Effects of oxytocin-antagonist injections on luteal regression in the goat

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1 Intra-arterial administration of 0.25 ml physiological saline to the non-pregnant goat between days 12 and 20 of the oestrous cycle did not affect luteal regression, which was characterized by decreasing peripheral plasma progesterone concentration, beginning on day 13 of the oestrous cycle, and an increase in the plasma concentration of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (PGFM) as oestrus approached on about day 20.

2 Intra-arterial administration of oxytocin antagonist (OA) in saline at a dose of $0.2 \mu\text{g kg}^{-1}$ body weight to goats between days 12 and 20 of the cycle significantly ($P < 0.001$) delayed luteal regression beyond day 20 (to day 26).

3 Injection of OA maintained plasma progesterone secretion at $4\text{--}5 \text{ ng ml}^{-1}$ till day 23 of the cycle and suppressed the increase in PGFM concentration.

4 Corpus luteum extract (100 μl) of OA-treated animals released a significant ($P < 0.001$) amount of $\text{PGF}_{2\alpha}$ from rat uterus *in vitro* as did authentic oxytocin. This oxytocic material failed to release $\text{PGF}_{2\alpha}$ during luteolysis in the goat, suggesting that oxytocin receptors for $\text{PGF}_{2\alpha}$ release may be occupied by OA.

5 It is concluded that oxytocin-receptor interaction in the uterus may be the stimulus for $\text{PGF}_{2\alpha}$ release which triggers luteal regression in the goat.

Introduction

There is evidence now to suggest that the corpus luteum of non-pregnant women, cows, sheep and goats contains oxytocin (Wathes & Swan, 1982; Flint & Sheldrick, 1982; Wathes *et al.*, 1982; 1983; Homeida, 1986). Oxytocin (OT) given exogenously in pharmacological doses interrupted the oestrous cycle of the goat and cow and caused luteolysis (Armstrong & Hansel, 1959; Cooke & Knifton, 1981). The physiological role of luteal OT, though uncertain, may be to facilitate the control of luteal function; the best evidence to date for such a role is that obtained from sheep and goats immunized against OT in which luteal regression is delayed (Sheldrick *et al.*, 1980; Cooke & Homeida, 1985). However, the response to OT immunization is quite variable both within and between the animals, and antisera produced might have significant cross-reactions with related compounds (Pavel *et al.*, 1979; Sheldrick *et al.*, 1980; Schams *et al.*, 1983; Cooke & Homeida, 1985). For this reason an attempt was made to inhibit the action of endogenous OT on the release of prostaglandin $F_{2\alpha}$ ($\text{PGF}_{2\alpha}$) from the uterus and hence prevent luteolysis, in the goat by the use of an oxytocin antagonist (OA).

Methods

Animals and treatments

The 12 non-pregnant Nubian goats that were used were 3–4 years old, clinically normal and experiencing regular cycles (19–21 days). On day 5 of the oestrous cycle, the animals were anaesthetized with pentobarbitone sodium (Sagatal, May & Baker Ltd. England), injected intravenously at a dose of 0.45 g kg^{-1} body weight, and maintained with halothane (Fluothane, ICI Ltd, England) and oxygen in a closed system. At laboratory, a femoral artery was catheterized with a polyvinyl catheter. The catheter was fed 20 cm into the vessel so that the tip lay in the abdominal aorta 6–10 cm above the origin of the uterine artery.

On day 12 of the cycle the animals were divided randomly into two equal groups. Animals in group A received 0.25 ml saline (9 g NaCl per litre) injected through the femoral arterial catheter over a period of 90 s daily between days 10 and 20 of cycle. Animals in group B were treated exactly as in group A but with an oxytocin antagonist (OA), (1-(B-mercapto-B, B-

diethyl-propionic acid), 2-O-ethyl tyrosine, 8-ornithine) vasotocin (dEt₂ Tyr(Et).OVT, code KB-IV-24; a gift from Professor Manning). The synthesis and pharmacological properties have been described by Bankowski *et al.* (1980). OA was given at a dose of 0.2 µg kg⁻¹ body weight in 0.25 ml saline (a total dose of about 8 µg). This dose was selected because in a preliminary study it inhibited the release of PGF_{2α} from the rat uterus *in vitro*, delayed luteal regression and produced no ill effects in the goats. The solution of OA was prepared fresh and kept in ice until used.

Corpora lutea (CL) were removed under halothane anaesthesia from three animals from each group on day 18 of the cycle. CL extracts were prepared as previously described (Homeida, 1986).

Jugular vein blood (5 ml) was collected every 2 h between 07 h 00 min and 19 h 00 min by venepuncture using 23 gauge needles between days 12 and 23 of the cycle. All blood samples were collected into chilled heparinized nylon syringes, stored in ice for a short period and centrifuged at 1500 g for 10 min. Plasma samples were stored at -30°C until analysis.

All females were checked for oestrus (day 0) at least twice daily and considered to be in oestrus when they allowed mounting by a male goat.

Hormone assays

The concentration of progesterone and the PGF_{2α} metabolite, 13,14-dihydro-15-keto prostaglandin F_{2α} (PGFM) in the plasma were estimated by radioimmunoassay (RIA) as previously described (Cooke & Homeida, 1985; Homeida, 1986). The intra- and inter-

assay coefficients of variation were 4.6% (*n* = 12) and 10.6% (*n* = 15), respectively for progesterone and 8.0% (*n* = 9) and 11.2% (*n* = 11), respectively for PGFM. Assay sensitivity was 75 pg ml⁻¹ for progesterone and 45 pg ml⁻¹ for PGFM.

Bioassay

The freeze-dried luteal extracts were pooled and re-constituted in Dejalon's solution. These were tested on *in vitro* rat uterus on day 1 post partum. The experimental protocol PGF_{2α} extraction and RIA were as described elsewhere (Homeida, 1986). PGF_{2α} intra- and inter-assay coefficients of variation were 5.2% (*n* = 12) and 8.4% (*n* = 14), respectively.

Data were analysed by Student *t* test (Snedecor & Cochran, 1967).

Results

The mean (± s.e.mean) length of the oestrous cycle in the 3 goats injected with OA (group B) was 26 ± 1.3 days (*n* = 3) and this was significantly (*P* < 0.001) longer than that of 19.2 ± 0.5 days (*n* = 3) in goats injected with saline (group A).

Plasma concentration of progesterone and PGFM are summarized in Table 1.

Progesterone concentration declined on day 15 of the cycle and thereafter till the day of oestrus (19–20 days after the first oestrus) in group A, whereas progesterone concentration was maintained at about 4–5 ng ml⁻¹ throughout the sampling period (day 23)

Table 1 Mean (± s.d.) plasma concentration of progesterone and 13,14-dihydro-15-keto-prostaglandin F_{2α} (PGFM) in goats injected with saline (group A) or oxytocin antagonist (OA, group B) between days 12 and 23 of the oestrous cycle

Days of oestrous cycle	Progesterone (ng ml ⁻¹)		PGFM (pg ml ⁻¹)	
	Group A (<i>n</i> = 3)	Group B (<i>n</i> = 3)	Group A	Group B
12	5.3 ± 0.3	5.0 ± 0.4	108 ± 10	110 ± 11
13	4.8 ± 0.4	5.4 ± 0.4	150 ± 10	118 ± 11
14	4.4 ± 0.3	4.8 ± 0.4	320 ± 18	201 ± 12*
15	3.3 ± 0.3	4.6 ± 0.3*	390 ± 20	115 ± 11*
16	2.4 ± 0.2	5.1 ± 0.4*	328 ± 28	110 ± 11*
17	1.2 ± 0.2	4.8 ± 0.3*	510 ± 32	125 ± 12*
18	0.5 ± 0.2	4.3 ± 0.3*	560 ± 30	130 ± 14*
19	0.1 ± 0.1	4.7 ± 0.3*	410 ± 26	115 ± 13*
20	0.1 ± 0.1	4.6 ± 0.4*	320 ± 19	108 ± 13*
21	0.1 ± 0.1	4.8 ± 0.4*	230 ± 14	116 ± 12*
22	0.5 ± 0.2	4.4 ± 0.3*	125 ± 10	120 ± 12
23	1.2 ± 0.4	4.5 ± 0.3*	103 ± 10	103 ± 11

**P* < 0.001 significantly different from controls.

Numbers in parentheses are the number of animals.

Table 2 Release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from rat uterine horns ($ng\ g^{-1}$ wet wt. h^{-1}) after treatment with Dejalon's solution (control), $1\ \mu g$ oxytocin (OT) or corpus luteum (CL) extract of non-pregnant goat

Control	OT-treated	CL-group A (n = 3)	CL-group B (n = 3)
28 ± 3.1	$63 \pm 6^*$ (8)		
29 ± 3.2		27 ± 2.9 (11)	
26 ± 2.7			$72 \pm 8^*$ (9)

Values are mean $\pm$ s.e.mean.

* $P < 0.001$ significantly different from controls.

Numbers in parenthesis are the number of experiments.

in group B. The values of progesterone in group B were significantly higher ($P < 0.001$) on days 15 to 23 than in group A.

Basal levels of PGFM varied between 100 – $125\ pg\ ml^{-1}$. Peaks greater than $300\ pg\ ml^{-1}$ were observed during days 14 to 20 in group A and were significantly ($P < 0.001$) higher than in group B. The concentration of PGFM released between days 12 and 23 in group B remained at the basal level.

The effects of oxytocin and CL extract on the release of $PGF_{2\alpha}$ from the rat uterus are summarized in Table 2.

The addition of $100\ \mu l$ of CL extract from group B released significant ($P < 0.001$) levels of $PGF_{2\alpha}$ from the rat uterus compared to control or group A.

Discussion

This study demonstrates that systemic administration of OA to goats around luteolysis maintained a functional CL, as indicated by the high level of peripheral plasma concentration of progesterone, and suppressed the increase in PGFM concentration. Similar effects using immunization against oxytocin have been reported in goats and sheep (Sheldrick *et al.*, 1980; Schams *et al.*, 1983; Cooke & Homeida, 1985). However, in contrast to the immunization studies, very little varia-

tion in the degree of cycle length was observed in this study.

Although oxytocin was present in CL of goats treated with OA on day 18 of the cycle, its ability to release uterine $PGF_{2\alpha}$, as indicated by its metabolite PGFM, was impaired. Similar effects were demonstrated *in vitro* when oxytocin co-administered with OA failed to release $PGF_{2\alpha}$ from rat uterus (Homeida, 1986). OA used in this study was also shown to be a potent antagonist to oxytocin in rat uterus and milk ejection responses (Bankowski *et al.*, 1980) and antagonists other than dEt_2 Tyr(Et)-OVT given intravenously to rats have been shown to inhibit both the uterotonic and prostaglandin-releasing action of oxytocin (Chan *et al.*, 1982). It has been reported that the degree of stimulation of $PGF_{2\alpha}$ synthesis by the endometrium of ewe is paralleled by the increased concentration of oxytocin binding sites or receptors (Soloff *et al.*, 1977). OA competes specifically with oxytocin for receptors in proportion to their biological activity *in vitro* (Soloff, 1979; Bankowski *et al.*, 1980); these receptors are the main factor controlling the uterine responsiveness to oxytocin. It would seem likely that the oxytocin-receptor interaction plays a major stimulus for the synthesis and release of uterine $PGF_{2\alpha}$, the luteolysin, and triggers luteolysis in the goat, since exogenous administration of oxytocin induced luteal regression via $PGF_{2\alpha}$ release (Cooke & Homeida, 1982; 1983) and active immunization of goats against oxytocin (Cooke & Homeida, 1985) and injection of OA suppressed $PGF_{2\alpha}$ release and delayed luteal regression. It should be noted that the role of oestrogen and progesterone in the synthesis of oxytocin receptors in the uterus is a prerequisite for the effects of oxytocin on that organ (Roberts *et al.*, 1975; Poyser, 1984; Sheldrick & Flint, 1985). However, oxytocin has also been shown to have a local role in the control of steroidogenesis (Tan *et al.*, 1982).

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