Effect of intra-ovarian infusion of oxytocin on plasma progesterone concentrations in pregnant ewes

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Summary. The function of oxytocin receptors in the corpus luteum of pregnant ewes was investigated by infusing saline or oxytocin (100 ng/min) into the utero-ovarian artery of pregnant ewes (62 ± 5 days, n = 12). During a 4-h infusion, plasma oxytocin (OT) concentration increased to 268 ± 80 pg OT/ml in the OT-infused group and remained unchanged at 2.5 ± 1.5 pg OT/ml in the saline-infused group. Progesterone concentration in jugular venous plasma (17 ± 9 ng/ml) rapidly decreased during oxytocin infusion to 59 ± 10% and 26 ± 9% of control at 1.5 and 2 h, respectively; the utero-ovarian venous concentration of 64 ± 38 ng/ml decreased by a similar magnitude during oxytocin infusion. Electron microscopy of corpora lutea, removed at the end of the experiments, showed no indication of luteolytic changes following oxytocin infusion. It was concluded that oxytocin markedly and rapidly reduces progesterone secretion in pregnant ewes.

Keywords: Oxytocin; corpus luteum; sheep; pregnancy; progesterone

Introduction

The octapeptide hormone oxytocin is produced by the hypothalamus and released from the posterior pituitary into the general circulation, where it acts primarily on smooth muscle of the uterus and mammary glands. It is now apparent that the hypothalamus is not the only site of oxytocin synthesis. Oxytocin has been identified in the Leydig cells of the testis (Gemmell & Sernia, 1989; Pickering et al., 1990), in the adrenal gland (Nussey et al., 1987) and in the corpus luteum (Wathey et al., 1986). A major function of ovarian oxytocin, at least in domestic ruminants, appears to be the stimulation of uterine prostaglandin F-2α (PGF-2α), which in turn acts on the corpus luteum to initiate luteolysis (Flint & Sheldrick, 1986; Wathey et al., 1986; Homeida & Khalafalla, 1987). An alternative role postulated by Schwall et al. (1986) is that oxytocin secreted by the large luteal cells influences the metabolism of the small luteal cells. Some studies have reported that oxytocin influences progesterone production from the human, bovine, and porcine corpus luteum (Tan et al., 1982a, b; Bennegard et al., 1987), but other studies on the same species (Mares & Casida, 1963; Richardson & Masson, 1985) failed to show an effect of oxytocin on progesterone production. In these latter studies, the role of the corpus luteum as a target for oxytocin was uncertain. Since any action of oxytocin would probably be mediated via receptors, corpora lutea from pregnant and nonpregnant sheep were examined (Sernia et al., 1989). Specific oxytocin binding was not observed in luteal tissue during the oestrous cycle, but it was in the corpora lutea of pregnant ewes. Thus, a direct action of oxytocin on the corpus luteum was possible after the first months of pregnancy, but not in the oestrous cycle. In this study, this hypothesis was examined further by measuring the effect of intra-ovarian infusion of oxytocin on plasma progesterone concentration in pregnant ewes.
Materials and Methods

Twelve adult Merino ewes were kept at pasture and inspected daily. The day of mating was detected by observing the crayon marking on the rump of the ewe, placed there from a raddle on the chest of the ram.

Experimental procedure. One day prior to surgery, the pregnant ewes were starved. Anaesthesia was induced with intravenous pentobarbitone and maintained with halothane. The reproductive organs were displayed by a midline incision and a polyvinyl catheter inserted into a small branch of the ovarian artery and tied into position (Thorburn & Nicol, 1971; Stacy et al., 1976). To confirm that the infusate could reach the corpus luteum, a small bolus of bromo-phenol green dye was infused retrogradely down the branch into the ovarian artery, whilst the blood flow to the corpus luteum was arrested for 20 s. With the return of the blood, the corpus luteum momentarily coloured green and then returned to a normal pink. Catheters were also inserted in the jugular and the utero-ovarian veins for the collection of blood samples. The infusion consisted of 1 h of isotonic saline (6 ml/h) followed by 4 h of saline or oxytocin (Peninsula Laboratories, Belmont, CA, USA) at 100 ng/min. Blood samples were obtained from the jugular and utero-ovarian catheters every 15 min for 2.5 h, and every 30 min for the remaining 2.5 h. Seven pregnant ewes at Day 43, 52, 60, 61, 80, 85 and 90, respectively, of gestation were infused with oxytocin. Five pregnant ewes at Day 40, 47, 50, 60 and 80, respectively, of gestation were infused with saline.

Morphological examination. At the termination of the infusion, the ewe was killed and the corpus luteum removed and fixed in a paraformaldehyde and glutaraldehyde mixture. The corpus luteum was processed for electron microscopy by techniques described by Gemmell et al. (1974).

Radioimmunoassay of progesterone and oxytocin. Plasma progesterone concentration was determined by a radioimmunoassay (RIA) procedure described by Gemmell (1979) using sheep antiprogestrone-11-hemisuccinate-bovine serum albumin (BSA) (antiserum no. 334) kindly donated by Dr R. J. Cox (CSIRO, Prospect, New South Wales, Australia). The antiserum is highly specific, the only significant cross-reactivity being with 11-hydroxyproges-terone (11-9%). The sensitivity of the assay was 20 pg/tube and the intra- and interassay coefficients of variation were 13.5% (n = 5) and 17% (n = 10), respectively.

Oxytocin was also measured by RIA. Plasma (2–5 ml) was diluted with an equal volume of distilled water and applied to phenyl silica minicolumns (Bond-Elut, Analytichem International, Harbour City, CA, USA), which had been preconditioned with 1 ml methanol and 1 ml of distilled water. The minicolumns were then washed with 2 ml of distilled water followed by the elution of peptides with 1 ml of methanol. The samples were dried and resuspended in 0.5 ml of RIA buffer (100 mmol phosphate-buffered saline/l with 0.2% BSA, pH 7.4). Loss of oxytocin during extraction and separation procedures was determined by adding 2000 c.p.m. of 125I-oxytocin to the plasma samples immediately before processing. Recovery was in the range of 70–85%. The RIA mixture consisted of 200-μl samples, 100 μl containing 20,000 c.p.m. of 125I-oxytocin and 200 μl of anti-oxytocin antibodies at a dilution of 1:500 000. After incubation for 16 h, antibody-bound peptide was precipitated with 22% polyethylene glycol, the radioactivity in the pellet was counted and the oxytocin concentration calculated from a standard curve. Oxytocin was radioiodinated with chloramine-T and separated by reverse-phase high-performance liquid chromatography to an approximate activity of 2000 Ci/mmol. The antibody (SOT-45) was raised in sheep using limpet haemocyanin conjugated to oxyto-cin with glutaraldehyde (Gemmell & Sernia, 1989). The cross-reactivity profile of SOT-45 is oxytocin = 100%, deamino-oxytocin = 12.5%, mesotocin = 2%, arginine-vasopressin <0.01%, isotocin = 1%, vasotocin = 0 and angiotensin = 0. The sensitivity of the RIA was 1 pg/tube; intra-assay and interassay variability was 3.7% and 15%, respectively.

Statistics. Data are expressed as means ± s.e.m. An analysis of variance for repeated measures was used to test the effect of saline or OT infusion. Comparison of means at corresponding times in the 2 groups was made by Duncan's multiple-range test (Winer, 1971).

Results

The ewes in our experiments were at Day 62 ± 5 (s.e.m.) of pregnancy. The jugular plasma concentra-tion of oxytocin did not change significantly from a control value of 2.5 ± 1.5 pg OT/ml (Fig. 1), while in the oxytocin-infused group the concentration increased from 9.6 ± 12 to 202 ± 50 pg OT/ml at 1 h and to 268 ± 80 pg OT/ml at 4 h. A striking, but not unusual (Flint et al., 1989; Sheldrick & Flint, 1990), feature of progesterone concentration for each ewe was its high variability over the 5-h sampling period. Consequently the mean value of the 5 samples obtained over the 60 min before the experimental period was used as a control for each ewe and subsequent values were expressed as a percentage of that control.

Jugular plasma progesterone concentrations (Fig. 2) in the control period were similar for saline-infused (16.8 ± 8.8 ng/ml, n = 5 ewes) and oxytocin-infused (16.6 ± 10.9 ng/ml, n = 7)
groups. In the saline-infused group, no significant changes in concentration were observed over the 4 h of infusion, while a significant decrease (ANOVA, $P < 0.01$) occurred in the oxytocin-infused group. The effect of the oxytocin was evident at 1-5 h, when the progesterone dropped to $59 \pm 10\%$ of control ($P < 0.05$) while the corresponding value in the saline-infused group remained at $100 \pm 20\%$. At 2 h, the progesterone in the oxytocin-infused group was $26 \pm 9\%$ of control and it stayed low for the remaining 2 h of infusion.

Progestrone concentrations in the utero-ovarian vein in the pretreatment period were higher than the corresponding jugular vein concentrations. Control values were $76.7 \pm 43.9$ ng/ml ($n = 5$) for the saline-infused group and $63.9 \pm 38.2$ ng/ml ($n = 7$) for the oxytocin-infused group. The infusion of oxytocin reduced progesterone concentration in a similar way to that seen in the jugular vein (Fig. 3). Thus no significant differences between the groups were seen until 1-5 h, and maximum suppression of progesterone secretion was reached at 2-5 h.

The ultrastructure of the corpus luteum in saline-infused and oxytocin-infused ewes was similar (Fig. 4). No increase was observed in lipid droplets or vacuoles in oxytocin-infused ewes to indicate the onset of luteolysis.

**Discussion**

Previously we reported that corpora lutea of ewes at Day 40, or later, of pregnancy contained specific oxytocin receptors, whereas corpora lutea of nonpregnant ewes showed no detectable binding of oxytocin (Sernia et al., 1989). In this study, the function of these receptors was tested by infusing oxytocin into a branch of the ovarian artery supplying blood to the corpus luteum (CL). Maximal plasma oxytocin concentrations were reached within 1-1.5 h and were comparable to those observed after stimulation of the CL in nonpregnant ewes with the prostaglandin analogue cloprostenol (Sheldrick & Flint, 1983). A significant decrease in both jugular and utero-ovarian progesterone could be seen as early as 1.5 h, about the same time as oxytocin reached maximum values. This coincidence between high oxytocin and low progesterone suggests that progesterone
secretion is being inhibited by a direct action of oxytocin. However, there are several indirect mechanisms which could lead to similar observations. For example, oxytocin could stimulate the release of sufficient prostaglandin F-2α to initiate luteolysis, even though uterine oxytocin receptor concentration is very low in early pregnancy (Roberts et al., 1976; Sheldrick & Flint, 1985). The luteolytic effect of PGF-2α causes distinct morphological changes, characterized by numerous lipid

Fig. 2. Plasma progesterone concentration in the jugular vein as a percentage of the controls in 5 saline-infused (■) and 7 oxytocin-infused (●) ewes. Duncan's multiple comparison test was used to compare the means of saline-infused and oxytocin-infused groups; *P < 0.05, **P < 0.01.

Fig. 3. Plasma progesterone concentration in the utero-ovarian vein as a percentage of the controls in 5 saline-infused (■) and 7 oxytocin-infused (●) ewes; *P < 0.05, **P < 0.01.
Fig. 4. Electron micrographs of (a) a large granulosa cell from a ewe at Day 52 of gestation infused with saline alone and (b) a similar view of a corpus luteum cell from an oxytocin-infused ewe at Day 60 of gestation. There was no evidence in (b) of an accumulation of lipid droplets or vacuoles to indicate luteolytic changes. \( N = \) nucleus, \( M = \) mitochondria; \( \times 10000 \).
droplets and vacuoles, within a short time of being administered to ewes (Thorburn & Nicol, 1971; Stacy et al., 1976). In our experiments, the corpora lutea removed after oxytocin infusion did not show ultrastructural luteolytic changes indicative of mediation by uterine prostaglandin (Fig. 4). Moreover, the infusion of 40 µg PGF-2α/h into the utero-ovarian artery appears to require longer than the 1-5 h observed by us to inhibit progesterone secretion significantly (Thorburn & Nicol, 1971; Stacy et al., 1976). These two observations suggest that uterine prostaglandins are unlikely to be a major consideration in our results, although their involvement cannot be dismissed at this stage.

Another plausible mechanism to explain our results would be the inhibition of gonadotrophin secretion by oxytocin. Current evidence suggests that oxytocin stimulates rather than inhibits gonadotrophin secretion (Robinson & Evans, 1990). Moreover, in recent experiments in which cyclic ewes were infused with oxytocin for 5 days, there was no indication of a direct effect on luteinizing hormone (LH) or progesterone secretion (Sheldrick & Flint, 1990). Yet another explanation of our results would be vasoconstriction of the ovarian vasculature by the high concentration of oxytocin. However, responses to vasoactive peptides are almost immediate (Montani et al., 1980), while the effect observed had a latency period of >1 h. We therefore conclude that a direct inhibitory action of oxytocin on progesterone secretion is the explanation most consistent with our data. A significant adjunct to this conclusion is the possibility that the placenta, which secretes significant amounts of progesterone after Day 50 of pregnancy (Ricketts & Flint, 1980), may be an additional target tissue for oxytocin in our experiments, although the placing of the infusion catheter in the ovarian artery maximized oxytocin delivery to the corpus luteum.

There is substantial support from in-vitro studies for an inhibitory action of oxytocin on progesterone secretion. In the study of Tan et al. (1982a) with bovine corpora lutea of early pregnancy, high concentrations of oxytocin inhibited gonadotrophin-stimulated, but not basal, secretion of progesterone in vitro. In contrast, luteal progesterone secretion in pseudopregnant rats (Mukhopadyay et al., 1984) and nonpregnant cows (Mares & Casida, 1963) and pigs (Duncan et al., 1961) is not reduced when incubated with oxytocin. In nonpregnant women (Richardson & Masson, 1985) and sheep (Rodgers et al., 1985), basal as well as gonadotrophin-stimulated progesterone secretion from the corpus luteum, in vitro, remained unaffected by oxytocin. Furthermore, there is evidence that oxytocin infusion has no effect on progesterone secretion in cyclic sheep (Sheldrick & Flint, 1990), cattle (Gilbert et al., 1989) or monkeys (Wilks, 1983). These results are in agreement with our earlier study on oxytocin receptors in sheep (Sernia et al., 1989). However, there are reports in pigs (Einspanier et al., 1986; Przala et al., 1986a, b; Pitzel et al., 1988, 1990), cattle (Schams, 1987, 1989) and women (Tan et al., 1982b; Bennegard et al., 1987) which, while supporting an inhibitory role, shows oxytocin effects in the nonpregnant condition. These observations need to be reconciled with the comparable studies by Richardson & Masson (1985), Duncan et al. (1961) and Gilbert et al. (1989), which show no oxytocin effect on progesterone secretion. Differences in the purity of oxytocin preparations have already been suggested as a source of variation (Richardson & Masson, 1985; Flint et al., 1989), but this view is not based on persuasive experimental evidence.

In our view, a satisfactory explanation of the apparent inconsistencies between studies is probably in one or more of the following observations. Firstly, oxytocin concentrations ≥100 ng/ml have generally been used to show inhibition in vitro, while low doses have occasionally given stimulation rather than inhibition (Tan et al., 1982a, b). The failure of Gilbert et al. (1989) to show an effect in cattle may be explained by the 70–500 pg oxytocin/ml plasma achieved in their experiments compared with ~1 µg/ml of incubation medium used by Tan et al. (1982a). Schams (1989) observed effects at much lower doses than Tan et al. (1982a), but, curiously, the magnitude of the inhibition was independent of oxytocin concentration. Secondly, the inhibitory action of oxytocin may differ in unstimulated and gonadotrophin-stimulated luteal tissue, greater inhibition usually being achieved in stimulated tissue (Tan et al., 1982a, b; Einspanier et al., 1986; Pitzel et al., 1988). Thirdly, the reproductive status may have a bearing on the responsiveness of luteal tissue to
oxytocin. We have already emphasized the distinction between corpora lutea from pregnant and cyclic animals in relation to their responsiveness to oxytocin. However, finer distinctions may be appropriate since sensitivity may differ with the stage of pregnancy or day of luteal phase, as reported by Schams (1987) for bovine corpora lutea. Fourthly, observations in vitro may not be comparable to those in vivo. There are reports of oxytocin inhibiting progesterone secretion in vitro and stimulating it in vivo (Pitzel et al., 1988, 1990; Jarry et al., 1990). Finally, there are probably species differences which are presently complicating the current data and perhaps leading to an erroneous perception of conflict between reports. Thus, while the present study, in conjunction with all the other reports on sheep (Sernia et al., 1989; Rodgers et al., 1985; Sheldrick & Flint, 1990), indicates that oxytocin does not have a luteal action in nonpregnant animals, there are sufficient data at least in women, pigs and cattle to raise the possibility of differences from sheep. The elucidation of this particular problem would be greatly assisted by investigating luteal tissue from various species for the presence of oxytocin receptors.

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References


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