Inhibitory effect of PGF-2 α on hCG-stimulated progesterone production *in vitro* by luteal cells from guinea-pigs at different stages of the oestrous cycle

M. C. Richardson and M. J. Peddie*

*Department of Human Reproduction and Obstetrics, University of Southampton, Princess Anne Hospital, Coxford Road, Southampton SO9 4HA, and * Department of Physiology and Pharmacology, School of Biochemical and Physiological Sciences, University of Southampton, Bassett Crescent East, Southampton SO9 3TU, U.K.*

Summary. Suspensions of luteal cells were prepared by collagenase dispersion of guinea-pig corpora lutea obtained at specific times during the oestrous cycle. Luteal cells incubated with hCG produced increased amounts of progesterone. For Days 3–13 of the oestrous cycle, the concentrations of hCG required for 50% of the maximum response were within the range, 1×10^{-3} to 7×10^{-3} i.u./ml, showing no marked loss of sensitivity to hCG with increasing luteal age. PGF-2 α (1 μ mol/l), had no effect on basal production of progesterone but significantly inhibited hCG-stimulated progesterone production by luteal cells isolated on Days 7, 9, 10, 12 and 13 of the cycle. This concentration of PGF-2 α had no significant effect on progesterone production by luteal cells prepared earlier in the cycle (Days 3 and 5). It is concluded that (a) the luteolytic action of PGF-2 α in the guinea-pig is mediated, at least in part, by direct action on luteal cells, and (b) the cells from newly formed corpora lutea are resistant to the direct inhibitory action of PGF-2 α .

Introduction

There is considerable evidence to support the concept that prostaglandin (PG) F-2 α is the uterine factor responsible for luteolysis in the guinea-pig (reviewed by Poyser, 1976). However, the luteolytic effect of PGF-2 α in the guinea-pig, well established *in vivo* (Blatchley & Donovan, 1972; Dwyer & Church, 1979), has not, hitherto, been investigated *in vitro*, where PGF-2 α may be added directly to suspensions of luteal cells.

In a number of species, newly formed corpora lutea have been shown to be refractory to the action of PGF-2 α *in vivo* (reviewed by Karim & Hillier, 1979). Such an early resistance to prostaglandin has been observed *in vivo* in the guinea-pig (Tso & Tam, 1977). If the effect of PGF-2 α *in vitro* were truly to reflect the action of this agent *in vivo*, luteal cells might be expected to differ in their response to PGF-2 α depending on the age of the corpora lutea from which they were prepared. Such a finding would also confirm the view that the effects of PGF-2 α measured *in vitro* are specific actions, requiring the same onset of responsiveness as that necessary for the initiation of luteolysis *in vivo*.

The present study was therefore undertaken to develop an *in-vitro* dispersed luteal cell system for the guinea-pig. Responses to a range of concentrations of hCG were measured at

specific stages during the life-span of the corpus luteum and the effect of luteal age on the action of PGF-2 α was also investigated.

Part of this study has been published in preliminary form (Richardson & Peddie, 1980).

Materials and Methods

Animals

Adult female guinea-pigs weighing 500–750 g were used. The animal house conditions and the method for monitoring oestrous cycles were as previously described (Peddie, 1980), the day of oestrus (designated as Day 1) being taken as the day on which maximum cornification of the vaginal epithelium was seen before the post-ovulatory influx of leucocytes.

Preparation and incubation of cell suspensions

Animals were anaesthetized with pentobarbitone sodium (40 mg/kg, i.p.) and then killed by cervical dislocation. The ovaries were removed and placed immediately in Dulbecco's modified Eagle's medium (Flow Laboratories, Irvine) containing glutamine (0.584 g/l), sodium bicarbonate (3.7 g/l) and bovine serum albumin (5 g/l). Corpora lutea were then excised under a dissecting microscope. For each day of the oestrous cycle studied, 6–8 corpora lutea from two animals were pooled and then dissociated in medium containing collagenase according to a method previously described for the preparation of cell suspensions from biopsies of human corpus luteum (Richardson & Masson, 1980). A sample of the dispersed cell preparation was examined under the light microscope and the cells were counted in the presence of methylene blue (0.01%, w/v). Many of the cells (total yield, $1-2 \times 10^6$) remained in small clumps of about 5–20 cells. The viability of the dispersed cells was about 80% as judged by trypan blue exclusion (Tennant, 1964). No change in viability was noted after incubating the cells (2 h, 37°C) in the presence or absence of PGF-2 α (1 μ mol/l).

Samples (0.3 ml) of the final suspension, each containing about 3×10^4 cells, were incubated at 37°C for 2 h with various hormonal additions. A preparation of the 2nd International Standard for hCG (National Institute for Biological Standards and Control, Hampstead, London) was serially diluted in culture medium before addition to the cell suspension. Samples of a stock ethanolic solution of PGF-2 α -tromethamine salt (donated by The Upjohn Co., Kalamazoo, Michigan, U.S.A.) were evaporated under nitrogen and then thoroughly dissolved in culture medium before addition. After incubation, the cell suspensions were stored frozen (–20°C) before radioimmunoassay.

Radioimmunoassay of progesterone

Cell suspensions (cells with medium) were assayed for progesterone, after extraction with hexane, using the radioimmunoassay described by Richardson & Masson (1980). The antiserum, raised in sheep against progesterone-11 α -hemisuccinate-ovalbumin, was from Guildhay Antisera, Guildford. The cross-reactivity of this antiserum at 50% displacement was: progesterone, 100%; 20 α -hydroxypregn-4-en-3-one, 2.5%; 20 β -hydroxypregn-4-en-3-one, 1.0%; 11-deoxycorticosterone, 0.9%; corticosterone, 0.8%; 17 α -hydroxyprogesterone, 0.3%; cortisol, 0.01%; testosterone, 0.01%; oestradiol-17 β , 0.01%. The intra- and interassay coefficients of variation were 7 and 15% respectively and the sensitivity of the assay was 0.022 pmol per assay tube. In each assay, allowance was made for extraction efficiency (usually > 90%) by extracting and assaying a standard solution of progesterone in culture medium.

Progesterone production was calculated following correction for progesterone values obtained for non-incubated samples of cell suspension. Levels of significance for differences in

progesterone estimates between sets of incubated samples were calculated using Student's *t* test for unpaired values.

Results

Text-figure 1 shows the effect of hCG on progesterone production by dispersed luteal cells from guinea-pigs on Day 12 of the oestrous cycle. The relationship between hCG concentration and progesterone production was similar for a number of cell suspensions prepared at different stages of the oestrous cycle: the concentrations of hCG required to elicit 50% of the maximum response were 7, 1, 3, 6, 4, 2 and 5 milliunits/ml for the particular experiments performed with cells prepared on Days 3, 5, 7, 9, 10, 12 and 13 of the cycle respectively. There was therefore no marked loss of sensitivity to hCG with increasing luteal age. Attempts to produce a cell suspension preparation from Day 15 corpora lutea were unsuccessful, presumably because of advanced luteolysis.

Text-fig. 1. Effect of PGF-2 α (1 μ mol/l) on hCG-stimulated progesterone production by dispersed cells from guinea-pig corpora lutea on Day 12 of the oestrous cycle. Luteal cells were incubated for 2 h. Mean values (with s.e.m.) refer to estimates on quadruplicate incubations. **P* < 0.02, ***P* < 0.001 compared with value for incubation without PGF-2 α .

The effect of direct addition of PGF-2 α (1 μ mol/l) to dispersed luteal cells from 12-day old corpora lutea is also illustrated in Text-fig. 1. PGF-2 α alone caused no significant change in progesterone production (this was also true for other luteal ages studied). However, PGF-2 α caused a marked inhibition of hCG-stimulated progesterone production which was significant for each stimulatory concentration of hCG used. A time-course study (data not shown) suggested that the effect of PGF-2 α was to reduce the rate of progesterone production (roughly constant in the presence of hCG, 1×10^{-2} i.u./ml, alone) to a lower value maintained throughout the 2 h incubation period.

The extent of inhibition of hCG-stimulated progesterone production by PGF-2 α (1 μ mol/l) at various stages of the oestrous cycle is shown in Table 1. The concentration of hCG used to make these comparisons (1×10^{-2} i.u./ml) was specifically chosen to give a clear response (more than 50% of maximum) without going beyond the concentration sufficient to elicit a maximum response. Gonadotrophin-stimulated progesterone production by luteal cells from newly formed

corpora lutea (Days 3 and 5) was not inhibited significantly by PGF-2 α . However, progesterone formation by preparations from older corpora lutea (Days 7, 9, 10, 12 and 13) was markedly diminished in the presence of the prostaglandin.

Table 1. The effect of PGF-2 α on hCG-stimulated progesterone production by luteal cells from guinea-pigs at various stages of the oestrous cycle

Day of cycle*	hCG-stimulated progesterone production† in the presence of PGF-2 α (1 μ mol/l) as % of value in absence of PGF-2 α	Significance‡ of inhibitory action of PGF-2 α
3	101.5 $\pm$ 14.2	N.S.
5	84.4 $\pm$ 5.0	N.S.
7	52.5 $\pm$ 9.8	$P < 0.05$
9	48.7 $\pm$ 10.9	$P < 0.01$
10	52.0 $\pm$ 5.3	$P < 0.001$
12	51.5 $\pm$ 7.0	$P < 0.001$
13	53.5 $\pm$ 8.4	$P < 0.01$

* For each day of cycle, results were obtained for one cell suspension prepared from 6–8 corpora lutea of 2 guinea-pigs.

† Progesterone produced in the presence of hCG (1×10^{-2} i.u./ml) minus basal production. Mean values ($\pm$ s.e.m.) refer to estimates on quadruplicate incubations. Variation (s.e.m.) between replicate incubations in the absence of PGF-2 α was 6.9, 5.4, 11.5, 6.5, 6.8, 3.0 and 1.6% for Days 3, 5, 7, 9, 10, 12 and 13 respectively.

‡ Calculated for differences between values for hCG-stimulated progesterone production in the presence and absence of PGF-2 α . N.S. = not significant.

Discussion

The present studies demonstrate that dispersed guinea-pig luteal cells respond in a dose-dependent manner to hCG by increasing progesterone production. It is assumed that the response to hCG mimics the action of LH since both hormones have been shown to interact with the same receptor population (Papaionannou & Gospodarowicz, 1975).

The present report also establishes that the dispersed luteal cell system, prepared at particular stages of the oestrous cycle, may be used to study variations in the responsiveness to hormones throughout most of the natural life-span of the corpora lutea in non-pregnant cycles. Using this approach, it was evident that the sensitivity to gonadotrophin (in terms of the concentration of hCG required for 50% of the maximum response *in vitro*) remained quite stable (within a log (dose) unit) during a major part of the life of the guinea-pig corpus luteum.

The effects of PGF-2 α were investigated using a concentration (1 μ mol/l) which was 5–6-fold greater than that observed for utero-ovarian vein blood on Day 15 of the oestrous cycle (Blatchley, Donovan, Horton & Poyser, 1972). This concentration of PGF-2 α inhibits progesterone production by rat luteal cells (Thomas, Dorfinger & Behrman, 1978). The finding that this concentration of PGF-2 α partly inhibited gonadotrophin-stimulated progesterone production by guinea-pig luteal cells is in agreement with observed inhibitory effects of this prostaglandin on the production of progesterone by guinea-pig corpora lutea *in vivo* (Dwyer & Church, 1979). Present observations are also in agreement with similar direct effects of PGF-2 α on dispersed luteal cells (Thomas *et al.*, 1978; Hall & Robinson, 1979) and luteal explants (Lahav, Freud & Lindner, 1976; Khan *et al.*, 1979) of the rat, and superfused preparations from pig corpora lutea (Watson & Maule-Walker, 1977). The observed lack of inhibition of basal

progesterone production in the presence of PGF-2 α (also noted for rat luteal cells by Thomas *et al.*, 1978) suggests that prostaglandin specifically inhibits gonadotrophin-stimulated steroid production. Such an inhibitory effect demonstrated *in vitro* clearly indicates that the luteolytic effect of PGF-2 α in the guinea-pig is mediated, at least in part, at the level of the individual luteal cell. This view is further supported by evidence of an absence of acute alteration in ovarian blood flow following administration of a luteolytic dose of PGF-2 α to guinea-pigs (Wehrenberg, Dierschke & Wolf, 1979).

The observation that hCG-stimulated progesterone production by cells from newly formed corpora lutea (Days 3 and 5) was resistant to the inhibitory action of PGF-2 α provides confirmation of a previous report which describes a refractory period *in vivo* for PGF-2 α action on luteal volume in guinea-pigs (Tso & Tam, 1977). A similar onset of responsiveness to PGF-2 α measured *in vivo* and *in vitro* has also been observed for corpora lutea of rats pretreated with PMSG (Khan *et al.*, 1979). It remains to be established whether the delayed onset of responsiveness to PGF-2 α is associated with changes in PGF-2 α receptors in the corpus luteum. Although a gradual increase in PGF-2 α receptors with luteal age has been demonstrated for bovine corpora lutea (Rao, Estergreen, Carman & Moss, 1979), the binding capacity and affinity of PGF-2 α receptors appeared to be similar at all stages of pseudopregnancy in the rat, despite changes in susceptibility to the prostaglandin (Wright, Pang & Behrman, 1980). The biochemical events between prostaglandin reception and inhibition of adenylate cyclase (observed for rat corpora lutea by Thomas *et al.*, 1978) may also be subject to change during maturation of the corpus luteum.

In summary, it would appear that the luteolytic action of PGF-2 α in the guinea-pig is manifest *in vitro* in terms of a rapid inhibitory effect on gonadotrophin-stimulated progesterone production. Responsiveness, acquired by Day 7 of the oestrous cycle, prepares the luteal cells for reception of the luteolytic signal provided by the uterine release of PGF-2 α beginning on Day 11 in the non-pregnant cycle (Blatchley *et al.*, 1972).

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Received 6 November 1981