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EFFECTS OF PROSTAGLANDIN E_2 ON ADENOSINE-3′,5′-MONOPHOSPHATE ACCUMULATION AND PROGESTERONE SYNTHESIS IN HUMAN CORPORA LUTEA *IN VITRO*

MASAO MAEYAMA, BUZOU SAITA, KENKICHI ICHIHARA, MASAHIDE MUNEMURA and NORIMASA MORI

Department of Obstetrics and Gynecology, Kumamoto University Medical School, Kumamoto, Japan

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SUMMARY

Accumulation of adenosine-3′,5′-monophosphate (cyclic AMP) and progesterone synthesis were studied *in vitro* in slices of human corpora lutea. On incubation of corpora lutea *in vitro*, prostaglandin E_2 (PGE_2) clearly stimulated progesterone synthesis, though its stimulatory effect was less than that of human chorionic gonadotropin (HCG). Conversely, accumulation of cyclic AMP induced by PGE_2 was markedly more than that induced by HCG. Exogenous cyclic AMP and dibutyryl cyclic AMP clearly stimulated progesterone synthesis. The present data suggest that the mechanism of action of PGE_2 on the human corpus luteum *in vitro* may be mediated by cyclic AMP.

INTRODUCTION

It has been reported that, *in vitro*, prostaglandin (PG)$F_2\alpha$ and PGE_2 stimulated progesterone synthesis in corpora lutea [1–4] which involved cyclic AMP as the second messenger [4, 5]. However, gonadotropins, such as luteinizing hormone (LH) and human chorionic gonadotropin (HCG) *in vivo* and *in vitro* stimulated progesterone synthesis in corpora lutea and were mediated by cyclic AMP [6–8]. Recently, Marsh and LeMaire[9] studied the effects of gonadotropin and PGs on cyclic AMP accumulation and steroidogenesis in the human corpus luteum. Using slices of human corpus luteum, they found that PGE_2 stimulated the accumulation of [8-^{3}H]-adenine into cyclic AMP in this *in vitro* system. The present paper studies the effects of PGE_2 and HCG on cyclic AMP accumulation and progesterone synthesis in human corpora lutea *in vitro*.

MATERIALS AND METHODS

Tissues. Human corpora lutea were obtained at laparotomy, and were at the luteal phase of the menstrual cycle or in early pregnancy (from cases of tubal ligation, ectopic pregnancy, myoma uteri or cancer of the cervix). Each experiment was performed with tissue from only one woman and usually started within 1 h after removing the tissue.

Chemicals. Prostaglandins (PGs) were donated by Ono Pharmaceutical Company, Japan. Human chorionic gonadotropin (HCG) was supplied by Organon Oss, Holland. [^{3}H]-Cyclic AMP (20·7 Ci/mmol) and [1,2-^{3}H]-progesterone (53·0 Ci/mmol) were purchased from the Radiochemical Centre, Amersham, England, and [^{3}H]-arachidonic acid (18·2 Ci/mmol) from New England Nuclear Corporation. Non-radioactive steroids, NAD, cyclic AMP, and dibutyryl cyclic AMP were obtained from Sigma Chemical Co. Reagent grade organic solvents were redistilled before use.

Incubation. The corpus luteum was removed, chilled and sliced with a hand microtom (approx. 100 mg wet wt.). For incubation with pregnenolone corpus luteum tissue was homogenized in 0·1 M phosphate buffer, pH 7·4, and centrifuged at 800 *g*. Tissues were incubated as described in the legends to tables and figures.

Measurement of progesterone synthesis. Progesterone was measured as described previously [10, 11].

In brief: The incubation was terminated by addition of 5 vol. of acetone, and slices were homogenized with the medium used for incubation. Progesterone was isolated and purified by extraction of an homogenate of the tissue and medium with ethyl acetate followed by chromatography of the steroid extract in column and thin-layer chromatographic system. The progesterone was determined quantitatively from its O.D. at 220, 240 and 260 nm in ethanol. The value for the O.D. at 240 nm was corrected using Allen's formula [12]. Tracer amounts of [1,2-^{3}H]-progesterone were added to each sample prior to homogenization to correct for losses.

Endogenous progesterone isolated and measured by U.V. absorption was identified on the following criteria [10]: (1) the absorption maximum at 240 nm in ethanol, (2) its absorption maximum at 295 nm in H_2SO_4 chromogen, (3) the absorption maximum at

380 nm of its 2,4-dinitrophenylhydroazone in chloroform, (4) its identical retention time with that of an authentic sample on gas chromatography, (5) its identical R_F value with that of an authentic sample on thin-layer chromatography.

Assay of cyclic AMP by protein binding. Cyclic AMP was estimated by assay of protein binding, using a slight modification of the method of Gilman[13]. Cyclic AMP-binding protein used was prepared from fresh bovine muscle according to the methods of Walsh *et al.*[14] and Miyamoto *et al.*[15], and its activity was 0·0325 pmol/μg protein. The standard binding reaction was conducted in 100 μl of 50 mM acetate buffer, pH 4·0, for 60 min at 0°C. Then the mixture was diluted to 1 ml with cold 20 mM potassium phosphate buffer, pH 6·0, and after 5 min was applied to a Millipore filter. The filter was then washed with 10 ml of the same buffer and placed in a counting vial with 1 ml of cellosolve, in which it readily dissolved. Radioactivity was counted in a liquid scintillation spectrometer. Protein in homogenates was determined by the method of Lowry *et al.*[16].

Estimation of prostaglandins (PGs) Prostaglandins (PGs) were estimated by the method of Ungar[17]. To measure incorporation of [^{3}H]-arachidonic acid into PGs, slices of corpora lutea (0·3–0·5 g) were incubated in 3 ml of 0·1 M phosphate buffer, pH 7·4, with [^{3}H]-arachidonic acid (0·5 μCi) at 37°C for 30 min. Then the slices were homogenized with the incubation medium and extracted twice with 3 vol. of butanol. The butanol extracts were pooled, evaporated to dryness, and applied to a silicic acid column (1·5 g). The column was eluted successively with benzene and benzene containing 30, 60 and 80% ethyl acetate. The eluate with 60% ethyl acetate, containing PGE_2, and the eluate with 80% ethyl acetate, containing $PGF_{2\alpha}$ were chromatographed on silica gel thin layer chromatograms in chloroform–ethanol–ethyl acetate–glacial acetic acid (200:40:200:10 by vol.). Standard samples of PGs were located on the chromatograms by the phosphomolybdic acid reaction. The labelled metabolites eluted from the thin-layer chromatogram were counted in a liquid scintillation spectrometer.

RESULTS

Dose response of PGE_2 and HCG on progesterone synthesis. Figures 1 and 2 show results of experiments in which PGE_2 and HCG were added to the incubation medium. The results show that progesterone synthesis increased with increase in the concentration of these substances. The maximum effective doses of PGE_2 and HCG appear to be approx. 10–50 μg/ml and 50–100 IU/ml, respectively.

Effects of PGE_2 and HCG on progesterone synthesis. Table 1 shows results obtained on incubation of slices of corpus luteum with PGE_2 (10 μg/ml), or HCG (100 IU/ml) or without either (control). Both PGE_2 and HCG significantly increased the synthesis of progesterone. The values for progesterone synthesis (mean ± S.E., μg/g tissue) were: control, 23·8 ± 4·1; PGE_2, 44·1 ± 6·7 ($P < 0·01$); HCG, 73·2 ± 11·7 ($P < 0·001$).

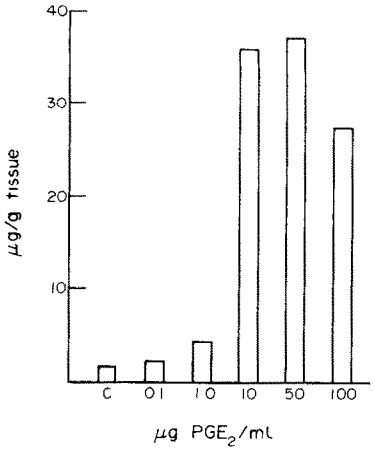

Fig. 1. Dose response of PGE_2 on progesterone synthesis. Flasks contained 5 ml of Krebs–Ringer phosphate buffer, pH 7·4, and 0·2–0·3 g of corpus luteum slices. Slices were incubated for 10 min in buffer alone (preincubation) and then transferred to fresh buffer with or without PGE_2 (0·1–100 μg/ml) and incubated at 37°C for 2 h in air. Corpus luteum used: 9th week of pregnancy. Values were corrected for the initial quantity in the tissue (determined on a sample of the tissue before incubation).

Effect of cyclic AMP on progesterone synthesis. To see whether cyclic AMP affects progesterone synthesis in the corpus luteum, tissue slices were preincubated with 0·0002–0·04 M cyclic AMP or with 0·005–1·0 mM dibutyryl cyclic AMP at 37°C for 10 min, and then incubated in fresh medium for 2 h. Both compounds stimulated progesterone synthesis in the slices, and their minimum effective concentrations

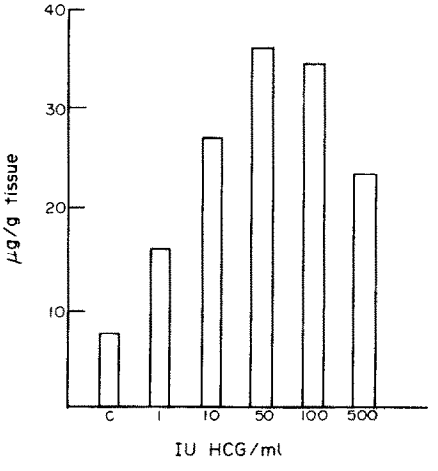

Fig. 2. Dose response of HCG on progesterone synthesis. Flasks contained 5 ml of Krebs–Ringer phosphate buffer, pH 7·4, and 0·2–0·3 g of corpus luteum slices. Slices were incubated for 10 min in buffer alone (preincubation) and then transferred at 37°C for 2 h in air. Corpus luteum used: 10th week of pregnancy. Results are expressed as in Fig. 1.

Table 1. Effect of PGE_2 on progesterone synthesis in human corpora lutea *in vitro*

Day of cycle or Week of pregnancy	Progesterone synthesis de novo* (ug/g, wet tissue) Control	PGE_2(10 μg/ml)	HCG(100 IU/ml)
25 d.	33.9	59.3	94.4
26 d.	7.5	35.9	72.1
32 d.	28.4	33.3	51.5
8 w.	27.7	64.6	100.7
8 w.	21.3	28.5	40.3
Mean ± S.E.	23.8 ± 4.1	44.1 ± 6.7**	73.2 ± 11.7***

* Incubation of slices and measurement of progesterone were carried out as for Fig. 1. Results are expressed as in Fig. 1.
** $P < 0.01$.
*** $P < 0.001$.

were 2×10^{-3} M and 1×10^{-5} M, respectively (Figs. 3 and 4).

Effect of theophylline on stimulation of progesterone synthesis by PGE_2 and HCG. To see whether the actions of PGE_2 and HCG on progesterone synthesis in the corpus luteum *via* cyclic AMP were due to activation of the adenyl cyclase system, 0·04 M of theophylline, which inhibits phosphodiesterase, was added to the incubation medium with PGE_2 or HCG. As seen in Fig. 5, PGE_2 and HCG still increased progesterone synthesis by PGE_2 and HCG in the presence of theophylline.

Accumulation of cyclic AMP. Time course of accumulation of cyclic AMP was observed. Corpus luteum slices were preincubated for 10 min, and then PGE_2 or HCG was added, and accumulation of cyclic AMP was measured at intervals. The amount of cyclic AMP, expressed in pmol per mg protein, showed a peak in the first 5 min under all conditions used (Table 2). Accordingly, in subsequent experiments accumulation of cyclic AMP was measured after 5 min.

The stimulatory effects of different concentrations of PGE_2 and HCG on accumulation of cyclic AMP were determined. As seen in Fig. 6, increasing the concentration of PGE_2 to 0·1 and 10 μg per ml resulted in definite increase in the amount of cyclic AMP. As for progesterone synthesis a concentration of 10 μg per ml of PGE_2 appeared to have the maximum effect, since there was no further increase in the amount of cyclic AMP on increasing the concent-

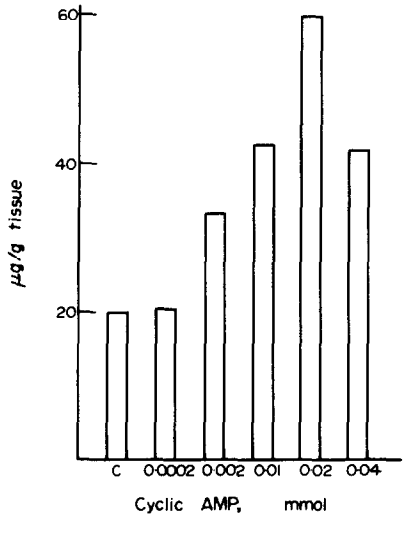

Fig. 3. Effect of cyclic AMP on progesterone synthesis. Flasks containing 0·2–0·3 g of corpus luteum slices in 5 ml of Krebs–Ringer phosphate buffer, pH 7·4, with or without cyclic AMP (0·0002–0·04 M), were incubated at 37°C for 10 min (preincubation). Then the slices were transferred to fresh buffer only and incubated at 37°C for 2 h in air. Corpus luteum used: 10th week of pregnancy. Results are expressed as in Fig. 1.

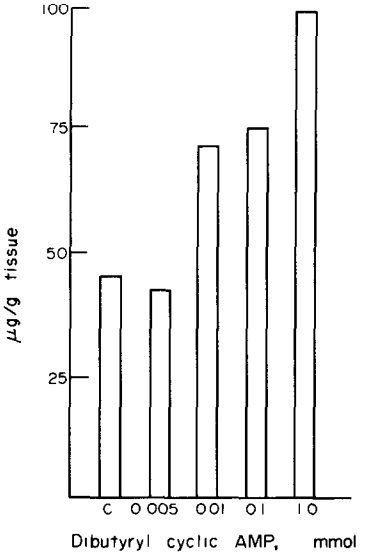

Fig. 4. Effect of dibutyryl cyclic AMP on progesterone synthesis. Incubation of slices and measurement of progesterone were carried out as for Fig. 1, except that the concentration of dibutyryl cyclic AMP was varied. Corpus luteum used: 8th week of pregnancy. Results are expressed as in Fig. 1.

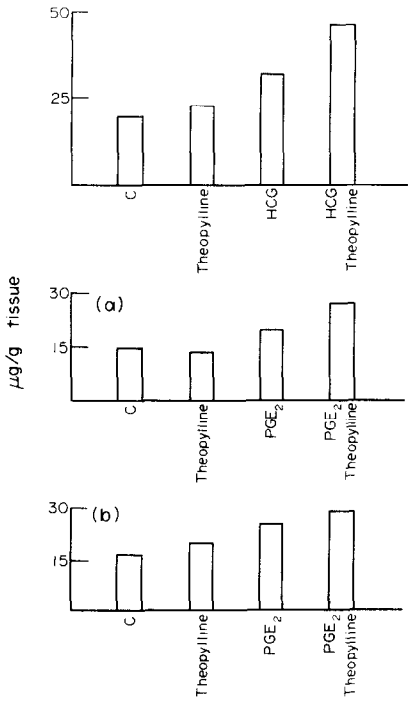

Fig. 5. Effect of theophylline on progesterone synthesis induced by PGE_2 and HCG. Incubation of slices was carried out as for Fig. 1. PGE_2 used, 10 μg/ml; HCG used, 100 IU/ml; theophylline used, 0·04 M. Corpora lutea used: PGE_2(A), 8th week of pregnancy, and PGE_2(B), 24th day of cycle; HCG, 22nd day of pregnancy. Results are expressed as in Fig. 1.

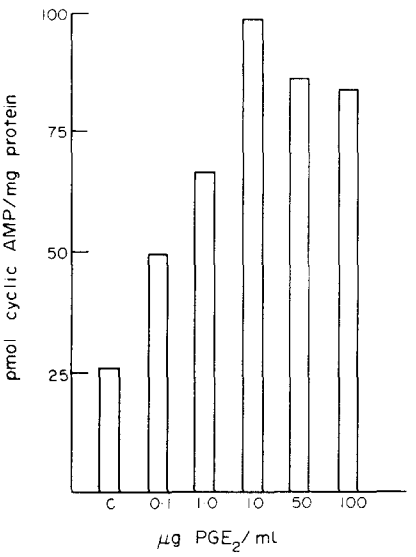

Fig. 6. Effect of PGE_2 concentration on cyclic AMP accumulation. Incubation of slices and measurement of cyclic AMP were carried out as for Table 2. Corpus luteum used: 22nd day of cycle. Results are expressed as in Table 2. Values are average of two determinations.

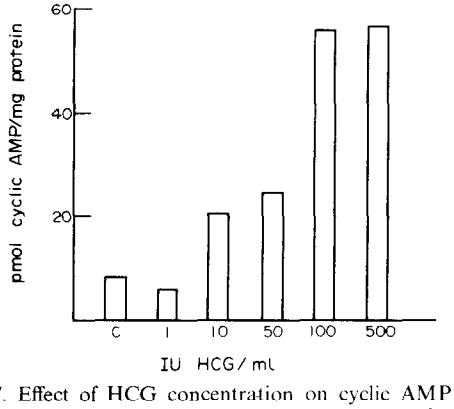

Fig. 7. Effect of HCG concentration on cyclic AMP accumulation. Incubation of slices and measurement of cyclic AMP were carried out as for Table 2. Corpus luteum used: 25th day of cycle. Results are expressed as in Table 2. Values are average of two determinations.

ration to 100 μg of PGE_2 per ml. A concentration of 100 IU per ml of HCG appeared to have the maximum effect, as for progesterone synthesis (Fig. 7).

Table 3 shows results obtained on incubation of slices of corpus luteum with PGE_2 (10 μg/ml), or

Table 2. Time course of cyclic AMP accumulation

Condition of incubation	Cyclic AMP accumulation* (pmoles/mg protein) 3 min	5 min	7.5 min	10 min	15 min
PGE_2	130.3	134.7	115.3	90.7	80.6
	121.7	128.3	100.9	83.3	68.0
	(126.0)	(131.5)	(108.1)	(87.0)	(74.3)
HCG	81.5	91.1	78.9		66.9
	70.3	83.7	70.3		61.5
	(75.9)	(87.4)	(74.6)		(64.2)
Control	30.3	33.9	29.5	25.1	18.0
	27.1	29.9	24.7	17.9	8.6
	(28.7)	(31.9)	(27.1)	(21.5)	(13.3)

Flasks contained 5 ml of Krebs–Ringer phosphate buffer, pH 7·4, and 0·1–0·15 g of corpus luteum slices. Slices were incubated for 10 min in buffer alone (preincubation), and then transferred to fresh buffer with or without 10 μg per ml of PGE_2 or 100 IU per ml of HCG and incubated for various times. Corpora lutea used: control, 20th day of cycle; PGE_2, 25th day of cycle; HCG, 22nd day of cycle.

* Cyclic AMP is expressed as pmoles per mg protein. Figures in parentheses represent averages of two determinations.

Table 3. Effect of PGE_2 on cyclic AMP accumulation in human corpora lutea *in vitro*

Day of cycle or Week of pregnancy	Cyclic AMP accumulation* (pmoles/mg protein) Control	PGE_2(10 μg/ml)	HCH(100 IU/ml)
19 d.	15.0	89.3	16.5
21 d.	10.8	61.3	18.7
23 d.	13.5	35.4	24.6
27 d.	10.9	97.5	65.6
30 d.	10.9	42.7	18.0
9 w.	13.1	97.8	42.7
Mean ± S.E.	12.4 ± 0.7	55.7 ± 10.9**	31.7 ± 7.1 ***

* Incubation of slices and measurement of cyclic AMP were carried out as for Table 2. Results are expressed as in Table 2. Values shown are averages of two determinations.

** $P < 0.001$.

*** $P < 0.001$.

Table 4. Effect of HCG on [^{3}H]-arachidonic acid incorporation into prostaglandins in human corpora lutea *in vitro*

Day of cycle	%Stimulation*	
	PGE_2	$PGF_{2\alpha}$
18 d.	110.9	121.5
20 d.	186.1	148.5
23 d.	144.8	132.9
24 d.	148.3	155.5
Mean ± S.E.	147.5 ± 15.3**	129.6 ± 7.3**

Each flask, containing 0·3–0·5 g of corpus luteum slices and 0·5 μCi of [^{3}H]-arachidonic acid in 3 ml of phosphate buffer, pH 7·4, were incubated with or without HCG (100 IU/ml) at 37°C for 30 min.

* Results are expressed as percentage increase over the control.

** $P < 0·05$.

** $P < 0·05$.

HCG (100 IH/ml) or without either (control). Both PGE_2 and HCG significantly increased the accumulation of cyclic AMP. The values for cyclic AMP accumulation (mean ± S.E., pmol/mg protein) were: control, 12·4 ± 0·7; PGE_2, 65·7 ± 10·9 ($P < 0·001$); HCG, 31·7 ± 7·1 ($P < 0·001$).

Effect of HCG on [^{3}H]-arachidonic acid incorporation into PGs. Corpus luteum slices were incubated with [^{3}H]-arachidonic acid at 37°C for 30 min in 3 ml of 0·1 M phosphate buffer with or without HCG. The incorporation of labelled arachidonic acid into PGE_2 and PGF_2 fractions increased by 47·5% ($P < 0·05$) and 29·6% ($P < 0·05$), respectively, in the presence of HCG (Table 4).

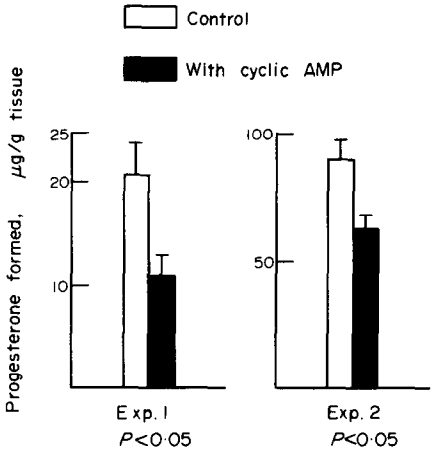

Fig. 8. Effect of cyclic AMP on conversion of pregnenolone to progesterone. Flasks containing 120 μg of pregnenolone, 100 mg of corpus luteum homogenate, and 0·5 μmol of NAD in 2 ml of 0·1 M phosphate buffer, pH 7·4, with or without 10 μmol of cyclic AMP were incubated at 37°C for 2 min in air. Corpora lutea used: Exp. 1, 25th day of cycle, and Exp. 2, 20th day of cycle. Values are averages of three determinations. Bars show standard errors of the means.

Effect of cyclic AMP on conversion of pregnenolone to progesterone. It has been reported that high levels of cyclic AMP inhibit the conversion of pregnenolone to progesterone in bovine corpus luteum [18] and adrenal [19], rat ovaries [20] and adrenal [21] and mouse ovaries [22]. To examine the effect of cyclic AMP on human corpus luteum, the conversion of pregnenolone to progesterone was measured in tissue homogenates under control conditions or in the presence of cyclic AMP (5×10^{-3} M). The results in Fig. 8 show that cyclic AMP inhibits the enzyme 3β-hydroxysteroid dehydrogenase, which converts pregnenolone to progesterone in homogenates of human corpus luteum ($P < 0·05$).

DISCUSSION

In the study on the prostaglandin stimulation of steroidogenesis in bovine corpora lutea *in vitro*, Speroff and Ramwell [3] have reported that all the PGs tested (PGE_2, PGE_1, $PGF_{2\alpha}$ and PGA_1) were found to be steroidogenic, stimulating both the production of progesterone as measured in micrograms and the incorporation of radioactivity from [1-^{14}C]-acetate and that PGE_2 gave the greatest effect, being approximately half effective as LH on a molar basis. They also reported that the time-response curves for PGE_2 and LH were similar and that there was no additive effect when PGs were added to luteal slices incubated with saturating doses of LH or HCG. The present results demonstrate that PGE_2, as well as HCG, stimulates progesterone synthesis *in vitro* in human corpus luteum slices, and confirm the findings of Marsh and LeMaire [9].

It is generally accepted that the mechanism of action of hormones involves two steps: first, the selective binding of the hormone to a specific receptor in the target cell and then stimulation of adenyl cyclase in the cell membrane to produce cyclic AMP. Many hormones, such as ACTH, LH and HCG, produce at least some of their effects *via* cyclic AMP. Moreover, many of the effects of PGs may be mediated directly or indirectly by cyclic AMP [23]. In this paper PGE_2 and HCG caused an increase in the accumulation of cyclic AMP in incubating slices of human corpora lutea. Furthermore, it has been shown here that exogenous cyclic AMP and its derivative, dibutyryl cyclic AMP, clearly stimulate progesterone synthesis in incubating corpus luteum slices, and that addition of theophylline, which inhibits phosphodiesterase, potentiates the steroidogenic actions of PGE_2 or HCG. The results suggest not only that the actions of PGE_2 or HCG on progesterone synthesis are mediated by cyclic AMP in the human corpus luteum, but also that the increase in the accumulation of cyclic AMP is due to stimulation of adenyl cyclase, rather than to inhibition of the phosphodiesterase enzyme system.

Although there are several problems in the methodology for estimation and identification of prosta-

glandins, it is interesting that HCG was found to stimulate the incorporation of [^{3}H]-arachidonic acid into PGE_2 and $PGF_{2\alpha}$ in slices of corpus luteum. Chasalow and Pharriss[24] have measured the effects of LH on prostaglandin synthetase activity in luteinized rat ovaries. Anti-LH antibodies were injected into pregnant rat at doses which interrupt progesterone secretion in this animal preparation, and the ovaries were harvested. Arachidonic acid incorporation into PGE fractions was measured, and it was found that the antibody significantly lowered the synthetase activity. Addition of LH to the animals or the incubation media reversed the effect of the antibody. However, from studies on bovine corpus luteum, Marsh[4] reported that the stimulatory effects of PGE_2 on steroidogenesis and on cyclic AMP accumulation in that tissue are separate phenomena and do not form part of the action mechanism of LH. Recently, Rao[25] suggested that there are specific receptors for PGE_1 and HCG in the cell membrane of the bovine corpus luteum and that these compounds bind to a "different" receptor molecule.

In the present work it was consistently observed that PGE_2 induced more accumulation of cyclic AMP and less progesterone synthesis than HCG. The reason for this is unknown. However, one reason may be related to the present findings that a high level of cyclic AMP inhibited the conversion of pregnenolone to progesterone *in vitro* in homogenates of corpus luteum.

Finally, in the present study it has been observed that both corpora lutea of menstrual cycle and early pregnancy respond to a similar extent to PGE_2 in terms of cyclic AMP accumulation and *in vitro* progesterone synthesis. These results do not agree with those of Marsh and LeMaire[9], probably largely due to differences in the conditions of the corpora lutea studied.

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