Br. J. Pharmac. (1986), **87**, 673–676

Evidence for the presence of oxytocin in the corpus luteum of the goat

A.M. Homeida

Department of Veterinary Medicine, Pharmacology and Toxicology, University of Khartoum P.O. Box 32, Khartoum North, Sudan

1 Purified acetic acid extracts of corpora lutea (CL) of non-pregnant goats were found to contain substantial amounts of oxytocin (OT) as measured by radioimmunoassay.

2 OT standard and the CL extracts released prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from rat isolated uteri in a quantitatively similar manner.

3 Treatment of both OT standard and CL extract with sodium thioglycolate, oxytocin antiserum or oxytocin antagonist abolished this biological activity.

4 Acid extracts of CL of pregnant goat were found to contain approximately 2% of levels during the cycle by day 21 after fertile mating and this had a reduced ability to release $PGF_{2\alpha}$ from rat uterus.

5 It is concluded that both the immunoreactivity and the biological activity of the CL extracts are due to the presence of an oxytocin-like substance and that tissue levels of oxytocin are low in pregnant compared to non-pregnant goats.

Introduction

Peripheral plasma concentrations of oxytocin (OT) are raised during the luteal phase of the oestrous cycle of the goat, and parallel those of progesterone (Homeida & Cooke, 1982; 1983). Furthermore, oxytocin will induce luteolysis in the goat via production of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Cooke & Homeida, 1982). These results suggest that oxytocin may be derived from the corpus luteum (CL) and be involved in the process of luteolysis. Oxytocin has been detected in the CL of sheep (Flint & Sheldrick, 1983), cows (Wathes *et al.*, 1983) sheep (Wathes & Swann, 1982) and women (Wathes *et al.*, 1982) by use of high performance liquid chromatography, milk ejection and rat uterus strip bioassays and radioimmunoassays (RIA) (Wathes & Swann, 1982). However the RIA employed are open to criticism on the grounds that immunological activity can be separated from biological activity, and the RIA may measure degraded and biologically inactive fragments of OT (Kumaresan *et al.*, 1969). In this paper a simple bioassay has been used to test the validity of results obtained by OT RIA of luteal extracts from the goat. This was used to demonstrate that the CL of the non-pregnant goat contains an oxytocin-like substance.

Methods

The USP Posterior Pituitary Reference Standard (a gift from Professor Manning) was used in all assays for agonistic activity.

CL were removed under halothane anaesthesia from six goats; three non-pregnant between days 8 and 9 of the oestrous cycle and three pregnant between days 20 and 21 after fertile mating. CL were weighed and acetic acid extracts were prepared as described by Flint & Sheldrick (1983). The crude acid extracts or oxytocin standard were added to separate columns (32 × 1 cm) of Sephadex G25–80 (Mitchell *et al.*, 1980) equilibrated and eluted with human serum albumin (1.25 mg ml^{-1}) in 0.25% v/v acetic acid. Fractions (1 ml) were collected, extracted on Vycor glass powder and assayed for oxytocin by RIA (Chard *et al.*, 1969; Cooke & Homeida, 1985). Oxytocin in the crude extract or oxytocin standard eluted between 16 and 20 ml fractions. Log dose-response curves for oxytocin standard (0, 10, 20, 30 and 40 μ) and CL extract (0, 100, 200, 300 and 400 μl) using the rat uterus bioassay were tested for parallelism by comparing their slopes (Tallarida & Murray 1981).

Jugular vein blood was obtained by direct venepuncture before removal of CL and centrifuged at 1500 *g* for 10 min. Plasma was stored at −30°C until analysis.

Oxytocin radioimmunoassay

The concentration of OT in the extracts and progesterone in the plasma were estimated by RIA as previously described (Homeida & Cooke, 1982; 1983). The OT anti-serum was raised in sheep against OT conjugated to bovine serum albumin (Sheldrick *et al.*, 1980) and the progesterone anti-serum (provided by Dr H. Dobson, Department of Veterinary Clinical Sciences, University of Liverpool) was raised in rabbits against progesterone-11-succinyl bovine serum albumin. Cross reactions have been described elsewhere (Cooke & Homeida, 1984). The intra- and inter-assay coefficient of variation were 10.9% ($n = 10$) and 12.2% ($n = 12$), respectively for oxytocin and 4.8% ($n = 7$) and 11.2% ($n = 9$), respectively for progesterone. Assay sensitivity was 2.1 pg ml^{-1} for oxytocin and 75 pg ml^{-1} for progesterone.

Bioassay

The freeze-dried luteal extracts were pooled and reconstituted in De Jalon's solution of the following composition (mM): NaCl 154, glucose 2.77, $NaHCO_3$ 5.95, $CaCl_2$ 0.06 and KCl 5.7. Individual uterine horns from 1-day post-partum Wistar rats (180–250 g) were suspended in a 7 ml tissue bath containing De Jalon's solution aerated with 5% CO_2: 95% O_2, at pH 7.4, 32°C. From each uterus, one horn was used as a test tissue, and the other as its paired control. After 60 min equilibration, during which time the bath fluid was changed every 15 min, the uterine horn was exposed to either 0.5 μ (1.1 ng), 1 μ (2.2 ng), 2 μ (4.4 ng) and 4 μ (8.8 ng) OT standard or equivalent amounts of CL extract based on the mean OT concentration obtained by RIA, or De Jalon's solution alone (control). The bath fluid was removed every 15 min for 1 h, snap-frozen in cardice-acetone and stored at −30°C until analysis. These experiments were repeated on fresh tissue after treatment of the OT standard (2 min) and CL extract with one of the following: (a) 20 μl 0.02 M sodium thioglycollate (Sigma, Poole, Dorset) at pH 7.5 for 30 min at room temperature (Ames *et al.*, 1950); (b) 0.1 ml of oxytocin antibody (dilution 1:30,000) and incubated for 8 h; (c) 0.1 ml of non-immune serum (obtained from sheep immunized with bovine serum albumin) and incubated for 8 h; (d) 1, 2 and 4 μg doses of oxytocin antagonist, (1- (β-mercapto-β, β-diethylpropionic acid), 2-0-ethyl tyrosine, 8- ornithine) vasotocin (d Et_2 Tyr (Et). OVT, code KB-IV-24; a gift from Professor Manning). The synthesis and pharmacological properties of the antagonist have been described by Bankowski *et al.* (1980). After use each uterine horn was lightly blotted and weighed.

Prostaglandin extraction and assay

Samples (3 ml) of pooled bath fluid from each experiment were acidified to pH 3 with 1 N HCl and extracted twice with an equal volume of ethyl acetate (Analar, B.D.H., Poole). The organic phase was evaporated to dryness under N_2 at 35°C and reconstituted in 0.2 ml of benzene/ethyl acetate/methanol (6:4:1). This was then subjected to column chromatography for separation of the different prostaglandins (Jaffe & Behrmann, 1974). The $PGF_{2\alpha}$ fractions were dried under N_2 at 50°C and the $PGF_{2\alpha}$ concentration estimated by use of a specific RIA (Cooke & Knifton, 1980). The antibody was used at a dilution of 1:9000; cross reactions were reported by Sharma (1972). The efficiency of extraction of 0.0013 μCi (3 pg) [^{3}H]-$PGF_{2\alpha}$ (Sp. Act. 160 Ci mmol^{-1}; Radiochemical Centre Amersham) added to the incubate was 76% ($n = 9$) and values were corrected for extraction losses. The intra- and inter-assay coefficients of variation were 5.4% ($n = 8$) and 8.8% ($n = 8$). Linear regression of the recovery of various amounts of $PGF_{2\alpha}$ added to tissue incubate gave by (amount recovered) = $0.92 \times - 3.11$ ($r = 98$).

The results were assessed by means of a paired *t* test.

Results

The mean (± s.e.mean) OT concentration in the CL of the non-pregnant goat as measured by RIA was 1445 ± 16 ng g^{-1} wet weight of tissue. As the average weight of caprine luteum is approximately 1 g and because there are normally 1–3 CL per animal, a non-pregnant goat in the peak luteal phase of the oestrous cycle has approximately 1.5–4.5 μg of ovarian oxytocin. The mean OT concentration in the CL of pregnant goats was 28 ± 3 ng g^{-1} wet weight of tissue (pregnancy was confirmed by peripheral plasma progesterone concentrations = 4.5 ng ml^{-1} on day 20 after fertile mating).

The slopes of log dose-response curves of the two lines (standard oxytocin versus CL extract) did not differ significantly ($t <$ tabular value 2.33; $P < 0.01$).

The effects of OT standard and CL extract on the uterine release of $PGF_{2\alpha}$ are summarized in Table 1. OT and CL extract stimulated release of $PGF_{2\alpha}$ from the rat uterus in a quantitatively similar manner. The biological activity of both was inhibited by pretreatment with thioglycollate and oxytocin antibody. The results of the addition of oxytocin standard, CL extract of non-pregnant goat or oxytocin antagonist at various doses to uterine tissue are summarized in Table 2. Addition of oxytocin standard (0.5–4 μ) or

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

Control	*OT-treated*	*CL extract-treated*
26 ± 2.1	61 ± 6* (9)	
27 ± 2.8		66.3 ± 8* (8)
25 ± 2.6	26.5 ± 3$ (8)	
29 ± 3.1		33.3 ± 4$ (8)
26 ± 3.0	24.2 ± 2.5† (10)	
28 ± 3.0		30 ± 3.3† (12)
29 ± 2.1	59 ± 6** (4)	
27 ± 3.1		62 ± 7** (4)

Values are mean ± s.e.mean.
Numbers in parentheses are the number of experiments.
*$P < 0.01$ significantly different from controls.
$OT and CL extract with sodium thioglycollate before the experiment.
†OT and CL extract treated with oxytocin antiserum before the experiment.
**OT and CL extract treated with non-immune serum before the experiment.

CL extract to uterine tissue resulted in a dose-dependent increase in oxytocin-stimulated release of $PGF_{2\alpha}$ from the uterus, whereas addition of the antagonist resulted in a dose-dependent decrease in oxytocin- or CL extract-stimulated release of $PGF_{2\alpha}$ from the uterus. As the CL extract of pregnant goat contained 2% of the levels of oxytocin during the cycle, similar dilutions to that of non-pregnant CL resulted in a negligible increase in $PGF_{2\alpha}$ release (i.e. maximum of 31 ng g^{-1} h^{-1}).

Discussion

Acid extracts of CL collected from non-pregnant goats were found, using RIA, to contain OT, in amounts similar to those reported for sheep (Flint & Sheldrick, 1983) and cows (Wathes *et al.*, 1983); and that oxytocin in corpora lutea falls during pregnancy in the goat, confirming earlier reports in sheep (Wathes & Swann 1982; Sheldrick & Flint 1983) and cow (Wathes *et al.*, 1983).

The results presented show that CL extract of non-pregnant goat was biologically active in releasing $PGF_{2\alpha}$ from rat uterus *in vitro* similar to oxytocin standard. The reduced ability of CL extract of pregnant goat to release $PGF_{2\alpha}$ suggests that the conceptus may be responsible for the reduction in luteal content of oxytocin. The post-partum rat uterus was used, as it is responsive to OT and releases substantial amounts of $PGF_{2\alpha}$; however the basal release is very low (Chan, 1977). It is generally believed that the release of prostaglandins is immediately preceded by their rapid synthesis and that degradation of $PGF_{2\alpha}$ by the *in vitro* uterus is low (Horton & Poyser, 1976); it is therefore possible to equate the amounts of $PGF_{2\alpha}$ released in response to OT with its synthesis. A one hour exposure period was chosen as OT has a slow onset of action (Roberts *et al.*, 1976). The observations that pretreatment of the luteal extract with thioglycollate, oxytocin antibody and oxytocin antagonist abolished the $PGF_{2\alpha}$-releasing activity is further proof that such activity is due to the presence of oxytocin-like material (Kumaresan *et al.*, 1969). Furthermore, parallelism of dose-response curves on rat uterus was observed between the oxytocin standard and CL extract. The biological activity in the superfused rat uterus bioassay (Fitzpatrick, 1961) was not caused by acetylcholine, 5-hydroxytryptamine, histamine or prostaglandins, being unaffected by both atropine and cyproheptidine (Cooke & Homeida, 1984). Therefore, although the peptide has been neither purified nor sequenced, there is substantial evidence to suggest that the caprine CL contains OT.

I thank Professor M. Manning, Medical College of Ohio for the gift of oxytocin antagonist and Dr A.P.F. Flint, ARC Institute of Animal Physiology, Babraham, for the oxytocin antiserum.

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 De Jalon's solution (control), 2 μ oxytocin (OT) standard or an equivalent amount of corpus luteum (CL) extract of non-pregnant goat or oxytocin antagonist (A)

Control	*Doses* (μ)	*OT-treated*	*OT + A* A = 1, 2 or 4 μg (8)	*CL-treated*	*CL + A* A = 1, 2 or 4 μg (9)
25 ± 2 (11)	0.5	42 ± 4* (5)		46 ± 4* (6)	
30 ± 2.8 (10)	1	59 ± 6* (5)		61 ± 5* (5)	
28 ± 2.5 (17)	2	69 ± 7* (8)	54 ± 5*, 39 ± 4* or 26 ± 3	72 ± 6* (9)	61 ± 6*, 52 ± 6* or 32 ± 8
26 ± 2.6 (13)	4	76 ± 8* (7)		80 ± 7* (6)	

*$P < 0.01$ significantly different from control.

References

AMES, R.G., MOORE, D.H. & VAN DYKE, H.B. (1950). Pituitary antidiuretic hormone in the urine and its detection in the blood. *Endocrinology*, **46**, 215.

BANKOWSKI, K., MANNING, M., SETO, J., HALDAR, J. & SAWYER, W.H. (1980). Design and synthesis *in vivo* of antagonists of oxytocin. *Int. J. Peptide Protein Res.*, **16**, 382–391.

CHAN, W.Y. (1977). Relationship between the uterotonic action of oxytocin and prostaglandins: Oxytocin action and release of PG – activity in isolated non-pregnant and pregnant rat uteri. *Biol. Reprod.*, **17**, 541–548.

CHARD, T., FORSLING, M.L., JAMES, M.A.R., KITAU, M.J. & LANDON, J. (1969). The development of a radioimmunoassay for oxytocin: sensitivity of the assay in aqueous buffer solution, specificity and dissociation of immunological and biological activity. *J. Endocr.*, **46**, 533–542.

COOKE, R.G. & HOMEIDA, A.M. (1982). Plasma concentration of 13, 14 – dihydro-15-keto prostaglandin $F_{2\alpha}$ and progesterone during oxytocin-induced oestrus in the goat. *Theriogenology*, **18**, 453–460.

COOKE, R.G. & HOMEIDA, A.M. (1984). Delayed luteolysis and suppression of the pulsatile release of oxytocin after indomethacin treatment in the goats. *Res. Vet. Sci.*, **36**, 48–51.

COOKE, R.G. & HOMEIDA, A.M. (1985). Suppression of prostaglandin $F_{2\alpha}$ release and delay of luteolysis after active immunization against oxytocin in the goat. *J. Reprod. Fert.*, **75**, 63–68.

COOKE, R.G. & KNIFTON, A. (1980). The effect of intra-aortic prostaglandin $F_{2\alpha}$ on uterine motility in pregnant goats. *J. Reprod. Fert.*, **59**, 347–350.

FLINT, A.P.F. & SHELDRICK, E.L. (1983). Evidence for a systemic role of ovarian oxytocin in luteal regression in sheep. *J. Reprod. Fert.*, **67**, 215–225.

FITZPATRICK, R.J. (1961). *Oxytocin*. ed. Caldeyro-Bracia R. & Heller, H. pp. 358–379. Oxford: Pergamon Press.

HOMEIDA, A.M. & COOKE, R.G. (1982). Peripheral plasma concentrations of 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ and progesterone around luteolysis and during early pregnancy in the goat. *Prostaglandins*, **24**, 313–321.

HOMEIDA, A.M. & COOKE, R.G. (1983). Suppression of pulsatile release of oxytocin during early pregnancy in the goat. *Prostaglandins*, **26**, 103–109.

HORTON, E.W. & POYSER, N.L. (1976). Uterine luteolytic hormone: A physiological role for prostaglandin $F_{2\alpha}$. *Physiol. Rev.*, **56**, 595–651.

JAFFE, B.M. & BEHRMAN, H.R. (1974). Prostaglandins E, A & F. In *Methods of Hormone Radioimmunoassay*. ed. Jaffe B.M. & Behrman H.R. pp. 19–34. New York: Academic Press.

KUMARESAN, P., KEGAN, A. & GLICK, S.M. (1969). Oxytocin: effects of degradation on radioimmunologic and biologic activity. *Science*, **166**, 1160–1161.

MITCHELL, M.D., MOUNTFORD, L.A., NATALE, R. & ROBINSON, J.S. (1980). Concentrations of oxytocin in the plasma and amniotic fluid of Rhesus monkeys (*Macaca mulata*) during the latter half of pregnancy. *J. Endocr.*, **84**, 473–478.

ROBERTS, J.S., McCRACKEN, J.A., GAVAGAN, J.E. & SOLOFF, M.S. (1976). Oxytocin-stimulated release of prostaglandin $F_{2\alpha}$ from ovine endometerium *in vitro*: Correlation with oestrous cycle and oxytocin binding. *Endocrinology*, **99**, 1107–1114.

SHARMA, S.C. (1972). A sensitive radioimmunoassay for prostaglandin F. *J. Physiol.*, **226**, 64P.

SHELDRICK, E.L., MITCHELL, M.D. & FLINT, A.P.F. (1980). Delayed luteal regression in sheep immunized against oxytocin. *J. Reprod. Fert.*, **59**, 37–42.

SHELDRICK, E.L. & FLINT, A.P.F. (1983). Luteal concentrations of oxytocin decline during early pregnancy in the ewe. *J. Reprod. Fert.*, **68**, 477–480.

TALLARIDA, R.J. & MURRAY, R.B. (1981). In *Manual Pharmacologic Calculations*. ed. Tallarida, R.J. & Murray, R.B. pp. 11–13. New York: Springer-Verlag.

WATHES, D.C. & SWANN, R.W. (1982). Is oxytocin an ovarian hormone? *Nature*, **297**, 225–227.

WATHES, D.C., SWANN, R.W., PICKERING, B.T., PORTER, D.G., HULL, M.G.R. & DRIFE, J.O. (1982). Neurohypophysial hormones in the human ovary. *Lancet*, **ii**, 410–412.

WATHES, D.C., SWANN, R.W., BIRKETT, S.D., PORTER, D.G. & PICKERING, B.T. (1983). Characterization of oxytocin, vasopresin and neurophysin from the bovine corpus luteum. *Endocrinology*, **113**, 693–698.

(*Received September 19, 1985.*
Revised December 2, 1985.
Accepted December 4, 1985.)