General and Comparative Endocrinology 166 (2010) 436–442

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Short Communication

The relationship between the production and the anti-gonadotrophic action of prostaglandin $F_{2\alpha}$ in luteal cells from the marmoset monkey (*Callithrix jacchus*) in the early and mid-luteal phase

G.E. Webley [a], A.E. Michael [a,b], D.R.E. Abayasekara [a,c,*]

[a] Institute of Zoology, Regent's Park, London NW1 4RY, UK
[b] Centre for Developmental and Endocrine Signalling, Academic Section of Obsterics and Gynaecology, Division of Clinical Developmental Sciences, St. George's University of London, Cranmer Terrace, London SW17 0RE, UK
[c] Reproduction and Development Group, Department of Veterinary Basic Sciences, Royal Veterinary College, Royal College Street, London NW1 0TU, UK

ARTICLE INFO

Article history:
Received 17 August 2009
Revised 30 November 2009
Accepted 5 January 2010
Available online 11 January 2010

Keywords:
Primate
Corpus luteum
Luteal cells
Luteolysis
Prostaglandin
Ovary
Progesterone
Indomethacin
Chorionic gonadotropin

ABSTRACT

To address the potential luteolytic role for prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in the corpus luteum of the common marmoset monkey (*Callithrix jacchus*), the ability of marmoset luteal cells, maintained in monolayer culture, to produce $PGF_{2\alpha}$ was determined *in vitro* in the presence and absence of human chorionic gonadotrophin (hCG) and other established pharmacological modulators of $PGF_{2\alpha}$ synthesis. We also assessed the effects of the $PGF_{2\alpha}$ analogue, cloprostenol, on progesterone output from luteal cells isolated in the early luteal phase versus the mid-luteal phase (days 3 and 14 post ovulation, respectively). Cloprostenol had no effect on progesterone output from luteal cells isolated on day 3 of the luteal phase, whereas it significantly inhibited both basal and hCG-stimulated progesterone synthesis by day 14 luteal cells during the culture period 48–72 h ($P < 0.001$). Intra-luteal $PGF_{2\alpha}$ concentrations were 5-fold higher in luteal cells isolated in the early luteal phase than in mid-luteal phase cells (16.5 ± 3.5 versus 3.5 ± 0.6 pmol/10^5 cells). While $PGF_{2\alpha}$ production was unaffected by hCG *in vitro*, it was decreased by indomethacin (1000 ng/ml) ($P < 0.05$) and stimulated by the calcium ionophore A23187 (10 μmol/l) ($P < 0.05$) in luteal cells from both stages of the luteal phase. Phospholipase A_2 did not influence $PGF_{2\alpha}$ production by day 3 luteal cells whereas at 10 IU/ml, it significantly stimulated $PGF_{2\alpha}$ production by day 14 luteal cells ($P < 0.05$). Hence, the timing of luteolysis in the common marmoset monkey appears to involve changes in both the luteal cell response to and production of $PGF_{2\alpha}$.

1. Introduction

A role for prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) as the endogenous luteolysin causing luteal regression in the primate has been difficult to define (Auletta and Flint, 1988; Patton and Stouffer, 1991; Michael et al., 1994). In non-primate mammal species, luteolysis is induced by the action of $PGF_{2\alpha}$ released from the uterus (reviewed by McCracken et al., 1972; Horton and Poyser, 1976; Silvia et al., 1991; Niswender et al., 2000, 2007; Weems et al., 2006). However, uterine $PGF_{2\alpha}$ does not appear to initiate luteolysis in primates as hysterectomy has no effect on cycle length (Neill et al., 1969; Beling et al., 1970; Metcalf et al., 1992) and different primate species differ in their responses to systemic administration of $PGF_{2\alpha}$. While administration of the $PGF_{2\alpha}$ analogue, cloprostenol, has a marked and rapid luteolytic action in the marmoset monkey (Summers et al., 1985; Webley et al., 1991a) it has no effect in the baboon (Eley et al., 1987). Furthermore, $PGF_{2\alpha}$ only exerts a transient luteolytic effect when administered systemically to women (Wentz and Jones, 1973; Karim and Hillier, 1979) and requires multiple injections to induce functional luteolysis in the rhesus macaque (Wilks, 1983).

These species-specific responses to $PGF_{2\alpha}$ and cloprostenol have called into question the role for $PGF_{2\alpha}$ as a luteolytic agent in primates and have lead to the suggestion that luteolysis may be an autonomous, pre-programmed event in women and non-human primates (Lenton and Woodward, 1988; Fisch et al., 1989). An alternative explanation is that there is an intraovarian (rather than uterine) source of $PGF_{2\alpha}$ (Olofsson and Leung, 1994; Davis and Rueda, 2002; Wiltbank and Ottobre, 2003) which acts as a luteolytic agent (Rothchild, 1981; Davis and Rueda, 2002) and that such a paracrine or autocrine action of $PGF_{2\alpha}$ may be difficult to mimic in primate species by systemic administration (Olofsson and Leung, 1994). Intra-luteal infusion or perfusion of $PGF_{2\alpha}$ results in luteolysis (Auletta et al., 1984; Hearn and Webley, 1987; Bennegard et al., 1991) and receptors for $PGF_{2\alpha}$ have been demon-

* Corresponding author. Address: Reproduction and Development Group, Department of Veterinary Basic Sciences, Royal Veterinary College, Royal College Street, London NW1 0TU, UK. Fax: +44 207 468 5204.
E-mail address: rabayase@rvc.ac.uk (D.R.E. Abayasekara).

0016-6480/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygcen.2010.01.009

strated in human corpora lutea (CL) (Powell et al., 1977; Tanaka et al., 1983). *In vitro*, $PGF_{2\alpha}$ can act on luteal cells to inhibit the luteotrophic actions of LH or hCG in all the primate species studied to date, including luteal tissue isolated from the mid-luteal phase for human (Dennefors et al., 1982; Abayasekara et al., 1993), rhesus macaque (Stouffer et al., 1979) and marmoset monkey (Michael and Webley, 1993), as well as in cultured human granulose-lutein cells (McNatty et al., 1975; Webley et al., 1991c; Abayasekara et al., 1993).

In terms of evidence for local prostaglandin synthesis, luteal tissue from the human and rhesus macaque can produce $PGF_{2\alpha}$ although reports of changes in concentrations during the luteal phase are inconsistent. A mid-luteal phase peak has been recorded in human corpora lutea (Patwardhan and Lanthier, 1985) coinciding with the onset of luteal regression whereas others found no relationship between $PGF_{2\alpha}$ concentrations and the timing of human luteal regression (Challis et al., 1976; Swanston et al., 1977). In the rhesus macaque, higher luteal concentrations of $PGF_{2\alpha}$ were recorded in the early and late luteal phase with a decrease in the mid-luteal phase (Houmard and Ottobre, 1989). Recent molecular investigations of the rhesus macaque CL have revealed a progressive decline in expression of prostaglandin endoperoxide synthase 2 (PTGS-2) accompanied by an increase in prostaglandin $F_{2\alpha}$ receptor (PTGFR) expression in the mid to late luteal phase (Bogan et al., 2008a,b). Moreover, there is a dramatic decline in expression of prostaglandin E synthase (PTGES) enzyme in the late luteal phase which increases availability of prostaglandin G_2 as substrate for the local synthesis of luteolytic $PGF_{2\alpha}$, rather than the luteotrophic PGE_2.

The marmoset CL shows a clear response, both *in vivo* and *in vitro*, to the luteolytic action of cloprostenol but the question still remains as to whether $PGF_{2\alpha}$ is the endogenous luteolysin in this New World primate and what determines the timing of its luteolytic actions. In the present study we set out to establish whether the marmoset CL secretes $PGF_{2\alpha}$ and, if so, to investigate whether the net output of $PGF_{2\alpha}$ from marmoset luteal cells, cultured for up to 3 days, relates to the ability of the $PGF_{2\alpha}$ analogue, cloprostenol, to exert anti-gonadotrophic actions in this non-human primate. Previous results demonstrated that prior to day 8 of a 20 day luteal phase, the marmoset CL does not respond to the luteolytic action of cloprostenol either *in vivo* (Summers et al., 1985) or *in vitro* (Michael and Webley, 1993) and that the *in vitro* response depends on the culture system used (Michael et al., 1994). In the present study we have also investigated the ability of hCG to influence $PGF_{2\alpha}$ production and determined whether known pharmacological modulators of $PGF_{2\alpha}$ production [calcium ionophore (A23187), phospholipase A_2 (PLA_2) and indomethacin] can influence $PGF_{2\alpha}$ output from marmoset luteal cells.

2. Methods

2.1. Animals

Adult female marmoset monkeys of proven fertility were maintained in family groups, as described previously (Hearn, 1980), in a self-sustaining colony at the Institute of Zoology. The marmoset monkey has an ovarian cycle of 28 days with a luteal phase length of 19–20 days (Harlow et al., 1983). Implantation starts on days 11–12 after ovulation (Moore et al., 1985). The start of the ovarian cycle was controlled by administration of a single i.m. injection of 0.5 μg cloprostenol (Estrumate: ICI Pharmaceuticals Division, Macclesfield, Cheshire, UK) (Summers et al., 1985). Animals were bled every other day for the analysis of progesterone levels by specific enzyme-linked immunosorbent assay. The day of ovulation was defined as the day preceding a rise in plasma progesterone concentrations above 10 ng/ml and the day of the luteal phase was the number of days after ovulation.

Laparotomy was performed on animals anaesthetized with an i.m. injection of a steroid anaesthetic (Saffan, 12 mg in 1.0 ml: Glaxo, Ware, Herts, UK). Luteal tissue was obtained by partial lutectomy from animals on day 3 ($n = 4$) and day 14 ($n = 4$) after ovulation. The days of the luteal phase chosen for study were early in the luteal phase when cloprostenol does not appear to have a luteolytic action (Summers et al., 1985) and the mid-luteal phase when cloprostenol has been established to exert both a luteolytic action *in vivo* (Summers et al., 1985) and an anti-gonadotrophic action *in vitro* (Michael and Webley, 1993). Measurement of plasma progesterone concentration in the day 14 animals confirmed that they were non-pregnant (Webley et al., 1991b).

2.2. Cell preparation and culture

The method of preparation of dispersed luteal cells was as described previously (Webley et al., 1989). In brief tissue from 2 CL from each marmoset were pooled, cut into small fragments and subjected to collagenase dispersion (Boehringer Mannheim GmbH, Lewes, Sussex, UK; 10 mg/ml) for 2 periods each of 25 min. After centrifugation the cells were pooled in Dulbecco's modified Eagle's medium (DMEM; Flow laboratories, Rickmansworth, Herts, UK) with BSA (5.0 g/l) and containing deoxyribonuclease (0.001% w/v; Sigma Chemicals, Poole, Dorset, UK). The cells were then washed a further 2 times in DMEM containing BSA and counted in a haemocytometer. Using this method, we have previously identified two distinct populations of luteal cells: larger steroidogenic cells of ~20 μm in diameter which stained positive for 3β-hydroxysteroid dehydrogenase (3β-HSD) and smaller cells (<10 μm in diameter) which did not stain with antibodies raised to the steroidogenic 3β-HSD enzyme (Webley et al., 1989). For the present series of studies, cell numbers were estimated on the basis of counts of the larger 3β-HSD positive cells.

The luteal cells were cultured in 96-well plates in DMEM containing penicillin (50 mg/ml), streptomycin (60 mg/ml), glutamine (0.584 g/l) and 10% (v/v) foetal calf serum at a cell concentration of $0.5–2.0 \times 10^4$ cells/ml. Replicates of 4 wells were incubated at 37 °C under an atmosphere of 5% (v/v) CO_2 in air and cultured for up to 72 h with daily changes of media. Samples of the cell preparation were frozen at the onset of culture (time 0 h) to allow estimation of $PGF_{2\alpha}$ concentrations prior to culture. Progesterone and $PGF_{2\alpha}$ production were each measured following two 24 h culture periods: 0–24 h and 48–72 h.

Luteal cells were exposed to the following treatments; hCG (1–1000 ng/ml; CR 127, biological potency 14,900 IU/mg; NIH, Bethesda, MD, USA) with and without cloprostenol (500 ng/ml), indomethacin (10–1000 ng/ml), calcium ionophore (0.1–10 μmol/l) and PLA_2 (from *Naja naja* venom – 0.1 to 10 IU/ml). The last three reagents were purchased from Sigma Chemicals. Indomethacin (10 mg/ml) and calcium ionophore (1 mmol/l) were each stored as stock solutions in ethanol but dilution with culture medium reduced the final ethanol concentration to a maximum of 0.01% (v/v). All the treatments were prepared at 10 times final concentrations required, added to culture wells in 30 μl volumes and diluted with media to give a final volume of 300 μl per well. After incubation the media samples were stored at −20 °C until assay.

2.3. Assays

2.3.1. Progesterone

Progesterone concentrations in marmoset plasma and culture medium were measured using a direct enzyme-linked immunosorbent assay as described previously (Hodges et al., 1988). For plasma, the sensitivity of the assay was 4 nmol/l with a working