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Evidence for the pulsatile release of PGF-2α inducing the release of ovarian oxytocin during luteolysis in the ewe*

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Summary. Frequent blood samples were removed from a utero-ovarian vein, a jugular vein and a femoral artery of 5 ewes during luteolysis. Analysis of these samples for oxytocin-associated neurophysin revealed a significant venous–arterial difference across the ovary and uterus but not across the head. This occurred during the pulsatile surges as well as when levels were basal and confirms the corpus luteum as a major source of the pulsatile surges of oxytocin-associated neurophysin and oxytocin that occur during CL regression and also of the elevated luteal phase concentrations of both hormones. The pulsatile surges of oxytocin-associated neurophysin measured in the utero-ovarian vein were accompanied by the release of an approximately equimolar amount of oxytocin.

The concentration of PGF-2α in the utero-ovarian vein samples began to increase before the levels of oxytocin and oxytocin-associated neurophysin started to increase. This suggests that uterine PGF-2α initiates the release of ovarian oxytocin and oxytocin-associated neurophysin during luteolysis in the ewe.

Introduction

The corpus luteum (CL) of the sheep contains high concentrations of oxytocin-like immunoreactive material (Wathes & Swann, 1982; Watkins, 1983) which is secreted together with neurophysin I/II (the oxytocin-associated neurophysin) into the ovarian vein (Flint & Sheldrick, 1982; Watkins, Moore, Flint & Sheldrick, 1984b). During the luteal phase of the oestrous cycle in the ewe, oxytocin and oxytocin-associated neurophysin concentrations are elevated in the peripheral circulation (Sheldrick & Flint, 1981; Webb, Mitchell, Falconer & Robinson, 1981; L. G. Moore, W. B. Watkins & R. J. Fairclough, unpublished observation) and are released in pulsatile peaks during luteolysis (Fairclough *et al.*, 1980; Flint & Sheldrick, 1983). Venous–arterial differences of oxytocin and oxytocin-associated neurophysin concentrations across the ovary in anaesthetized ewes (Flint & Sheldrick, 1982; Watkins *et al.*, 1984b) and higher levels of oxytocin in the vena cava than in the jugular vein of cows during the luteal phase (Walters, Schams & Schallenberger, 1984) suggest that the elevated luteal-phase values of oxytocin originate from the CL. This conclusion is supported by the observations that the luteal-phase oxytocin concentration is positively correlated with the number of CL present (Schams, Lahlou-Kassi & Glatzel, 1982; Schams, Prokopp & Barth, 1983) and that the plasma oxytocin concentration can be reduced by cloprostenol-induced CL regression (Flint & Sheldrick, 1983). It is not known whether the pulsatile surges of oxytocin and oxytocin-associated neurophysin during CL regression originate from the CL (Flint & Sheldrick, 1983) or from the posterior pituitary gland (Fairclough *et al.*, 1980; Mitchell, Kraemer, Brennecke & Webb, 1982).

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We have suggested that pulsatile surges of oxytocin induce the release of uterine prostaglandin (PG) F-2α which in turn causes CL regression (Fairclough *et al.* (1980). This is supported by the observation that coincident surges of the PGF-2α metabolite, 13,14-dihydro-15-keto-PGF-2α (PGFM) and oxytocin-associated neurophysin (Fairclough *et al.*, 1980) or oxytocin (Flint & Sheldrick, 1983) occur when hourly plasma samples are taken during luteolysis. Further support for oxytocin causing uterine PGF-2α release during luteolysis is given by the fact that exogenous oxytocin can stimulate the release of uterine PGF-2α, particularly at the end of the cycle when there is a large increase in the number of endometrial oxytocin receptors (Roberts, Barcikowski, Wilson, Skarnes & McCracken, 1975; Roberts, McCracken, Gavagan & Soloff, 1976; Roberts & McCracken, 1976). An alternative inter-relationship between oxytocin and PGF-2α within the utero-ovarian systems is indicated from the finding that cloprostenol (an analogue of PGF-2α) can cause the release of oxytocin and its associated neurophysin into the utero-ovarian vein (Flint & Sheldrick, 1982; Watkins *et al.*, 1984b).

The present experiments were undertaken to determine whether the pulsatile surges of oxytocin-associated neurophysin and oxytocin originate from the posterior pituitary or the corpus luteum and to examine the temporal relationship between oxytocin and PGF-2α plasma concentrations during a pulsatile peak.

Materials and Methods

Animals. The oestrous cycles of 5 Romney ewes were synchronized by insertion, for 12 days, of intravaginal sponges containing 60 mg 6α-methyl-17α-acetoxyprogesterone. The day the sponge was removed was denoted as Day −2 of the oestrous cycle. On Day 10 or Day 12 of the oestrous cycle, the ewes were anaesthetized by an epidural administration of 0·25% bupivacaine hydrochloride and 1% lignocaine (Astra Chemicals, Sydney, Australia) and catheters were inserted into a utero-ovarian vein draining an ovary containing a CL. The operative procedure followed was similar to that described by Flint & Sheldrick (1983) with the catheter inserted down-stream into the ovarian vein and the top of the catheter being placed about 100 mm past the junction of the uterine and ovarian veins. Jugular vein and femoral artery catheters were also introduced.

Blood (1·5 ml) was collected in tubes containing EDTA every 30 min for 12 h from all 3 catheters and about every 6 min for 8 h from the utero-ovarian catheter on Days 13, 14 and 15 of the oestrous cycle. The samples were collected on ice, centrifuged within 20 min and stored at −20°C until assayed.

Hormone assays. Plasma concentrations of oxytocin-associated neurophysin were determined using a radioimmunoassay (RIA) based upon that previously described (Moore & Watkins, 1983). The assay cross-reacts 2·5% with ovine neurophysin-III, the vasopressin-associated neurophysin. Amino acid and analysis of the purified standards of ovine neurophysin-I, -II and -III, prepared according to Moore & Watkins, (1981), revealed that they consisted of 48, 61, 64% protein, respectively, which is comparable to results found for human neurophysins (North, LaRochelle, Melton & Mills, 1980). The quantities measured by RIA have been corrected to account for this discrepancy. The antiserum was raised against ovine neurophysin-II and neurophysin-I was iodinated as described previously (Moore & Watkins, 1983). Each assay tube contained 300 μl antiserum to neurophysin-II at a dilution of 1 : 10 000, 2 μl non-immunized rabbit serum and 100 μl plasma or 100 μl of the reference standard neurophysin-I dissolved in phosphate buffer (Moore & Watkins, 1983). After incubation for 3 days at 4°C, 50 μl ^{125}I-labelled neurophysin-I (120 pg, ~20 000 c.p.m.) were added and the incubation was continued for a further 2 days at 4°C. Separation of antibody-bound and free ^{125}I-labelled neurophysin-I was achieved by the addition of sheep anti-rabbit γ-globulin antiserum (1·5 ml) at a dilution of 1 : 300 in assay buffer containing 3% (w/v) polyethylene glycol. After incubating overnight at 4°C the tubes were centrifuged at 2000 ***g*** for 35 min, and the radioactivity of the precipitate was counted.

The assay had a sensitivity of 63 pg/ml and a range from 330 pg/ml (80% of the zero standard) to 5700 pg/ml (20% of the zero standard). Intra-assay and inter-assay variations were 5·7% and 5·3% respectively for a plasma sample which had a mean value of 1·07 ng/ml ($n = 13$). Parallelism between standard and unknowns was achieved when samples were diluted or had additional neurophysin added to them.

PGF-2α was measured using the RIA described by Liggins, Campos, Roberts & Skinner (1980) adapted for plasma samples. Each assay tube contained: 200 μl PGF-2α antiserum diluted 1 : 4000 in Tris–HCl buffer (pH 8·0); 50 μl utero-ovarian vein plasma; 50 μl Tris–HCl buffer (pH 8·0) or 50 μl plasma obtained from a hysterectomized ewe; standard PGF-2α in 50 μl Tris–HCl buffer. After incubation for 4 h at 4°C, 50 μl tritiated PGF-2α (3000 c.p.m.) in Tris–HCl buffer were added and the mixture was incubated for a further 20 min at 4°C. Bound and free hormone were separated by the addition of 2·0 ml 60% (w/v) ammonium sulphate (pH 8·0) and centrifugation for 15 min at 1500 ***g***. The precipitate was dissolved in 0·8 ml distilled water and transferred to a scintillation vial for measurement of radioactivity. The RIA had a sensitivity of 0·1 ng/ml and a range from 0·5 ng/ml (80% of zero standard) to 13 ng/ml (20% of the zero standard). Intra-assay and inter-assay variations were 7·9% and 6·1% respectively for a plasma sample which had a mean value of 1·41 ng-ml ($n = 4$). Parallelism between standard and unknown was achieved when samples were diluted or had additional PGF-2α added to them. Plasma oxytocin assays were performed using the antiserum and protocol described by Robinson (1980). The intra- and inter-assay variation in the assay was 3·8% and 7·4% respectively. Progesterone was measured using an RIA with ^{125}I-labelled progesterone (RSL : Carson, California) but with plasma from an ovariectomized sheep added to the reference standards.

Analyses. Statistical evaluation of the venous–arterial differences was performed using the Mann–Whitney test.

Results

Concentrations of oxytocin-associated neurophysin in utero-ovarian vein plasma collected at 30-min intervals from 5 ewes between Days 13 and 15 of the oestrous cycle (mean 1·98 ng/ml) were significantly higher ($P < 0·0001$) than the arterial values (mean 0·96 ng/ml). Jugular vein concentrations (mean 0·97 ng/ml) were not significantly different from the arterial values.

Many pulses of oxytocin-associated neurophysin were observed in the utero-ovarian plasma but these were classified as pulsatile peaks only when coordinate increases in the utero-ovarian vein, jugular vein and femoral artery oxytocin-associated neurophysin were observed. Four pulsatile peaks were observed in 3 of the ewes examined, with these occurring just before or when the plasma progesterone concentration was decreasing.

During the pulsatile peaks, utero-ovarian oxytocin-associated neurophysin concentrations (mean 4·33 ng/ml) were significantly higher ($P < 0·001$) than the arterial values (mean 1·56 ng/ml). No significant difference was found between jugular vein (mean 1·60 ng/ml) and femoral artery concentrations of oxytocin-associated neurophysin (Fig. 1).

Analysis of the pulsatile peak samples taken at 6-min intervals revealed that there was a concurrent release of oxytocin and oxytocin-associated neurophysin (Fig. 1). During the pulsatile peaks the hormones were released in a mean molar ratio of 1 : 1·2 ($n = 4$).

Utero-ovarian vein plasma PGF-2α concentrations increased during the pulsatile peaks an average of 17 min before the levels of oxytocin began to rise to form a peak of sufficient magnitude and duration to cause an increase in systemic plasma concentrations of oxytocin-associated neurophysin (Fig. 2).

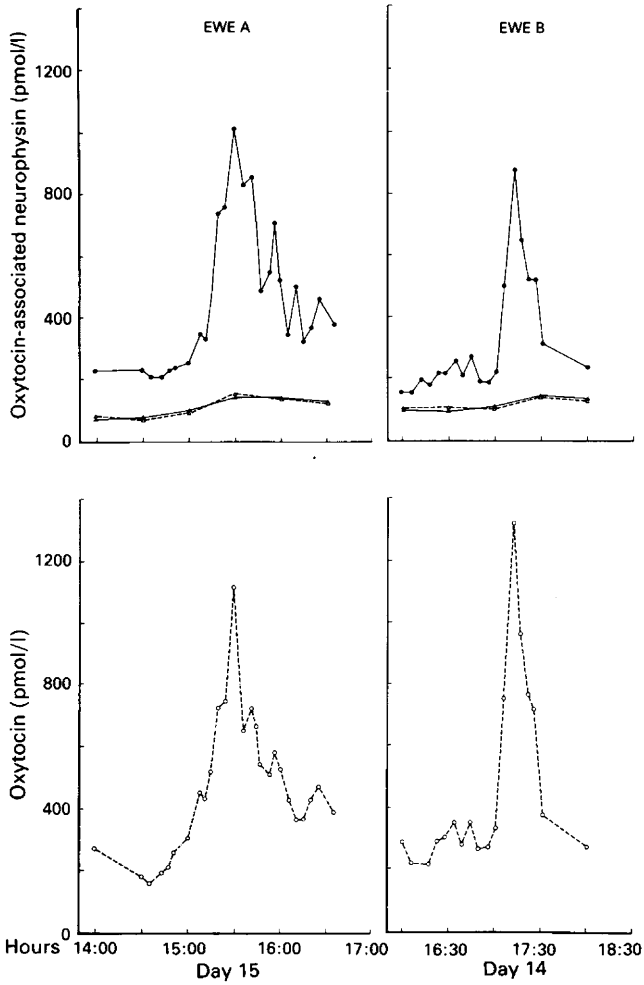

Fig. 1. Plasma concentrations of oxytocin-associated neurophysin in the femoral artery (▲——▲), jugular vein (△ – – – △) and utero-ovarian vein (●——●) and of oxytocin in the utero-ovarian vein (○ – – – ○) during two pulsatile peaks in two ewes (A and B) on Days 15 and 14, respectively of the oestrous cycle.

Discussion

Our present results show that, during the late luteal phase, pulsatile peak concentrations of oxytocin-associated neurophysin in the utero-ovarian vein exceed arterial levels and that no venous–arterial difference was found across the head. This indicates that the CL is the source of the pulsatile peaks of oxytocin and oxytocin-associated neurophysin that occur during luteolysis.

It is unlikely that the elevated utero-ovarian concentrations of oxytocin and oxytocin-associated neurophysin do not originate from the CL because, relative to the CL, non-luteal ovarian tissues and uterine tissues contain little oxytocin (Flint & Sheldrick, 1983). Further support for a luteal origin of the elevated utero-ovarian oxytocin and oxytocin-associated neurophysin values comes from the work of Sheldrick & Flint (1984). They found that, in anaesthetized ewes, basal and

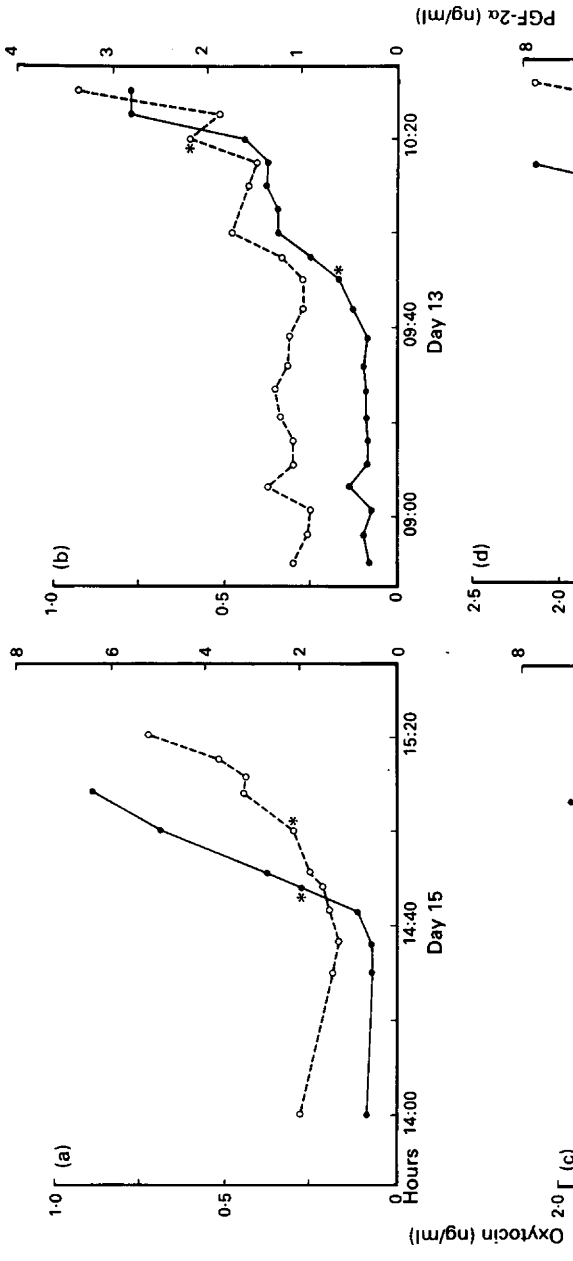

Fig. 2. Utero-ovarian vein plasma concentrations of PGF-2α (●——●) and oxytocin (○ - - - ○) during the early stages of 4 (a–d) pulsatile peaks. *The first of two consecutive samples that are at least 2 standard deviations higher than the mean of the previous samples.

cloprostenol-stimulated ovarian vein concentrations of oxytocin were higher than those in the jugular vein or uterine artery. These differences were only observed when the ovary contained a CL (Flint & Sheldrick, 1983).

We have previously reported that oxytocin and oxytocin-associated neurophysin were released in approximately equimolar ratios into the utero-ovarian vein after cloprostenol stimulation of ewes (Watkins *et al.*, 1984b). We have now shown that during the late luteal phase of the sheep oestrous cycle spontaneous pulsatile release of oxytocin into the ovarian vein is accompanied by the release of an approximately equimolar amount of oxytocin-associated neurophysin.

That PGF-2α is released into the utero-ovarian vein before either oxytocin or oxytocin-associated neurophysin (Fig. 2) would support the proposal that PGF-2α induces the pulsatile release of oxytocin and oxytocin-associated neurophysin during luteolysis in the sheep. This is consistent with the inhibition of pulsatile oxytocin-associated neurophysin release in ewes (Watkins, Moore, Fairclough, Peterson & Tervit, 1984a) and oxytocin release in goats (Cooke & Homeida, 1984) after systemic treatment of the animals with the prostaglandin inhibitor indomethacin.

Ovarian oxytocin or PGF-2α does not appear to be necessary for the release of uterine PGF-2α because the pulsatile release of PGFM has been observed in ovariectomized ewes with oestrogen replacement (Lye, Sprague & Challis, 1983). In cows that have received exogenous progesterone, luteal oxytocin or PGF-2α do not seem to be involved in the pulsatile release of PGFM which occurs after the CL has regressed (Smith, Fairclough & Peterson, 1979; Kindahl, Edqvist & Lindell, 1980). However, until oxytocin or oxytocin-associated neurophysin has been measured in animals, without CL, that are releasing PGFM in a pulsatile manner, the possibility that oxytocin is being released from the other sites cannot be excluded.

A previous objection to PGF-2α causing oxytocin release during luteolysis was that PGF-2α could not reach the brain to cause oxytocin release because it is rapidly metabolized by the lungs (Piper, Vane & Wyllie, 1970; Davis, Fleet, Harrison & Maule Walker, 1980; Fairclough *et al.*, 1980). Our present results, which suggest an ovarian source of the oxytocin, overcome this objection because PGF-2α can reach the CL by the countercurrent mechanism operating between the utero-ovarian vein and the ovarian artery (McCracken *et al.*, 1972). However, it is still possible that a PGF-2α metabolite may cause oxytocin release by a systemic route.

Oxytocin appears to be important for CL regression because immunization against oxytocin prolongs the luteal phase (Sheldrick, Mitchell & Flint, 1980; Schams *et al.*, 1983). The findings that exogenous oxytocin does not cause CL regression in goats treated with the prostaglandin synthetase inhibitor meclofenamate (Cooke & Knifton, 1981) or in hysterectomized cows (Armstrong & Hansel, 1959) and sheep (Hatjiminaoglou, Alifakiotis & Zervas, 1979) and that oxytocin can stimulate PGF-2α release, particularly at the end of the oestrous cycle when there is a large increase in the number of uterine oxytocin receptors (Roberts *et al.*, 1975, 1976; Roberts & McCracken, 1976), suggest that the role of oxytocin in luteolysis involves the release of uterine PGF-2α.

However, the present results, which show that PGF-2α increases before oxytocin at the beginning of a pulsatile peak, indicate that an increase in plasma oxytocin does not initiate the pulsatile release of PGF-2α. McCracken, Schramm & Okulicz (1984) have suggested that basal levels of oxytocin interact with uterine oxytocin receptors, thus initiating PGF-2α release. The ability of oxytocin to provide positive feedback on PGF-2α release and cause down-regulation of uterine oxytocin receptors suggests that oxytocin may fine-tune the PGF-2α pulses so that they can efficiently cause CL regression (Schramm, Bovaird, Glew, Schramm & McCracken, 1983).

Whether luteal oxytocin also has a role in modulating the release of hormones from other endocrine tissues during the oestrous cycle should be the subject of further investigation (Donaldson, Hansel & Van Vleck, 1965; Tan, Tweedale & Biggs, 1982; Froehlick, Ben-Jonathan & Neill, 1984).

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