Suppression of prostaglandin F-2α release and delay of luteolysis after active immunization against oxytocin in the goat

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Summary. Active immunization against oxytocin significantly prolonged the oestrous cycle in 3 out of 4 goats; the mean (± s.e.m.) cycle length was 29.1 ± 1.7 days (n = 12) compared to 19.4 ± 0.6 days (n = 9) in control animals. During Days 10–21 of the cycle in the 3 responsive goats, peripheral plasma concentrations of progesterone and oxytocin were steady and those of 13,14-dihydro-15-keto-prostaglandin F-2α were very low (50–100 pg·ml⁻¹) with no marked pulsatile activity. The major effect of immunization would appear to be suppression of the synthesis of the uterine luteolysin PGF-2α, thus confirming that endogenous oxytocin has a facilitatory role in luteolysis via prostaglandin production.

Introduction

Prostaglandin (PG) F-2α and oxytocin are intimately involved in the mechanism of luteolysis in ruminant animals. In sheep, luteolysis is associated with the synchronous pulsatile release of the PGF-2α metabolite 13,14-dihydro-15-keto-PGF-2α (PGFM), and oxytocin (Flint & Sheldrick, 1983) or oxytocin-associated neurophysins (Fairclough et al., 1980); similarly, in the goat, luteolysis is characterized by surges of PGFM and oxytocin (Cooke & Homeida, 1984). There is evidence for both species (Flint & Sheldrick, 1983; Cooke, Homeida & Watkins, 1984) that this oxytocin is derived from the ovary. Exogenous oxytocin will induce luteolysis in goats via production of PGF-2α (Cooke & Homeida, 1982, 1983) but whether ovarian oxytocin has a similar physiological role is not certain. The best evidence to date of a role for endogenous oxytocin in the control of luteal function is probably that obtained from sheep immunized against oxytocin, in which luteal regression is delayed (Sheldrick, Mitchell & Flint, 1980; Schams, Prokopp & Barth, 1983). In this study an attempt was made to control endogenous oxytocin activity in the goat by active immunization and to observe the effects on luteolysis and the release of PGF-2α.

Materials and Methods

Seven mature female goats were used between July and December 1983; all animals had had a normal pattern of oestrous cycles (19–21 days) in the previous breeding season (1982/83). The goats were divided randomly into two groups: in Group 1 (controls), the goats were injected subcutaneously (s.c.) or intradermally (i.d.) with bovine serum albumin and Freund’s complete adjuvant, and in Group 2 (immunized) they were injected s.c. or i.d. with oxytocin–bovine serum albumin conjugate and Freund’s complete adjuvant.

Oxytocin was conjugated to bovine serum albumin with glutaraldehyde (Kagan & Glick, 1974). Briefly, 20 mg bovine serum albumin (Sigma, Poole, U.K.) were mixed with 2 mg oxytocin (Sandoz Inc., Basel, Switzerland) and dissolved in 1 ml 0.1 M-phosphate buffer (pH 7.5); 1 ml
0.2 M-glutaraldehyde (Sigma) was added drop by drop with constant stirring. The conjugate was separated from un conjugated oxytocin by dialysis. The conjugation ratio, determined by incorporation of 125I-labelled oxytocin, was in the range 4.2–5.6 mol oxytocin/mol bovine serum albumin.

This material (1 mg) was emulsified in 2 ml 0.9% NaCl (w/v) and 4 ml Freund’s complete adjuvant (Sigma). Each animal in Group 2 was injected with this material i.d. into multiple sites along the back at 2-week intervals between 22 June and 26 August, then at 3-week intervals between 21 September and 10 December. These injections caused severe abscessation and so s.c. injection was sometimes used. The 3 control goats (Group 1) were injected according to the above schedule with bovine serum albumin and Freund’s complete adjuvant only. Blood was collected 14 days after each booster injection and sera were separated and stored at –30°C until analysis. Antibody titres were evaluated at dilutions of 1:200 and 1:2000 by incubating samples of diluted serum (0.1 ml) with 125I-labelled oxytocin (0.1 ml) overnight at 4°C in 0.05 M-phosphate-buffered saline containing 0.1% sodium azide and 0.2% bovine γ-globulin (pH 7.5). Gamma globulins were precipitated using polyethylene glycol 6000 (0.2 ml 30% w/v) solution in water; bound 125I-labelled oxytocin was measured in the resulting pellet (Sheldrick et al., 1980). Specificities of the antisera were assessed by comparing the amounts of oxytocin standard and various related compounds needed to displace 50% 125I-labelled oxytocin from the antibody.

Throughout the experimental period the goats were housed in individual pens under conditions of natural daylength and temperature; they were checked 3 times daily for signs of oestrus using a fertile buck. Oestrous cycle lengths were recorded. Near the end of the experimental period intensive jugular blood sampling was carried out in each animal; heparinized samples were obtained by direct venepuncture each hour from 09:00 to 17:00 h between Days 10 and 21 of the oestrous cycle. Plasma was snap-frozen in solid CO2-acetone and stored at –30°C until analysis. Concentrations of oxytocin, PGFM and progesterone were determined in each plasma sample.

Radioimmunoassay of hormones

Oxytocin. Oxytocin (450 i.u. mg−1; Sandoz) was iodinated using the chloramine-T method and purified by column chromatography using Sephadex G-25 (Mitchell, Mountford, Natale & Robinson, 1980). The specific activity of the 125I-labelled oxytocin was about 200 Ci·g−1.

The extraction on Vycor glass powder and the assay procedure used were as described by Sheldrick & Flint (1981). The oxytocin antibody (GJ/137/1, provided by Dr A. P. F. Flint) was raised in sheep against oxytocin conjugated to bovine serum albumin (Sheldrick et al., 1980). It was used at a dilution of 1:30 000. The cross-reactions obtained (relative to oxytocin = 100%) were arginine vasopressin 0–125%; lysine vasopressin, arginine vasotocin, angiotensin, LH, PGFM and progesterone < 0.02%. Assay sensitivity (calculated from 2 x s.d. below the zero-point binding) was 2·1 pg/ml. The intra- and inter-assay coefficients of variation were 10-9% (n = 10) and 12.6% (n = 12), respectively, for a sample with a low concentration of oxytocin (20 pg·ml−1), and 11.2% (n = 13) and 14.2% (n = 10), respectively, for a sample with a high concentration (100 pg·ml−1). Extraction recovery was between 88 and 94%, and this was taken into account in calculating concentrations. The accuracy of the assay was determined by extracting and assaying plasma samples to which various amounts of oxytocin had been added; regression analysis of the resulting data gave y (amount recovered) = 0.90x – 1.03 (r = 0.98).

Selected plasma samples showing high oxytocin immunoreactivity also exhibited marked biological activity in the superfused rat uterus bioassay (Fitzpatrick, 1961). This biological activity was not caused by acetylcholine, 5-hydroxytryptamine or prostaglandins, being unaffected by both atropine and cyproheptadine. However, activity was abolished by treatment of the plasma extract with oxytocin antibody (100 µl of 1:1000 dilution) or with sodium thioglycollate (Sigma; 20 µl of 0.2 M solution at pH 7.5) which reduces the disulphide bond in the oxytocin ring structure causing inactivation (Kumaresan, Kagan & Glick, 1969).
**Oxytocin and luteolysis in the goat**

PGFM. PGFM was extracted from plasma with diethyl ether and assayed as previously described (Cooke & Knifton, 1981). The antibody (Upjohn, Kalamazoo, MI, U.S.A.) was used at a final dilution of 1:1000 and cross-reactions (relative to PGFM = 100%) were: 15-keto-PGF-2α, 7-5%; 13,14-dihydro-PGF-2α, 0-7%; PGE-2, 0-5%, and PGF-2α < 0-01%. The sensitivity of the assay was 45 pg/ml. The intra- and inter-assay coefficients of variation were 8·1% (n = 10) and 12% (n = 11), respectively, for a sample with a low concentration of PGFM (100 pg·ml⁻¹), and 8-2% (n = 11) and 11-6% (n = 9), respectively, for a sample with a high concentration (800 pg·ml⁻¹). Extraction efficiency was 84·1 ± 7·1 (s.d.)% and results were not corrected for extraction losses.

**Progesterone.** Progesterone was extracted in hexane and assayed using the method of Hotchkiss, Atkinson & Knobil (1971). The antibody (provided by Dr H. Dobson) was raised in rabbits against progesterone-11-succinyl-BSA and used at a final dilution of 1:7000. Cross-reactions (relative to progesterone = 100%) were: 20α-dihydroprogesterone, 20β-dihydroprogesterone, corticosterone and 11-deoxycorticosterone < 0·1%. The sensitivity of the assay was 75 pg·ml⁻¹. The intra- and inter-assay coefficients of variation were 4·8% (n = 7) and 12·2% (n = 9), respectively, for a plasma sample of low progesterone concentration (500 pg·ml⁻¹) and 5·1% (n = 9) and 14·6% (n = 11), respectively, for a sample with a high concentration (4 ng·ml⁻¹). Extraction efficiency was 82·4 ± 6·4 (s.d.)%, and the results were corrected for extraction losses.

Data were analysed using Student’s t test and the Mann–Whitney test (Snedecor & Cochran, 1967).

**Results**

The mean (± s.e.m.) length of the oestrous cycle in the 4 goats immunized against oxytocin (Group 2) was 29·1 ± 1·7 days (n = 12) and this was significantly (P < 0·001) longer than that of 19·4 ± 0·6 days (n = 9, range 17–22 days) in goats immunized against bovine serum albumin (Group 1). No oxytocin binding activity was detected in sera of the control animals. Responses to immunization of Group 2 differed between animals both in the rapidity and degree of antibody production (see Text-fig. 1). In Goat A, antibody concentration remained extremely low (< 10%) throughout the experimental period and cycles were not prolonged (20–21 days). In Goat B, however, antibody concentration rose very quickly, being 20% (at 1:200 dilution) in August, rising rapidly to 60% thereafter; cycle lengths were between 35 and 36 days. In Goat C the rise in antibody concentration was much slower, being 20% in September, 45% in October, 60% in November but finally reaching 75% at the end of the experiment; oestrous cycles varied between 27 and 29 days. Goat D occupied an intermediate position in terms of antibody concentration (20% in August, 50% in September and 70% from October onwards) and cycle length (32–33 days).

Antisera produced by 3 of the 4 goats immunized against oxytocin showed no significant cross-reactions with related peptides, other than with arginine vasotocin (2-2–5·9%). The antisera also failed to cross-react with other compounds involved in cyclic ovarian activity.

The effects of immunization of animals against bovine serum albumin and oxytocin on plasma concentrations of progesterone, PGFM and oxytocin are illustrated in Text-figs 2(a) & 2(b), respectively. In those goats that responded to immunization with the production of oxytocin antibodies, progesterone concentrations remained steady and in excess of 4 ng/ml throughout the sampling period. Oxytocin concentrations, which approximately paralleled those of progesterone, remaining steady, rather than pulsatile, were significantly (P < 0·001) raised relative to those in control goats (mean ± s.e.m. 70·1 ± 0·7 pg/ml, n = 312, vs 35·5 ± 1·0 pg/ml, n=263). However, this high immunological activity was not reflected in high biological activity in the superfused rat uterus bioassay; biological activity was abolished. PGFM concentrations remained at low levels (50–100 pg/ml) with no marked pulsatile activity.
From the treatment group the hormone profiles of Goat A, which did not respond to immunization, resembled those of the 3 control goats; i.e. a decline in progesterone concentrations starting on Days 12–14 indicating luteolysis, peaks of PGFM from Days 12–14 of 200 pg/ml, rising in magnitude (600–800 pg/ml) until oestrus, and pulses of oxytocin from Day 13 onwards superimposed on declining concentrations.

**Discussion**

These results show that, in the goat, as in sheep (Sheldrick et al., 1980; Schams et al., 1983), active immunization against oxytocin causes a significant prolongation of the oestrous cycle. Peripheral progesterone concentrations indicate that this increase in length of the cycle reflects a lengthening of the functional life-span of the corpus luteum.

A different immunization schedule was used in this study from those used for sheep. Sheldrick et al. (1980) began immunization during the breeding season and found that the degree of cycle prolongation could generally be related to the concentration of circulating antibodies; mean cycle length was extended by approximately 4 days. Schams et al. (1983) immunized sheep regularly for
12 months before cycle lengths were observed; mean cycle length was extended by about 10 days, but variability both within and between animals was very high. Goats were immunized regularly 3 months before the start of the breeding season, and while 1 animal failed to respond at all, in those that did there was relatively little variation in the degree of cycle extension.

As in sheep (Sheldrick et al., 1980) immunization against oxytocin raised plasma concentrations of the peptide; also, these plasma concentrations did not fall at the expected time of oestrus (Days 17–21). Such effects are probably due to a reduction in the metabolic clearance of oxytocin.

Antisera from the 3 goats with prolonged cycles showed no significant cross-reactions with related compounds, other than with arginine vasotocin. This has been shown to interfere with LH release in male rats when administered into the cerebral ventricles (Pavel, Luca, Calb & Goldstein, 1979); there is the possibility, therefore, that prolongation of the cycle may have been due to a
central effect. However, apart from the fact that Sheldrick et al. (1980) found negligible levels of antibody in the cerebrospinal fluid, the hormone profiles over Days 10–21 show that probably the major effect of immunization against oxytocin in the goat is to suppress the synthesis of PGF-2α, the uterine luteolysin. It would appear, therefore, that the pharmacological effect of oxytocin (Cooke & Homeida, 1982, 1983) is mimicked physiologically during luteolysis. These results confirm the earlier suggestions (Sheldrick et al., 1980; Schams et al., 1983), that luteal oxytocin plays a facilitatory role in the control of luteal function, through uterine prostanaglandin production.

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References


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