Roles of cyclic AMP and inositol phosphates in the luteolytic action of cloprostenol, a prostaglandin $F_{2\alpha}$ analogue, in marmoset monkeys (*Callithrix jacchus*)

A. E. Michael* and G. E. Webley

MRC/AFRC Comparative Physiology Research Group, Institute of Zoology, Regent's Park, London, NW1 4RY, UK

The luteolytic response to a prostaglandin $F_{2\alpha}$ analogue, cloprostenol, was investigated *in vivo* and *in vitro* at defined stages of the luteal phase. *In vivo* administration of cloprostenol to female marmoset monkeys on day 3 after ovulation had no effect on plasma progesterone concentrations, whereas administration on day 14 after ovulation reduced plasma progesterone to preovulatory concentrations within 4 h. To identify the cellular basis for this luteolytic action, marmoset luteal tissue obtained on days 3, 6 and 14 after ovulation was incubated *in vitro* and progesterone production, cAMP accumulation and phosphoinositide (PI) turnover measured in response to cloprostenol, human chorionic gonadotrophin (hCG) with or without cloprostenol, or dibutyryl-cAMP with or without cloprostenol. Progesterone production was stimulated by both hCG and dbcAMP at all stages of the luteal phase. Although neither hCG nor dbcAMP had any significant effects on PI turnover, hCG also increased cAMP accumulation. In marmoset luteal tissue obtained on day 3 after ovulation, cloprostenol had no significant effect on basal or hCG/dbcAMP-stimulated progesterone production but significantly stimulated PI turnover. In contrast, on days 6 and 14 after ovulation, cloprostenol significantly inhibited hCG- and dbcAMP-stimulated progesterone production and the cAMP response to hCG, but had no significant effect on PI turnover. Since progesterone production by the marmoset corpus luteum depends on the luteotrophic support of luteinizing hormone (LH), these observations suggest that the luteolytic action of cloprostenol *in vivo* involves the inhibition of LH/hCG action at sites both prior and subsequent to cAMP accumulation. However, such luteolytic effects do not appear to require the generation of inositol phosphates by increased PI turnover.

Introduction

In non-primate species, luteolysis is induced by the eicosanoid hormone, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Horton and Poyser, 1976). However, the luteolytic role for endogenous $PGF_{2\alpha}$ in primates remains unclear. Several *in vitro* studies have identified receptors for $PGF_{2\alpha}$ on primate luteal cells (Powell *et al.*, 1974) and have confirmed that the primate corpus luteum can both synthesize and secrete $PGF_{2\alpha}$ (Challis *et al.*, 1976; Swanston *et al.*, 1977). In humans, exogenous $PGF_{2\alpha}$ induces a transient decline in plasma progesterone concentrations for the duration of $PGF_{2\alpha}$ administration (Wentz and Jones, 1973; Karim and Hillier, 1979). Furthermore, repeated injection of the $PGF_{2\alpha}$ analogue, cloprostenol, had no significant effect on peripheral plasma progesterone concentrations in olive baboons (*Papio cynocephalus*) (Eley *et al.*, 1987). Conversely, in rhesus macaques (*Macaca mulatta*), intraluteal infusion of $PGF_{2\alpha}$ induced a rapid decline in plasma progesterone (Auletta *et al.*, 1984), whereas in common

marmoset monkeys (*Callithrix jacchus*) luteolysis can be induced by a single intramuscular injection of cloprostenol (0.5 μ g) (Summers *et al.*, 1985; Webley *et al.*, 1991a). Cloprostenol could induce luteolysis consistently only in marmosets if it was administered later than day 8 after ovulation (Summers *et al.*, 1985).

In agreement with studies of other primate species (Hutchinson and Zeleznik, 1984; Fraser *et al.*, 1986), the requirement for LH in maintaining normal luteal progesterone production has been demonstrated in the marmoset monkey by the luteolytic action of a luteinizing hormone-releasing hormone (LHRH) antagonist (Hodges *et al.*, 1988; Webley *et al.*, 1991a). The luteolytic response induced by the LHRH antagonist commences 2 h after antagonist administration, coincident with the significant reduction in circulating bioactive LH (Hodges *et al.*, 1988; Webley *et al.*, 1991a). In comparison, the luteolytic effect of cloprostenol in the marmoset is immediate and occurs without a significant change in the circulating bioactive LH concentration (Hodges *et al.*, 1988; Webley *et al.*, 1991a).

At the cellular level, the ability of LH to maintain progesterone production is mediated largely via cyclic adenosine-3',5'-monophosphate (cAMP). While $PGF_{2\alpha}$ has no effect on basal cAMP accumulation, this luteolytic agent inhibits the luteotrophic

*Present address: Department of Biochemistry, Royal Free Hospital School of Medicine, Rowland Hill Street, London, NW3 2PF, UK.
Received 3 April 1992.

actions of LH and hCG at sites both prior and subsequent to cAMP accumulation in non-primate corpora lutea (Evrard *et al.*, 1978; Thomas *et al.*, 1978; Jordan, 1981; Fletcher and Niswender, 1982; Pate and Condon, 1984). PGF_{2α} has also been shown to stimulate the generation of inositol phosphates via increased phosphoinositide (PI) turnover in non-primate luteal cells (Leung *et al.*, 1986; Veldhuis, 1987; Davis *et al.*, 1989; Lahav *et al.*, 1989). The objectives of the present study were therefore to establish whether the luteolytic action of cloprostenol in the marmoset monkey involves an inhibition of gonadotrophin action at the cellular level, to determine the roles for cAMP and inositol phosphates in the antigonadotrophic actions of cloprostenol, and to relate the luteolytic effects of cloprostenol *in vivo* to its antigonadotrophic effects *in vitro* at defined stages of the luteal phase.

Materials and Methods

Animals

The common marmoset monkeys used in the present study were from the self-sustaining colony housed at the Institute of Zoology as described previously by Hearn (1983). Females exhibited an ovarian cycle length of 28.6 ± 1.0 days (mean $\pm$ SEM) of which the luteal phase constituted 19.2 ± 0.6 days (Harlow *et al.*, 1983).

Ovarian cycles were monitored by the routine collection of venous blood samples (0.3 ml) from the femoral vein into 1.0 ml heparinized syringes. The day of ovulation was defined as the day preceding a rise in the peripheral progesterone concentration above 32 nmol l^{-1} (Harlow *et al.*, 1983). The start of the ovarian cycle was synchronized by the i.m. injection $0.5 \mu\text{g}$ cloprostenol (Estrumate: ICI Pharmaceuticals, Macclesfield, Cheshire) in isotonic saline between days 14 and 18 after ovulation; the next ovulation occurred 8–11 days after cloprostenol administration (Hodges *et al.*, 1988).

Progesterone responses to cloprostenol *in vivo*

On day 3 after ovulation, blood samples were taken from five female marmosets before the i.m. injection of $0.5 \mu\text{g}$ cloprostenol in 0.12 ml isotonic saline. Serial blood samples were then taken from the femoral vein of each individual at 0.5, 1.0, 1.5, 2, 4 and 24 h after cloprostenol administration. The same procedure was repeated with four females on day 14 after ovulation.

Progesterone production and cAMP accumulation by marmoset luteal tissue *in vitro*

Corpora lutea were collected from non-pregnant female marmosets on days 3 ($n = 5$ animals), 6 ($n = 2$) and 14 ($n = 3$) after ovulation by partial lutectomy with the animal under general anaesthesia (Saffan: Glaxo, Ware, Herts). Excised luteal tissue was collected into ice-cold Krebs–Ringer bicarbonate-buffered saline (KRB) containing $10 \text{ mmol glucose l}^{-1}$ and 1 g BSA l^{-1} (Sigma Chemical Co., Poole, Dorset), retained on ice until the completion of surgery. Isolated corpora lutea were then transferred onto filter paper and repeatedly bisected to obtain pieces of approximately 0.5 mg wet weight. Luteal tissue was weighed into 1.5 ml Eppendorf micro-test-tubes

(Anderman and Co., Surrey) such that each tube contained two pieces of tissue (preferably from different corpora lutea or different regions of the same corpus luteum) to give a combined tissue weight of approximately 1 mg per tube.

Tubes each received 100 μl KRB supplemented with $10 \text{ mmol glucose l}^{-1}$ and 1 g BSA l^{-1} and were gassed under 95% O₂: 5% CO₂ before being sealed and preincubated for 20 min at 37°C in a shaking water bath. After this initial preincubation, the medium was replaced and the tissue pieces were treated ($n = 3$ tubes per treatment) with medium alone (control), $1 \mu\text{g hCG ml}^{-1}$ (CR125: NIH, Bethesda, Maryland), $500 \text{ ng cloprostenol ml}^{-1}$, hCG plus cloprostenol, $500 \mu\text{g dibutyryl cyclic AMP ml}^{-1}$ (dbcAMP; Sigma), and dbcAMP plus cloprostenol. Tubes were then gassed and returned to the water bath for 90 min after which incubations were terminated by the removal of 50 μl medium for progesterone assay and the addition of ice-cold perchloric acid (HClO₄: BDH, Poole, Dorset) (final concentration = 107 mmol l^{-1}) to the remaining sample to extract cAMP. All samples were stored at -20°C until assayed.

Inositol phosphate production by marmoset luteal tissue *in vitro*

Luteal tissue, prepared as described above on days 3 ($n = 3$) and 14 ($n = 3$) after ovulation, was incubated for the determination of inositol phosphate production using a modification of the method previously described by Flint *et al.* (1986). Tissue was weighed and placed in tubes containing 100 μl KRB supplemented with $10 \text{ mmol glucose l}^{-1}$, $[2\text{-}^3\text{H}]\text{myo-inositol}$ ($1 \mu\text{Ci ml}^{-1}$: Amersham International, Amersham, Bucks) and $10 \mu\text{mol cold myo-inositol l}^{-1}$ (Sigma). All tubes were gassed under an atmosphere of 95% O₂: 5% CO₂, sealed and transferred to a shaking water bath for a 2 h preincubation at 37°C during which $8.7 \pm 1.9\%$ of the $[^3\text{H}]\text{inositol}$ was incorporated into the tissue. After 2 h, the preincubation buffer was removed and replaced with 200 μl KRB (maintained at 37°C) containing $10 \text{ mmol cold myo-inositol l}^{-1}$. Tubes were then gassed and returned to the water bath for a further 30 min to remove any unincorporated $[^3\text{H}]\text{inositol}$. After this second incubation, the buffer from each tube was replaced with KRB containing $10 \text{ mmol lithium chloride l}^{-1}$ (BDH) (to inhibit the breakdown of inositol phosphates) and tissue incubation was continued for 10 min. Tissue was then treated ($n = 3$ tubes per treatment) for 30 min with $1 \mu\text{g hCG ml}^{-1}$, $500 \mu\text{g dbcAMP ml}^{-1}$, $500 \text{ ng cloprostenol ml}^{-1}$, hCG plus cloprostenol, or dbcAMP plus cloprostenol. After 30 min, acid-soluble inositol phosphates were extracted by the addition of HClO₄ (final concentration 107 mmol l^{-1}) before storing all samples at -20°C .

Progesterone assay

Progesterone concentrations in unextracted plasma samples and in the incubation medium were measured by the direct enzymeimmunoassay (EIA) previously described by Hodges *et al.* (1988). The sensitivity of the assay for plasma samples was $4 \text{ pmol progesterone ml}^{-1}$ of plasma with a working range of $4\text{--}240 \text{ pmol ml}^{-1}$. Intra-assay and interassay coefficients of variation were 5.3 and 15.3%, respectively, at 20% binding. The use of this assay for the determination of progesterone in culture medium has been validated previously and gave a correlation coefficient of 0.94 with a direct radioimmunoassay

(Webley *et al.*, 1988). The working range of the EIA in culture medium was 0.1–8 pmol progesterone ml^{-1} of culture medium with an assay sensitivity of 0.1 pmol ml^{-1} . Intra- and interassay coefficients of variation were 11.4 and 13.6%, respectively, at 20% binding.

Cyclic AMP assay

Immediately before assay, thawed samples were neutralized by the addition of 154 mmol tripotassium phosphate l^{-1} (K_3PO_4 ; BDH) to each tube. The cAMP concentration in neutralized samples was then measured using the cAMP radioimmunoassay previously described by Steiner *et al.* (1972) with the acetylation step subsequently proposed by Harper and Brooker (1975). The working range of this assay was 0.25–20 pmol cAMP ml^{-1} of culture medium with an assay sensitivity of 0.25 pmol ml^{-1} . Intra- and interassay coefficients of variation were 8.3 and 15.0%, respectively, at 20% binding.

Inositol phosphate assay

Inositol phosphates were chromatographically separated using a modification of the anion exchange method previously described by Wregget and Irvine (1987). Amprep minicolumns, each containing 100 mg trimethylaminopropyl-SAX sorbent (Amersham International) were mounted on an Amprep vacuum manifold and conditioned before use by washing each with 10 ml 3 mol ammonium formate l^{-1} (BDH) in 100 mmol formic acid l^{-1} followed by 15 ml double-distilled water. Neutralized sample extracts were then applied to separate columns in 1 ml KRB containing unlabelled inositol phosphates in sodium phytate hydrolysate, prepared by the method of Wregget and Irvine (1987) and donated for use by D. Lander (IAPGR, Babraham, Cambridge, UK).

Unincorporated [^3H]inositol was washed from each column with 8 ml double-distilled water, drawn through at a flow rate of 3 ml min^{-1} by the application of a mild vacuum (10 mmHg). Glycerophosphatidylinositol (GPI) and all retained inositol phosphates were subsequently eluted using a step gradient of ammonium formate (20–2500 mmol l^{-1}) in 100 mmol formic acid l^{-1} (pH 4.2). Samples were decanted into glass scintillation vials, suspended in aqueous miscible scintillation cocktail (Hionic-Fluor: Canberra Packard, Pangbourne, Berks; 1 part sample: 5 parts scintillant) and radioactivity was determined for each fraction using a 1900-CA Tri-Carb liquid scintillation analyser (Canberra Packard). Sample recoveries ranged from 74.4 to 93.3% of [^3H]inositol phosphates loaded. Counts for each fraction were summated and results standardized as the [^3H]inositol activity (c.p.m.) incorporated into total inositol phosphates per mg wet weight luteal tissue.

Statistical analysis

In vivo measurements of plasma progesterone were subjected to one-way analysis of variance (ANOVA) for repeated measures followed by Duncan's multiple range test. Progesterone production, cAMP and [^3H]inositol phosphate accumulation *in vitro* were all standardized per mg wet weight luteal tissue. For each *in vitro* observation, the values of a representative experimental replicate are illustrated in an appropriate figure, accompanied by

the statistical significance of each response in that particular replicate as assessed by one-way ANOVA and unpaired *t* tests. However, values in the text represent means $\pm$ SEM (unless otherwise indicated) of the response for a series of *n* experimental replicates. These data were statistically analysed by one-way ANOVA followed by paired *t* tests. Probabilities of less than 0.05 were accepted as significant in all statistical tests.

Results

Progesterone responses to cloprostenol *in vivo*

Plasma progesterone concentrations prior to the administration of cloprostenol did not differ significantly between animals treated on day 3 (166 ± 20 pmol ml^{-1} ; $n = 5$) and day 14 (201 ± 17 pmol ml^{-1} ; $n = 4$) after ovulation (Fig. 1). When administered on day 3 after ovulation, cloprostenol (0.5 μg) had no significant effect on peripheral plasma progesterone concentrations over 4 h in any of the five monkeys tested (Fig. 1). However, on day 14 after ovulation, cloprostenol significantly ($P < 0.05$) reduced the plasma progesterone concentration in each of the four monkeys tested. Progesterone concentrations fell to 50% of the initial value within the first hour and reached preovulatory values (i.e. < 32 nmol l^{-1}) by 4 h after cloprostenol administration (Fig. 1).

Progesterone production and cAMP accumulation by marmoset luteal tissue *in vitro*

In luteal tissue obtained on day 3 after ovulation, basal progesterone production ranged from 20.9 to 708.4 pmol mg^{-1} luteal tissue. Progesterone production was stimulated significantly ($P < 0.05$) by both hCG ($280.6 \pm 96.5\%$ of basal; $n = 5$) (Fig. 2a) and dbcAMP ($364.3 \pm 106.4\%$ of control; $n = 4$) (Fig. 2a). Cloprostenol had no significant effect on basal or dbcAMP-stimulated progesterone production and did not influence hCG-stimulated progesterone production in four of five experiments (Fig. 2a). Basal cAMP accumulation in luteal tissue obtained on day 3 after ovulation ranged from 3.7 to 101.4 pmol mg^{-1} . hCG significantly ($P < 0.01$) stimulated cAMP accumulation ($389.2 \pm 49.4\%$ of basal; $n = 5$), whereas cloprostenol had no significant effect on basal cAMP accumulation (Fig. 3a). In three of the five experiments performed with day 3 luteal tissue, cloprostenol significantly ($P < 0.01$) inhibited hCG-stimulated cAMP accumulation ($73.1 \pm 5.1\%$ decrease; $n = 3$) (Fig. 3a). However, in only one case was the inhibition of hCG-stimulated cAMP accumulation associated with a significant ($P < 0.01$) inhibition of hCG-stimulated progesterone production.

In luteal tissue obtained on day 6 after ovulation, basal progesterone production ranged from 6.3 to 21.3 pmol mg^{-1} luteal tissue and was stimulated significantly ($P < 0.01$) by hCG ($241.0 \pm 74.9\%$ of basal; mean $\pm$ SD; $n = 2$) (Fig. 2b). Basal cAMP accumulation, which ranged from 5.5 to 5.9 pmol mg^{-1} , was also significantly ($P < 0.01$) stimulated by hCG ($270.5 \pm 41.4\%$ of basal; mean $\pm$ SD; $n = 2$) (Fig. 3b). Although cloprostenol had no significant effect on basal progesterone production or cAMP accumulation, it significantly ($P < 0.05$) inhibited both the progesterone and cAMP

Fig. 1. Peripheral plasma progesterone concentrations (means $\pm$ SEM) in blood samples taken from individual marmoset monkeys following a single i.m. injection of cloprostenol (0.5 μ g) on day 3 (●; $n = 5$) and day 14 (▽; $n = 4$) after ovulation. * $P < 0.05$, ** $P < 0.01$ versus concentration at time zero for each day (Duncan's multiple range test).

responses to hCG on day 6 after ovulation (54.3 ± 4.5 and $63.2 \pm 5.1\%$ decreases, respectively; mean $\pm$ SD; $n = 2$) (Figs 2b and 3b).

In luteal tissue obtained on day 14 after ovulation, basal progesterone production ranged from 4.1 to 8.2 pmol mg⁻¹ luteal tissue. Progesterone production was stimulated significantly ($P < 0.01$) by both hCG ($279.3 \pm 26.2\%$ of basal; $n = 3$) and dbcAMP ($238.8 \pm 9.9\%$ of control; mean $\pm$ SD; $n = 2$) (Fig. 2c). Furthermore, basal cAMP accumulation, which ranged from 1.2 to 6.8 pmol mg⁻¹, was also significantly ($P < 0.01$) stimulated by hCG ($262.7 \pm 2.4\%$ of basal; $n = 3$) (Fig. 3c). Although cloprostenol had no significant effect on basal progesterone production or cAMP accumulation, it significantly ($P < 0.05$) inhibited the progesterone responses to both hCG ($70.0 \pm 5.6\%$ decrease; $n = 3$; Fig. 2c) and dbcAMP ($60.0 \pm 9.5\%$ decrease; mean $\pm$ SD; $n = 2$) (Fig. 2c) and the cAMP response to hCG ($62.2 \pm 5.7\%$ decrease; $n = 3$) (Fig. 3c).

Inositol phosphate production by marmoset luteal tissue in vitro

Buffer samples from incubations of luteal tissue obtained on days 3 and 14 after ovulation were subjected to anion exchange chromatography in the same assay. Basal PI turnover, as assessed by the incorporation of [³H]inositol into total inositol phosphates after 30 min, did not differ significantly between days 3 and 14 after ovulation (7390 ± 511 versus 7050 ± 748 c.p.m. mg⁻¹ luteal tissue, respectively; $n = 3$; $P > 0.05$) (Fig. 4).

Neither hCG nor dbcAMP had any significant effect on PI turnover in marmoset luteal tissue collected on days 3 and 14 after ovulation (Fig. 4). In contrast, cloprostenol significantly ($P < 0.05$) stimulated PI turnover by marmoset luteal tissue

obtained on day 3 after ovulation ($249.0 \pm 28.2\%$ of basal; $n = 3$) (Fig. 4a), but had no significant effect on PI turnover by luteal tissue obtained on day 14 after ovulation (Fig. 4b). The responses to cloprostenol were not influenced by co-treatment with hCG or dbcAMP (Fig. 4).

Discussion

The study reported here has demonstrated that although cloprostenol had no effect on plasma progesterone concentrations in female marmoset monkeys if administered on day 3 after ovulation, this PGF_{2α} analogue induced a rapid decline in peripheral progesterone concentrations if injected on day 14 after ovulation. These observations are consistent with the study by Summers *et al.* (1985) which found that only four of six monkeys responded to cloprostenol between days 8 and 10 after ovulation, but that cloprostenol was luteolytic in all monkeys tested on or after day 11 of the luteal phase.

Basal progesterone production and cAMP accumulation by luteal tissue obtained on day 3 after ovulation showed far more variation than in day 14 luteal tissue. This may reflect the fact that the day 3 corpus luteum is a relatively immature gland still undergoing functional differentiation, whereas the day 14 corpus luteum is assumed to represent a fully differentiated gland. Regardless of the variation in basal progesterone output, hCG stimulated a 1.5- to 2.5-fold increase in progesterone production by pieces of marmoset luteal tissue at all stages of the luteal phase. In addition, hCG increased cAMP accumulation in marmoset luteal tissue, and it was possible to stimulate luteal progesterone production with the cAMP analogue, dbcAMP. These findings are in agreement with previous studies of isolated corpora lutea from rhesus macaques (Stouffer *et al.*, 1979) and humans (Hamberger *et al.*, 1979; Dennefors *et al.*, 1982; Patwardhan and Lanthier, 1984) and are consistent with a role for cAMP in mediating the luteotrophic actions of gonadotrophins in the marmoset corpus luteum. The failure of hCG to influence PI turnover in marmoset luteal tissue obtained in both the early and mid-luteal phase contrasts with previous observations in porcine and bovine luteal cells (Allen *et al.*, 1988; Davis *et al.*, 1989) and suggests that inositol phosphates do not mediate the luteotrophic actions of gonadotrophins in the primate corpus luteum.

In luteal tissue obtained on day 3 after ovulation, cloprostenol was generally unable to inhibit the luteotrophic action of hCG. However, in three of five experiments involving day 3 luteal tissue, cloprostenol significantly inhibited the cAMP response to hCG without inhibiting hCG-stimulated progesterone production. One explanation for this observation is that during the early luteal phase, the cAMP response to hCG might exceed that required to elicit a maximal steroidogenic response such that cloprostenol can significantly reduce hCG-stimulated cAMP accumulation without inhibiting the effect of any residual cAMP on progesterone production. Alternatively, cAMP may not be primarily responsible for mediating the luteotrophic action of hCG during the early luteal phase.

In corpora lutea obtained in the mid-luteal phase (i.e. on days 6 and 14 after ovulation), cloprostenol had no effect on basal progesterone production but inhibited the progesterone response to hCG. The ability of PGF_{2α} to inhibit the luteotrophic

Fig. 2. Progesterone production (mean $\pm$ SEM; $n = 3$ tubes per treatment) by marmoset luteal tissue obtained on (a) day 3, (b) day 6 and (c) day 14 after ovulation and treated for 90 min *in vitro* with (A) medium alone, (B) cloprostenol, (C) hCG, (D) hCG plus cloprostenol, (E) dbcAMP or (F) dbcAMP plus cloprostenol. Representative results of 5 (day 3), 2 (day 6) and 3 (day 14) experimental replicates. * $P < 0.05$, ** $P < 0.01$ versus control; ▼ $P < 0.05$, ▼▼ $P < 0.01$ cloprostenol plus hCG/dbcAMP versus hCG/dbcAMP alone for each day (unpaired t tests).

Fig. 3. Cyclic AMP accumulation (mean $\pm$ SEM; $n = 3$ tubes per treatment) by marmoset luteal tissue obtained on (a) day 3, (b) day 6 and (c) day 14 after ovulation and treated for 90 min *in vitro* with (A) medium alone, (B) cloprostenol, (C) hCG or (D) hCG plus cloprostenol. Representative results of 5 (day 3), 2 (day 6) and 3 (day 14) experimental replicates. ** $P < 0.01$ versus control; ▼▼ $P < 0.01$ cloprostenol plus hCG versus hCG alone for each day (unpaired t tests).

Fig. 4. Phosphoinositide turnover ([³H]inositol phosphate generation; mean $\pm$ SEM; $n = 3$ tubes per treatment) by marmoset luteal tissue obtained on (a) day 3 and (b) day 14 after ovulation and treated for 30 min *in vitro* in the presence of 10 mmol LiCl l⁻¹ with (A) medium alone, (B) cloprostenol, (C) hCG, (D) hCG plus cloprostenol, (E) dbcAMP or (F) dbcAMP plus cloprostenol. Representative results of three experimental replicates for each day. ** $P < 0.01$ versus control for each day (unpaired t tests).

actions of LH or hCG has been demonstrated previously in mid-luteal phase luteal tissue/cells from humans (Dennefors *et al.*, 1982; Patwardhan and Lanthier, 1984), rhesus macaques (Stouffer *et al.*, 1979), rats (Thomas *et al.*, 1978; Jordan, 1981; Dorflinger *et al.*, 1983; Lahav *et al.*, 1989), sheep (Fletcher and Niswender, 1982) and cows (Pate and Condon, 1984; Alila *et al.*, 1988), and in cultured human granulosa cells (McNatty *et al.*, 1975; Michael and Webley, 1991; Webley *et al.*, 1991b). In several of these species, the overall antigonadotrophic action of PGF_{2α} (or cloprostenol) involves an inhibition of LH-stimulated cAMP accumulation (Lahav *et al.*, 1976; Thomas *et al.*, 1978; Fletcher and Niswender, 1982; Dorflinger *et al.*, 1983; Michael and Webley, 1991) and of the steroidogenic response to cAMP (Jordan, 1981; Pate and Condon, 1984; Kenny and Robinson, 1986; Alila *et al.*, 1988; Michael and Webley, 1991). In agreement with these previous studies, the study reported here confirmed that in the marmoset corpus luteum, cloprostenol inhibits both the cAMP response to hCG and the progesterone response to dbcAMP. It would appear therefore that in marmoset monkeys, as in a range of other eutherian species, PGF_{2α} can inhibit the luteotrophic action of LH and hCG at sites both prior and subsequent to the generation of cAMP. Since progesterone production by the marmoset corpus luteum depends on the luteotrophic support of LH (Hodges *et al.*, 1988; Webley *et al.*, 1991a), the ability of cloprostenol to inhibit LH-stimulated progesterone production at pre- and post-cAMP sites may underlie its luteolytic action *in vivo* in marmoset monkeys.

Although cloprostenol has a profound luteolytic action in marmoset monkeys, this effect is not evident at all stages of the luteal phase. As previously observed in non-primate species (Hearnshaw *et al.*, 1973; Poyser, 1981), the marmoset corpus luteum is resistant to the luteolytic action of cloprostenol *in vivo* if administered during the early luteal phase (that is on day 3 after ovulation). In the present study, this variation in the luteolytic efficacy of cloprostenol *in vivo* corresponded to a variation in the antigonadotrophic effects of cloprostenol *in vitro*. On day 3 after ovulation, cloprostenol had no significant effect on peripheral plasma progesterone concentrations *in vivo* nor on hCG-stimulated progesterone production by luteal tissue *in vitro*. Conversely, on day 14 after ovulation cloprostenol was luteolytic *in vivo* and could inhibit hCG action *in vitro*. This correlation between the *in vivo* and *in vitro* actions of cloprostenol over the course of the luteal phase supports the hypothesis that the luteolytic action of cloprostenol *in vivo* involves an inhibition of LH support at the cellular level.

From the observations that PGF_{2α} can stimulate PI turnover in luteal cells from a range of non-primate species and that addition of phospholipase C inhibits LH-stimulated progesterone production by bovine luteal cells at both pre- and post-cAMP sites (Alila *et al.*, 1988; Benhaim *et al.*, 1990), it has been suggested that inositol phosphates might mediate the antigonadotrophic actions of PGF_{2α} in non-primate luteal cells. However, in the present study cloprostenol could only stimulate a significant increase in PI turnover by day 3 luteal tissue, despite being unable to influence hCG action *in vitro* or to induce luteolysis *in vivo* at this stage of the luteal phase. Conversely, on day 14 after ovulation, cloprostenol induced luteolysis *in vivo* and antagonized the luteotrophic action of

hCG *in vitro*, but had no significant effect on PI turnover. These findings are in agreement with previous studies of isolated corpora lutea from the pseudopregnant rat which showed that PI responses to PGF_{2α} are restricted to the early corpus luteum (Lahav *et al.*, 1989) in spite of the fact that PGF_{2α} can induce luteolysis *in vivo* only in the latter half of pseudopregnancy. Furthermore, treatment of ovine luteal cells with pertussis toxin inhibited the PI response to PGF_{2α} without influencing its ability to inhibit LH action (McCann and Flint, 1987). Such observations suggest that the inositol phosphates generated during increased PI turnover are not required for the luteolytic actions of PGF_{2α}/cloprostenol either *in vivo* or *in vitro*.

In the present study, the PI response to cloprostenol was confined to marmoset luteal tissue obtained in the early luteal phase, that is 3 days after the ovulatory LH surge. Recently, Houmard *et al.* (1991) demonstrated that in rhesus macaques, PGF_{2α} could stimulate a significant increase in PI turnover only in luteal cells obtained 24 h after the administration of exogenous hCG *in vivo*. Hence we would suggest that recent exposure to high concentrations of LH or hCG *in vivo* is required for PGF_{2α} or cloprostenol to stimulate PI turnover *in vitro* by the primate corpus luteum. Since pre-exposure to hCG and human LH *in vivo* or *in vitro* suppresses the subsequent antigonadotrophic actions of cloprostenol in cultured human granulosa cells (Michael and Webley, 1991; Webley *et al.*, 1991b), it is possible that recent exposure of the primate corpus luteum to LH or hCG *in vivo* confers resistance to the luteolytic actions of PGF_{2α}/cloprostenol in the early luteal phase and in early pregnancy, respectively, while facilitating the PGF_{2α}-induced stimulation of PI turnover.

Given the functional dependency of the marmoset corpus luteum on LH and the association between the *in vivo* and *in vitro* effects of cloprostenol, it would appear that the luteolytic action of cloprostenol in the marmoset involves inhibition of the luteotrophic stimulus of LH at sites both prior and subsequent to cAMP accumulation. Both the luteolytic action of cloprostenol *in vivo* and its antigonadotrophic effects *in vitro* appear to be inversely related to the cloprostenol-induced increase in PI turnover such that inositol phosphates do not appear to mediate the luteolytic/antigonadotrophic actions of cloprostenol. However, it is possible that increased PI turnover during the early luteal phase may be necessary to render the corpus luteum sensitive to the luteolytic/antigonadotrophic actions of cloprostenol in the mid-luteal phase. Indeed, the stimulation of PI turnover by cloprostenol may be functionally linked to its inability to inhibit gonadotrophin action (and hence to induce luteolysis) in the early luteal phase.

We would like to thank NIH for their kind donation of the hCG preparation used in this study. This work was funded by a joint MRC/AFRC programme grant. A. E. Michael was also supported by an MRC studentship.

References

- Alila HW, Dowd JP, Corradino RA, Harris WV and Hansel W (1988) Control of progesterone production in small and large bovine luteal cells separated by flow cytometry *Journal of Reproduction and Fertility* **82** 645–655

- Allen RB, Su HC, Snitzer J and Dimino MJ (1988) Rapid decreases in phosphatidylinositol in isolated luteal cell membranes after stimulation by luteinizing hormone *Biology of Reproduction* **38** 79–83
- Auletta FJ, Kamps DL, Pories S, Bisset J and Gibson M (1984) An intra-corpus luteum site for the luteolytic action of prostaglandin $\text{F}_{2\alpha}$ in the rhesus monkey *Prostaglandins* **27** 285–298
- Benhaim A, Bonnamy PJ, Mitre H and Leymaire P (1990) Involvement of phospholipase C second messenger system in the regulation of steroidogenesis in small bovine luteal cells *Molecular and Cellular Endocrinology* **68** 105–111
- Challis JRG, Calder AA, Dille A, Forster SS, Hillier K, Hunter DJS, McKenzie IZ and Thorburn GD (1976) Production of prostaglandins E and $\text{F}_{2\alpha}$ by corpus luteum, corpora albicantia and stroma from the human ovary *Journal of Endocrinology* **68** 401–408
- Davis JS, Alila HW, West LA, Corradino RA, Weakland LL and Hansel W (1989) Second messenger systems and progesterone secretion in the small cells of the bovine corpus luteum: effects of gonadotrophin and prostaglandin $\text{F}_{2\alpha}$ *Journal of Steroid Biochemistry and Metabolism* **55** 102–107
- Dennefors BL, Sjorgen A and Hamberger L (1982) Progesterone and adenosine 3',5'-monophosphate formation by isolated human corpora lutea of different ages: influences of human chorionic gonadotropin and prostaglandins *Journal of Clinical Endocrinology and Metabolism* **55** 102–107
- Dorflinger LJ, Luborsky JL, Gore SD and Behrman HR (1983) Inhibitory characteristics of prostaglandin $\text{F}_{2\alpha}$ in the rat luteal cell *Molecular and Cellular Endocrinology* **33** 225–241
- Eley RM, Summers PM and Hearn JP (1987) Failure of the prostaglandin $\text{F}_{2\alpha}$ analogue, cloprostenol, to induce functional luteolysis in the olive baboon (*Papio cynocephalus*) *Journal of Medical Primatology* **16** 1–12
- Evrard M, Lebouilleux P and Hermier C (1978) Role of prostaglandin $\text{F}_{2\alpha}$ in modulation of LH-stimulated steroidogenesis *in vitro* in different types of rat and ewe corpora lutea *Prostaglandins* **16** 491–502
- Fletcher PW and Niswender GD (1982) Effect of $\text{PGF}_{2\alpha}$ on progesterone secretion and adenylate cyclase activity in ovine luteal tissue *Prostaglandins* **23** 803–818
- Flint APF, Leat WMF, Sheldrick EL and Stewart HJ (1986) Stimulation of phosphoinositide hydrolysis by oxytocin and the mechanism by which oxytocin controls prostaglandin synthesis in the ovine endometrium *Biochemical Journal* **237** 797–805
- Fraser HM, Abbott M, Laird NC, McNeilly AS, Nestor JJ Jr and Vickery BH (1986) Effects of an LH-releasing hormone antagonist on the secretion of LH, FSH, prolactin and ovarian steroids at different stages of the luteal phase of the stump-tailed macaque (*Macaca arctoides*) *Journal of Endocrinology* **111** 83–90
- Hamberger L, Nilsson L, Dennefors B, Khan I and Sjorgen A (1979) Cyclic AMP formation of isolated human corpora lutea in response to hCG-interference by $\text{PGF}_{2\alpha}$ *Prostaglandins* **17** 615–621
- Harlow CR, Gems S, Hodges JK and Hearn JP (1983) The relationship between plasma progesterone and the timing of ovulation and early embryonic development in the marmoset monkey (*Callithrix jacchus*) *Journal of Zoology* **201** 273–282
- Harper JF and Brooker G (1975) Femtomole sensitive assay for cyclic AMP and cyclic GMP after 2'-O-acetylation by acetic anhydride in aqueous solution *Journal of Cyclic Nucleotide Research* **1** 207–218
- Hearn JP (1983) The common marmoset monkey (*Callithrix jacchus*). In *Reproduction in New World Primates* pp 181–216 Ed. JP Hearn. MTP Press, Lancaster
- Hearnshaw H, Restall BJ and Gleeson AR (1973) Observations on the luteolytic effects of prostaglandin $\text{F}_{2\alpha}$ during the oestrous cycle and early pregnancy in the ewe *Journal of Reproduction and Fertility* **32** 322–323
- Hodges JK, Green DI, Cottingham PG, Sauer MJ, Edwards C and Lightman SL (1988) Induction of luteal regression in the marmoset monkey (*Callithrix jacchus*) by a gonadotrophin-releasing hormone antagonist and the effects on subsequent follicular development *Journal of Reproduction and Fertility* **82** 743–752
- Horton EW and Poyser NL (1976) Uterine luteolytic hormone: a physiological role for prostaglandin $\text{F}_{2\alpha}$ *Physiological Reviews* **56** 595–561
- Houmar BS, Guan Z, Stokes BT and Ottobre JS (1991) Activation of the phosphatidylinositol (PI) system in the primate corpus luteum (CL) by prostaglandin ($\text{PGF}_{2\alpha}$) *Biology of Reproduction* **44** Supplement 1: Abstract 159
- Hutchinson JS and Zeleznik AP (1984) The rhesus monkey corpus luteum is dependent on pituitary gonadotrophin secretion throughout the luteal phase of the menstrual cycle *Endocrinology* **117** 1043–1049
- Jordan AW (1981) Effects of prostaglandin $\text{F}_{2\alpha}$ treatment on LH and dibutyryl cyclic AMP-stimulated progesterone secretion by isolated rat luteal cells *Biology of Reproduction* **25** 327–331
- Karim SMM and Hillier K (1979) Prostaglandins in the control of animal and human reproduction *British Medical Bulletin* **35** 173–180
- Kenny N and Robinson J (1986) Prostaglandin $\text{F}_{2\alpha}$ -induced functional luteolysis: interactions of LH, prostaglandin $\text{F}_{2\alpha}$ and forskolin in cyclic AMP and progesterone synthesis in isolated rat luteal cells *Journal of Endocrinology* **111** 415–423
- Lahav M, Freud A and Lindner HR (1976) The abrogation by prostaglandin $\text{F}_{2\alpha}$ of LH-stimulated cAMP accumulation in isolated rat corpora lutea of early pregnancy *Biochemical and Biophysical Research Communications* **68** 1294–1299
- Lahav M, Davis JS and Rennett H (1989) Mechanism of the luteolytic action of prostaglandin $\text{F}_{2\alpha}$ in the rat *Journal of Reproduction and Fertility Supplement* **37** 233–240
- Leung PCK, Minegishi T, Ma F, Zhou F and Ho-Youen B (1986) Induction of polyphosphoinositide breakdown in rat corpus luteum by prostaglandin $\text{F}_{2\alpha}$ *Endocrinology* **119** 12–18
- McCann TJ and Flint APF (1987) Effect of prostaglandin $\text{F}_{2\alpha}$ on cyclic adenosine monophosphate and inositol trisphosphate in sheep corpus luteum *Journal of Endocrinology* **115** Supplement: Abstract 102
- McNatty KP, Henderson KM and Sawers RS (1975) Effects of prostaglandins $\text{F}_{2\alpha}$ and E_2 on the production of progesterone by cultured human granulosa cells in tissue culture *Journal of Endocrinology* **67** 231–240
- Michael AE and Webley GE (1991) Prior exposure to gonadotrophins prevents the subsequent antigonadotrophic effects of cloprostenol by a cyclic AMP-dependent mechanism in cultured human granulosa cells *Journal of Endocrinology* **131** 319–325
- Pate JL and Condon WA (1984) Effects of prostaglandin $\text{F}_{2\alpha}$ on agonist-induced progesterone production in cultured bovine luteal cells *Biology of Reproduction* **31** 427–435
- Patwardhan VV and Lanthier A (1984) Effect of prostaglandin $\text{F}_{2\alpha}$ on hCG-stimulated progesterone production by human corpora lutea *Prostaglandins* **27** 465–473
- Powell WS, Hammarstrom S, Samuelsson B and Sjoberg B (1974) Prostaglandin $\text{F}_{2\alpha}$ receptor in human corpora lutea *Lancet* **i** 1120
- Poyser NL (1981) *Prostaglandins in Reproduction* Research Studies Press, New York
- Steiner AL, Parker CW and Kipnis DM (1972) Radioimmunoassay for cyclic nucleotides *Journal of Biological Chemistry* **247** 1106–1113
- Stouffer RL, Nixon WE and Hodgen GD (1979) Disparate effects of prostaglandins on basal and gonadotropin-stimulated progesterone production by luteal cells isolated from rhesus monkeys during the menstrual cycle and pregnancy *Biology of Reproduction* **20** 897–903
- Summers PM, Wennink CJ and Hodges JK (1985) Cloprostenol-induced luteolysis in the marmoset monkey (*Callithrix jacchus*) *Journal of Reproduction and Fertility* **73** 133–138
- Swanston I, McNatty KP and Baird DT (1977) Concentrations of prostaglandin $\text{F}_{2\alpha}$ and steroids in the human corpus luteum *Journal of Endocrinology* **73** 115–122
- Thomas JP, Dorflinger LJ and Behrman HR (1978) Mechanism of the rapid antigonadotrophic action of prostaglandins in cultured luteal cells *Proceedings of the National Academy of Sciences USA* **75** 1344–1348
- Veldhuis JD (1987) Prostaglandin $\text{F}_{2\alpha}$ initiates polyphosphatidylinositol hydrolysis and membrane translocation of protein kinase C in swine ovarian cells *Biochemical and Biophysical Research Communications* **149** 112–117
- Webley GE, Luck MR and Hearn JP (1988) Stimulation of progesterone secretion by cultured human granulosa cells with melatonin and catecholamines *Journal of Reproduction and Fertility* **84** 669–677
- Webley GE, Hodges JK, Given A and Hearn JP (1991a) Comparison of the luteolytic action of a gonadotrophin-releasing hormone antagonist and cloprostenol, and the ability of human chorionic gonadotrophin and melatonin to override their luteolytic effects in the marmoset monkey *Journal of Endocrinology* **128** 121–129
- Webley GE, Richardson MC, Given A and Hearn JP (1991b) Pre-incubation of human granulosa cells with gonadotrophin prevents the cloprostenol-induced inhibition of progesterone production *Human Reproduction* **6** 779–782
- Wentz AC and Jones GS (1973) Transient luteolytic effect of prostaglandin $\text{F}_{2\alpha}$ in the human *Obstetrics and Gynecology* **42** 172–181
- Wregget KA and Irvine RF (1987) A rapid separation method for inositol phosphates and their isomers *Biochemical Journal* **245** 655–660