$ inhibited this steroidogenic effect of indomethacin.

DISCUSSION

The aim of the present study was to examine the long-term effects of $PGF_{2\alpha}$ on P_4 production by bovine luteal cells. This was accomplished utilizing a previously established serum-free culture system that maintained the luteal cells in a hormone-responsive state for at least 11 days (Pate and Condon, 1982). In that system, freshly dissociated luteal cells responded to LH during the first 24 h of culture with an increase in P_4. This response then was lost during the period from Days 1 to 3, but the responsiveness to LH returned as the culture progressed. The cells in the present experiment also were not responsive to LH on Day 3, but the interaction between LH and $PGF_{2\alpha}$ could easily be studied on Day 1 and anytime after Day 3 of culture.

Thomas et al. (1978) reported that $PGF_{2\alpha}$ stimulated basal P_4 synthesis in a 2-h culture of rat luteal cells, but completely inhibited LH-stimulated steroidogenesis. This suggests that simultaneous stimulation by another hormone may influence $PGF_{2\alpha}$ action. In the present study, $PGF_{2\alpha}$ inhibited any LH-stimulated P_4 after Day 1, although it had no effect on basal steroidogenesis. However, $PGF_{2\alpha}$ was not able to overcome the LH stimulation during the first 24 h of culture. Similarly, Fletcher and Niswender (1982) reported that $PGF_{2\alpha}$ could inhibit LH-stimulated P_4 accumulation in ovine luteal tissue slices only after a 60-min preincubation with the prostaglandin. The difference between the 60-min preincubation necessary for $PGF_{2\alpha}$ action and the time lag in the present study could be due to different responses of the cells in a tissue slice incubation compared to cultured cells. Also, Henderson and McNatty (1977), using cultures of bovine granulosa cells, reported that $PGF_{2\alpha}$ had an inhibitory effect if added at

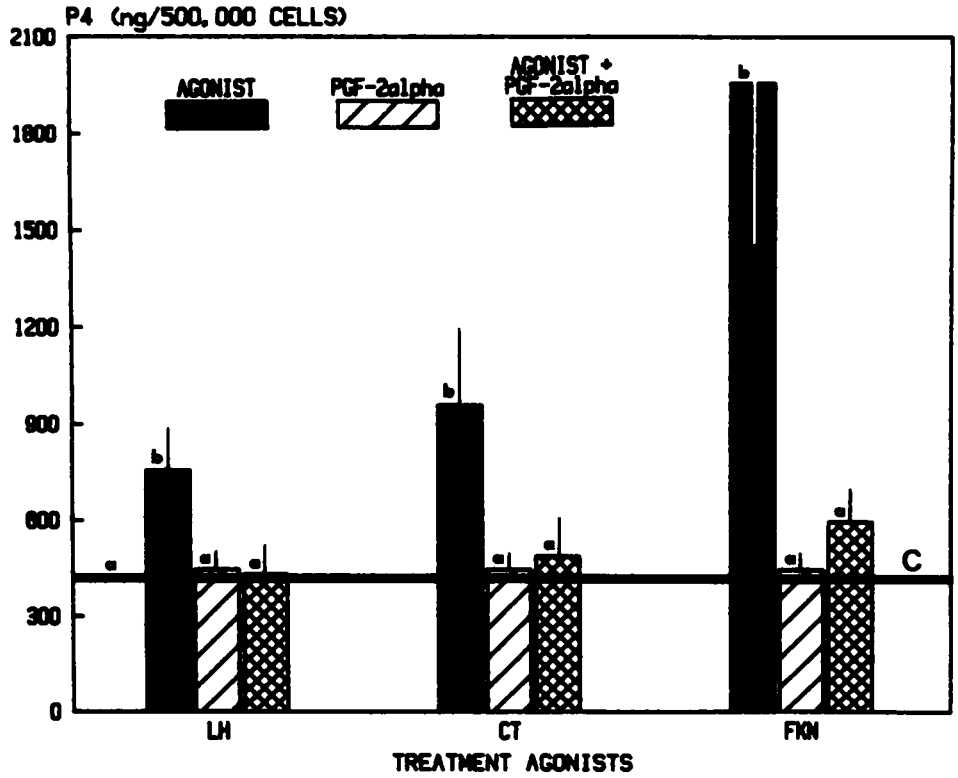

FIG. 3. P_4 production on Day 9 of culture. Treatments were as described in Fig. 1. Bars within a group with different *superscript letters* are significantly different ($P<0.05$, LH, CT; $P<0.01$, FKN). Mean ± SEM, n=5.

the start of the culture, but not if added later in the culture period. Cultured granulosa cells initially produce very low levels of P_4, and these levels increase as the culture progresses and the cells undergo "luteinization." These authors suggested that $PGF_{2\alpha}$ uptake and subsequent luteolytic action were inversely related to P_4 production. This would agree with the results reported here, since P_4 production was much greater on Day 1 in luteal cell cultures, and $PGF_{2\alpha}$ did not inhibit LH-stimulated steroidogenesis at this time. Unfortunately, Henderson and McNatty used 20% calf serum in their granulosa cell cultures and could get no LH response, so it is not possible to distinguish between $PGF_{2\alpha}$ inhibition of basal or LH-stimulated P_4 in their system.

The manner in which $PGF_{2\alpha}$ inhibits LH-stimulated steroidogenesis remains controversial. It is probably not due to a loss of LH receptors, since $PGF_{2\alpha}$ does not reduce gonadotropin uptake in the rat CL in vitro (Behrman et al., 1978; Pang and Behrman, 1981). To determine whether the $PGF_{2\alpha}$ inhibition occurs prior to the activation of adenylate cyclase, CT and FKN were used as stimulatory agents. Incubation of rat luteal cells with CT produces a dose-dependent increase in P_4 production (Azhar and Menon, 1981). FKN potentiates ACTH-induced steroidogenesis in adrenal cells (Moriwaki et al., 1982) and stimulates cAMP accumulation in thyroid membranes (Fradkin et al., 1982). Both CT- and FKN-stimulated steroidogenesis were inhibited by $PGF_{2\alpha}$ in the present study, indicating that it is not possible to overcome $PGF_{2\alpha}$ action by merely bypassing the LH receptor.

If $PGF_{2\alpha}$ was acting solely on the adenylate cyclase system to inhibit cAMP formation, the addition of dbcAMP would be expected to overcome the prostaglandin inhibition. In the present study, dbcAMP did not increase P_4 in the presence of $PGF_{2\alpha}$ after Day 1, suggesting that $PGF_{2\alpha}$ can inhibit steroidogenesis at a point distal to the accumulation of cellular cAMP.

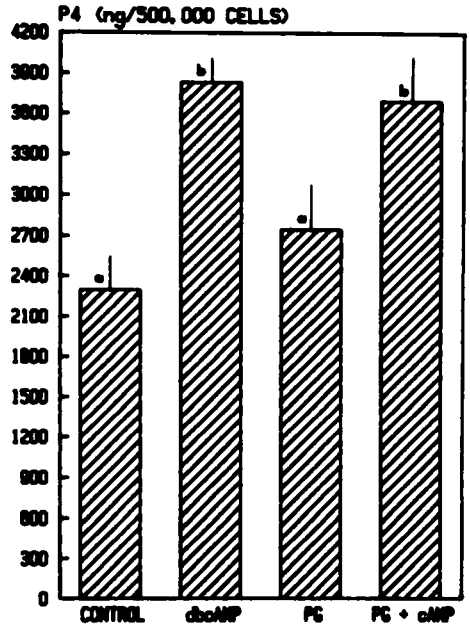

FIG. 4. P_4 production by luteal cells on Day 1 of culture. Cells were treated with dbcAMP (100 μM), alone and in combination with $PGF_{2\alpha}$ (*PG*) (10 ng/ml). Bars with different *superscript letters* are significantly different ($P<0.05$). Mean ± SEM, n=4.

These results support the work of Jordan (1981), but disagree with those of Thomas et al. (1978), and Dorflinger and Behrman (1979), who reported that dbcAMP could reverse the inhibitory effects of $PGF_{2\alpha}$ in rat luteal cells. These differences could be related to the duration of exposure to the prostaglandin. It is possible that chronic exposure to $PGF_{2\alpha}$ is required for the post-cAMP inhibition to occur. Also, $PGF_{2\alpha}$ might have multiple sites of action in luteal cells, thus insuring complete luteolysis.

The exact mechanism by which $PGF_{2\alpha}$ can suppress steroidogenesis at a post-cAMP site is not known. Khan and Rosberg (1979) have stated that LH-stimulated adenylate cyclase is inhibited only when $PGF_{2\alpha}$ is incubated with whole luteal cells, not with isolated membranes. This might suggest that $PGF_{2\alpha}$ can initiate (or suppress) an intracellular event that would subsequently influence enzyme activity in the plasma membrane.

There are many ways in which the P_4 biosynthetic pathway might be altered by $PGF_{2\alpha}$. The prostaglandin can affect the activity of enzymes such as 20α-hydroxysteroid dehydroge-

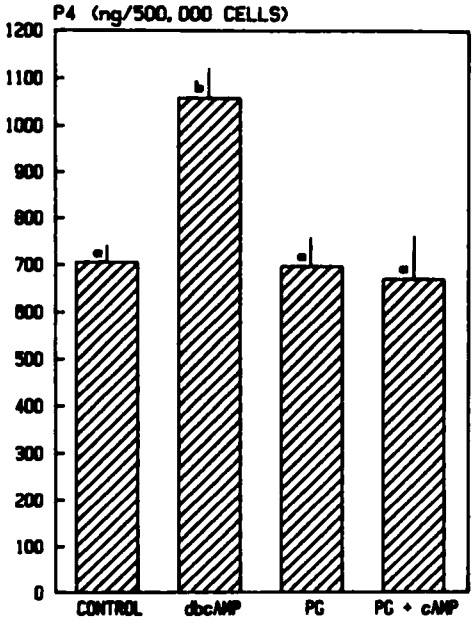

FIG. 5. P_4 production on Day 9 of culture. Treatments were as described in Fig. 4. Bars with different *superscript letters* are significantly different ($P<0.01$). Mean ± SEM, n=4.

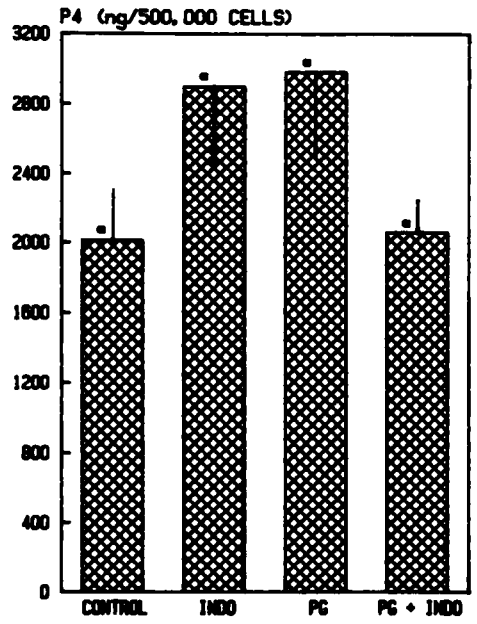

FIG. 6. P_4 production by luteal cells on Day 1 of culture. Treated cultures contained either indomethacin (*INDO*, 10 μg/ml), $PGF_{2\alpha}$ (*PG*, 10 ng/ml) or *INDO* + $PGF_{2\alpha}$. Mean ± SEM, n=5.

TABLE 1. Prostaglandin production by cultured bovine luteal cells.

Prostaglandin measured	Day of culture	Treatment			
		Control	LH	INDO[a]	INDO + LH
6-Keto-$PGF_{1\alpha}$[b]	1	36.17 ± 6.96[d]	52.38 ± 11.44[d]	2.25 ± 0.61[e]	2.44 ± 0.57[e]
	5	2.53 ± 0.45[d]	3.09 ± 0.33[d]	0.41 ± 0.21[e]	0.49 ± 0.19[e]
	11	0.61 ± 0.40	1.16 ± 0.41	0.15 ± 0.06	0.42 ± 0.15
$PGF_{2\alpha}$[c]	1	8031 ± 864	9157 ± 938	24 ± 0	24 ± 0
	5	3375 ± 920	3474 ± 1292	24 ± 0	24 ± 0
	11	2862 ± 744	4399 ± 1357	24 ± 0	24 ± 0

[a] *INDO*=indomethacin.

[b] ng/500,000 cells; mean ± SEM; n=3.

[c] pg/500,000 cells; mean ± SD; n=1.

[d,e] Means within a row with different superscript letters are statistically different (P<0.01).

nase (Jones and Hsueh, 1981), 3β-hydroxysteroid dehydrogenase (Dwyer and Church, 1981), cholesterol esterase and cholesterol synthetase (Behrman et al., 1971). Since $PGF_{2\alpha}$ had no effect on basal steroidogenesis in the present study, the post-cAMP site(s) of action is likely to be one which is stimulated in the presence of LH. One such site might be at the rate-limiting step of steroidogenesis, cholesterol side-chain cleavage, which is greatly augmented in the LH-stimulated cell. Perhaps $PGF_{2\alpha}$ can block the gonadotropin-induced increase in this reaction or related events. Endogenously synthesized prostaglandins might also be involved in the luteolytic process. As shown in Table 1, bovine luteal cells in culture are capable of syn-

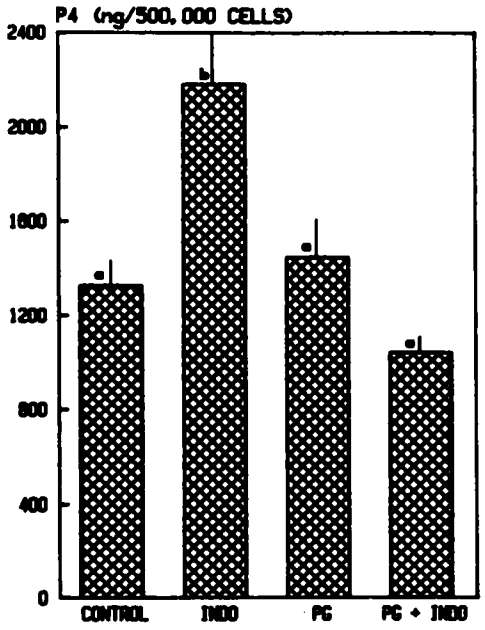

FIG. 7. P_4 production on Day 3 of culture. Treatments were as described in Fig. 6. Bars with different *superscript letters* are significantly different (P<0.01). Mean ± SEM, n=5.

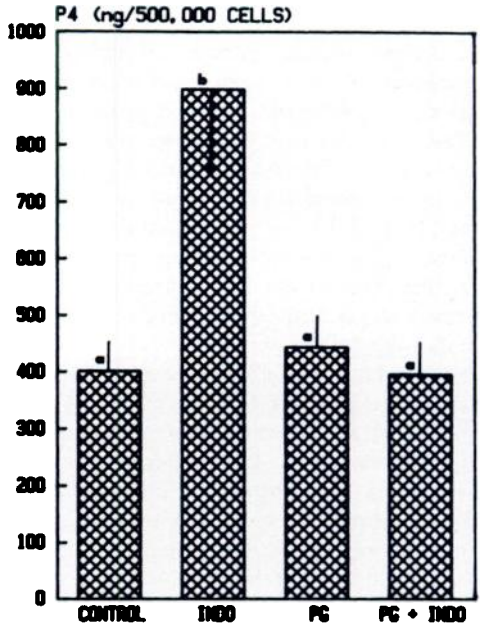

FIG. 8. P_4 production on Day 9 of culture. Treatments were as described in Fig. 6. Bars with different *superscript letters* are significantly different (P<0.01). Mean ± SEM, n=5.

thesizing 6-keto-$PGF_{1\alpha}$, the stable metabolite of prostacyclin. The slightly higher levels of 6-keto-$PGF_{1\alpha}$ observed in LH-treated versus control cultures is not surprising since Demers et al. (1973), and Shemesh and Hansel (1975) demonstrated an LH-stimulated rise in PGF synthesis by rat and bovine corpora lutea, respectively. Furthermore, rat granulosa cells in vitro synthesize 6-keto-$PGF_{1\alpha}$ and LH increases this synthesis (Koos and Clark, 1982; Veldhuis et al., 1982).

Prostaglandin levels decreased over the culture period (Table 1), similar to the decline in P_4 which is observed in cultured luteal cells (Pate and Condon, 1982). It is not known if luteal prostaglandin synthesis can influence exogenous $PGF_{2\alpha}$ action, but it is possible that the higher levels of endogenous prostaglandins early in the culture could have suppressed, or masked, the effect of the added $PGF_{2\alpha}$. The declining production of prostaglandins during the culture period would then allow $PGF_{2\alpha}$ to exert its inhibitory effects. A possible relationship between luteal prostaglandin production and $PGF_{2\alpha}$ action requires further investigation.

Evrard et al. (1978) showed that LH-stimulated P_4 synthesis was elevated in rat and ewe corpora lutea incubated in vitro in the presence of indomethacin. In the present study, treatment of bovine luteal cell cultures with indomethacin resulted in an increase in P_4 production above control levels. Since basal P_4 production declines during culture, the effect of indomethacin may have been to maintain steroidogenesis at a level more nearly approaching that of Day 1. In any case, P_4 production is greater in cultures treated with indomethacin. The high levels of indomethacin employed in this experiment (10 μg/ml) may have promoted steroidogenesis through a mechanism independent of its inhibitory effects on prostaglandin synthesis. However, regardless of the mechanism of P_4 stimulation, $PGF_{2\alpha}$ was able to overcome this increase even when endogenous prostaglandin synthesis was blocked. Therefore, $PGF_{2\alpha}$ probably did not inhibit the indomethacin-induced rise in P_4 through a stimulation of prostaglandin synthesis. The influence of indomethacin on $PGF_{2\alpha}$-inhibition of steroidogenesis in response to other agonists is currently being examined.

In conclusion, we have shown that $PGF_{2\alpha}$ had no effect on basal P_4 production by cultured bovine luteal cells, but inhibited steroidogenesis stimulated by either LH, CT, FKN, dbcAMP or indomethacin. This inhibitory effect was not apparent during the first 24 h in culture, but appeared after Day 1 and persisted throughout the remainder of the culture period.

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REFERENCES

Azhar, S. and Menon, K.M.J. (1981). Receptor-mediated gonadotropin action in the ovary. Rat luteal cells preferentially utilize and are acutely dependent upon the plasma lipoprotein-supplied sterols in gonadotropin-stimulated steroid production. J. Biol. Chem. 256:6548–6555.

Behrman, H. R., MacDonald, G. J. and Greep, R. O. (1971). Regulation of ovarian cholesterol esters: Evidence for enzymatic sites of prostaglandin-induced loss of corpus luteum function. Lipids 6:791–796.

Behrman, H. R., Grinwich, D. L., Hichens, M. and MacDonald, G. J. (1978). Effect of hypophysectomy, prolactin, and prostaglandin $F_{2\alpha}$ on gonadotropin binding in vivo and in vitro in the corpus luteum. Endocrinology 103:349–357.

Brooker, G., Pedone, C. and Barovsky, K. (1983). Selective reduction of forskolin-stimulated cAMP accumulation by inhibitors of protein synthesis. Science 220:1169–1170.

Cassel, D. and Pfeuffer, T. (1978). Mechanism of cholera toxin action. Covalent modification of the guanyl nucleotide-binding protein of the adenylate cyclase system. Proc. Natl. Acad. Sci. 75:2669–2673.

Demers, L. M., Behrman, H. R. and Greep, R. O. (1973). Effects of prostaglandins and gonadotrophins on luteal prostaglandin and steroid biosynthesis. Adv. Biosci. 9:701–707.

Dorflinger, L. J. and Behrman, H. R. (1979). Effects of cholera toxin, LH and $PGF_{2\alpha}$ on steroidogenesis in luteal cells. In: Ovarian Follicular and Corpus Luteum Function (C. P. Channing, J. M. Marsh and W. A. Sadler, eds.). Plenum Publ., New York, pp. 603–611.

Dwyer, R. J. and Church, R. B. (1979). Effect of prostaglandin $F_{2\alpha}$ on ovarian enzyme activity in the hysterectomized guinea pig. J. Reprod. Fertil. 56:85–88.

Evrard, M., Leboulleux, P. and Hermier, C. (1978). Role of prostaglandin $F_{2\alpha}$ in modulation of LH-stimulated steroidogenesis in vitro in different types of rat and ewe corpora lutea. Prostaglandins 16:491–502.

Fletcher, P. W. and Niswender, G. D. (1982). Effect of $PGF_{2\alpha}$ on progesterone secretion and adenylate cyclase activity in ovine luteal tissue. Prostaglan-

dins 23:803–818.

Fradkin, J. E., Hope Cook, G., Kilhoffer, M. C. and Wolff, J. (1982). Forskolin stimulation of thyroid adenylate cyclase and cyclic 3′,5′-adenosine monophosphate accumulation. Endocrinology 111:849–856.

Gibori, G., Antczak, E. and Rothchild, I. (1977). The role of estrogen in the regulation of luteal progesterone secretion in the rat after day 12 of pregnancy. Endocrinology 100:1483–1495.

Gill, D. M. and Meren, R. (1978). ADP-ribosylation of membrane proteins catalyzed by cholera toxin: basis of the activation of the adenylate cyclase. Proc. Natl. Acad. Sci. 75:3050–3054.

Hall, A. K. and Robinson, J. (1979). Functional luteolysis in the pseudopregnant rat: Effects of prostaglandin $F_{2\alpha}$ and 16-aryloxy prostaglandin $F_{2\alpha}$ in vitro. J. Endocrinol. 81:157–165.

Hansel, W., Concannon, P. W. and Lukaszewska, J. H. (1973). Corpora lutea of the large domestic animals. Biol. Reprod. 8:222–245.

Henderson, K. M. and McNatty, K. P. (1977). A possible interrelationship between gonadotrophin stimulation and prostaglandin $F_{2\alpha}$ inhibition of steroidogenesis by granulosa-luteal cells in vitro. J. Endocrinol. 73:71–78.

Hixon, J. E. and Hansel, W. (1979). Effects of prostaglandin $F_{2\alpha}$, estradiol and luteinizing hormone in dispersed cell preparations of bovine corpora lutea. In: Ovarian Follicular and Corpus Luteum Function (C. P. Channing, J. M. Marsh and W. A. Sadler, eds.). Plenum Publ., New York, pp. 613–620.

Inskeep, E. K. (1973). Potential uses of prostaglandins in control of reproductive cycles of domestic animals. J. Anim. Sci. 36:1149–1157.

Jones, P.B.C. and Hsueh, A.J.W. (1981). Regulation of progesterone-metabolizing enzyme by adrenergic agents, prolactin, and prostaglandins in cultured rat ovarian granulosa cells. Endocrinology 109: 1347–1354.

Jordan, A. W. (1981). Effects of prostaglandin $F_{2\alpha}$ treatment on LH and dibutyryl cyclic AMP-stimulated progesterone secretion by isolated rat luteal cells. Biol. Reprod. 25:327–331.

Khan, M. I. and Rosberg, S. (1979). Acute suppression by $PGF_{2\alpha}$ on LH, epinephrine and fluoride stimulation of adenylate cyclase in rat luteal tissue. J. Cyclic Nucleotide Res. 5:55–63.

Koos, R. D. and Clark, M. R. (1982). Production of 6-keto-prostaglandin $F_{1\alpha}$ by rat granulosa cells in vitro. Endocrinology 111:1513–1518.

Moriwaki, K., Itoh, Y., Lida, S. and Ichihara, K. (1982). Forskolin potentiates adrenocorticotropin-induced cyclic AMP production and steroidogenesis in isolated rat adrenal cells. Life Sci. 30: 2235–2240.

O'Grady, J. P., Kohorn, E. I., Glass, R. H., Caldwell, B. V., Brock, W. A. and Speroff L. (1972). Inhibition of progesterone synthesis in vitro by prostaglandin $F_{2\alpha}$. J. Reprod. Fertil. 30:153–156.

Ottobre, J. S., Vinson, D. L., Silvia, W. J. and Inskeep, E. K. (1984). Aspects of regulation of uterine secretion of prostaglandins during the estrous cycle. Anim. Reprod. Sci. 7:75–100.

Pang, C. Y. and Behrman, H. R. (1981). Acute effects of prostaglandin $F_{2\alpha}$ on ovarian and luteal blood flow, luteal gonadotropin uptake in vivo, and gonadotropin binding in vitro. Endocrinology 108: 2239–2244.

Pate, J. L. and Condon, W. A. (1982). Effects of serum and lipoproteins on steroidogenesis in cultured bovine luteal cells. Mol. Cell. Endocrinol. 28: 551–562.

Pexton, J. E., Ford, S. P., Wilson, L., Jr., Butcher, R. L. and Inskeep, E. K. (1975). Prostaglandins F in uterins tissue and venous plasma in ewes with intrauterine devices. J. Anim. Sci. 41:141–153.

Seamon, K. and Daly, J. W. (1981). Activation of adenylate cyclase by the diterpene forskolin does not require the guanine nucleotide regulating protein. J. Biol. Chem. 256:9799–9801.

Shemesh, M. and Hansel, W. (1975). Stimulation of prostaglandin synthesis in bovine ovarian tissues by arachidonic acid and luteinizing hormone. Biol. Reprod. 13:448–452.

Silvia, W. J., Ottobre, J. S. and Inskeep, E. K. (1984). Concentrations of prostaglandins E_2, $F_{2\alpha}$ and 6-keto-prostaglandin $F_{1\alpha}$ in the utero-ovarian venous plasma of nonpregnant and early pregnant ewes. Biol. Reprod. 30:936–944.

Speroff, L. and Ramwell, P. W. (1970). Prostaglandin stimulation of in vitro progesterone synthesis. J. Clin. Endocrinol. Metab. 30:345–350.

Stengel, D., Guenet, L., Desmier, M., Insel, P. and Hanoune, J. (1982). Forskolin requires more than the catalytic unit to activate adenylate cyclase. Mol. Cell. Endocrinol. 28:681–690.

Thomas, M.-P., Dorflinger, L. J. and Behrman, H. R. (1978). Mechanism of the rapid antigonadotropic action of prostaglandins in cultured luteal cells. Proc. Natl. Acad. Sci. 75:1344–1348.

Veldhuis, J. D., Klase, P. and Demers, L. M. (1982). Luteinizing hormone stimulates the production of prostacyclin by isolated ovarian cell in vitro. Prostaglandins 23:319–327.

Wright, K., Pang, C. Y. and Behrman, H. R. (1980). Luteal membrane binding of prostaglandin $F_{2\alpha}$ and sensitivity of corpora lutea to prostaglandin $F_{2\alpha}$-induced luteolysis in pseudopregnant rats. Endocrinology 106:1333–1337.