Effect of finadyne on oestradiol-induced ovarian oxytocin and uterine PGF_{2α} secretory systems on day 15 after oestrus in ovarian autotransplanted ewes

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This study was undertaken to determine whether induction of ovarian oxytocin after oestradiol treatment on day 15 after oestrus is mediated through prostaglandin secretion by blocking prostaglandin synthesis using finadyne, an inhibitor of the cyclo-oxygenase pathway. Nine ewes with ovarian autotransplants were assigned randomly to receive an i.m. injection of either oestradiol benzoate (50 µg) in peanut oil ($n = 5$) or oestradiol benzoate plus finadyne (2.2 mg kg^{-1}) ($n = 4$) at 3 h intervals starting at the time of oestradiol injection. Blood samples were collected from the ovarian and contralateral jugular veins at 30 min intervals for 6 h before and at 15 min intervals for up to 9 h after the oestradiol and finadyne injections. The secretion rate of ovarian progesterone remained high in all ewes, thus indicating the presence of a functional corpus luteum.

Peripheral oestradiol concentrations were significantly ($P < 0.001$) higher during the 9 h after oestradiol injection in both groups. None of the oestradiol–finadyne-treated ewes showed significant pulses in either ovarian oxytocin secretion or release of the prostaglandin F_{2α} metabolite 13,14-dihydro-15-keto PGF_{2α} (PGFM) after injections. In ewes treated with oestradiol only, at least one detectable pulse of ovarian oxytocin and jugular PGFM was observed with mean $\pm$ SEM amplitude of $17.7 \pm 7.29 \text{ ng min}^{-1}$ and $237.18 \pm 43.13 \text{ pg ml}^{-1}$, respectively. The areas under the curve for ovarian oxytocin and jugular PGFM pulses were significantly increased after oestradiol treatment. These findings demonstrate that initiation of the arachidonic acid cascade is important for the secretion of oxytocin after oestrogen treatment.

Introduction

In ruminants, the oestrous cycle occurs as a consequence of a positive feedback system between luteal oxytocin, which stimulates secretion of the uterine prostaglandin PGF_{2α}, and vice versa (Flint and Sheldrick, 1982). During luteolysis, pulses of oxytocin or its associated neurophysin occur simultaneously with pulses of PGF_{2α} or its metabolite 13,14 dihydro-15-keto prostaglandin F_{2α} (PGFM) (Hooper *et al.*, 1986).

Oestrogen also appears to play a role in the regulation of uterine function. Administration of oestradiol to ovarian autotransplanted (Al-Matubsi *et al.*, 1997) or intact (Zhang *et al.*, 1991) ewes during the late stages of the oestrous cycle altered the timing and pattern of uterine PGF_{2α} or PGFM release. Similar results have been reported by Jacobs *et al.* (1988) showing that the increase in secretion of endogenous PGF_{2α} that follows induction of luteolysis with cloprostenol, a synthetic prostaglandin, is suppressed by intravenous injections of tamoxifen, an oestrogen antagonist.

Oestrogen is also involved in regulation of the corpus luteum and influences luteolysis. Cook *et al.* (1974)

demonstrated that exogenous administration of oestrogen directly into the corpus luteum on day 10 after oestrus resulted in regression of the injected corpus luteum but had little effect on the contralateral corpus luteum. Moreover, removal of endogenous oestrogen by destroying ovarian follicles, the primary source of oestradiol, either via ablation (Karsch *et al.*, 1970) or X-irradiation (Karsch *et al.*, 1970; Zhang *et al.*, 1991), could prolong the lifespan of the corpus luteum for a few days. Subsequent treatment of the X-irradiated ewes with oestradiol resulted in normal pulsatile release of PGF_{2α} and the occurrence of a normal cycle (Zhang *et al.*, 1991). Furthermore, Al-Matubsi *et al.* (1997) demonstrated that oestradiol is also involved in induction of ovarian oxytocin secretion. As ovine large luteal cells, which are the source of oxytocin (Rodgers *et al.*, 1983), have been reported to contain oestrogen receptor (Glass *et al.*, 1984) and the rat gene for oxytocin contains an oestrogen response element upstream of the transcription start site (Richard and Zingg, 1990), it is also possible that the luteolytic effect of oestradiol may be mediated directly through induction of ovarian oxytocin release.

Thus, the present study was undertaken to investigate whether the stimulatory effect of oestradiol on ovarian oxytocin secretion is mediated through PGF_{2α} release, by

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inhibiting prostaglandin synthesis using flunixin, which is an inhibitor of the cyclo-oxygenase pathway.

Materials and Methods

Experimental animals

Border Leicester $\times$ Merino ewes ($n = 9$) were prepared with ovarian autotransplants as described by Goding *et al.* (1967). The ewes were housed individually in metabolic cages in a temperature-controlled room (20°C) and were fed once a day with 800 g of a pelleted ration consisting of hammer milled lucerne (60%) and oats (40%). Water was available *ad libitum*. The study was carried out at CSIRO Division of Animal Production, Australia. All protocols were approved by the Animal Experimentation Ethics Committees of Victoria University of Technology and the CSIRO Division of Animal Production.

Experimental design

As ewes with autotransplanted ovaries do not naturally undergo oestrous cycles, oestrus was induced synchronously by two injections of 125 µg synthetic PGF_{2α} (Estrumate; ICI, Sydney) given 15 days apart. After the second injection, oestrus was detected by inspection twice a day for the presence of crayon marks after mating with a ram fitted with a sire-o-sine harness (Radford *et al.*, 1960). The day that the ewes displayed oestrous behaviour was designated day 0. On day 15 of the cycle, all ewes were injected i.m. with 50 µg oestradiol benzoate (Intervet, Sydney) in peanut oil. In addition, four of these ewes were injected i.m. with 2.2 mg kg⁻¹ of the prostaglandin synthetase inhibitor, flunixin (Allhank Trading Company, Melbourne) at 3 h intervals starting at the time of oestradiol injection. The remaining five ewes received vehicle only.

Cannulation of jugular and ovarian veins

Cannulations were carried out under local anaesthesia (10% lignocaine hydrochloride spray: xylocaine; Astra Pharmaceuticals, Sydney) as described by McCracken *et al.* (1969) at least 24 h before the start of blood sampling. In brief, a polyvinyl catheter was inserted into the jugular vein exteriorized in the skin loop to cannulate the ovarian vein. The tip of the catheter was positioned at the junction of the ovarian and jugular veins. An additional polyvinyl catheter (50 cm) was inserted into the contralateral jugular vein. The catheters were filled with heparinized saline (1000 iu ml⁻¹).

Blood sampling

On day 15 after oestrus, 5 ml and 3 ml samples of blood were collected from the ovarian and contralateral jugular veins, respectively, at 30 min intervals for 6 h before oestradiol or flunixin injections and subsequently at 15 min intervals for up to 9 h after injection. The blood samples (3 ml) collected from the contralateral jugular vein

were placed in heparinized glass tubes and the catheter was refilled with heparinized saline (50 iu ml⁻¹).

Ovarian venous blood was collected using the method described by McCracken *et al.* (1969). Approximately 5 ml ovarian blood was allowed to drain freely every 30 min from the open end of the catheter into heparinized 15 ml graduated centrifuge tubes. The time taken to collect this sample was measured using a stopwatch. Three millilitres of ovarian venous blood were collected at alternate 15 min intervals after collection of each 5 ml sample after oestradiol injection. Thus, samples were collected every 15 min for determination of hormone concentrations and every 30 min for determination of blood flow (Lamsa *et al.*, 1989). The blood samples were centrifuged at 1900 g for 15 min. Plasma was collected and stored at -20°C until assayed for oxytocin and progesterone (ovarian venous plasma) or PGFM and oestradiol (jugular venous plasma) by radioimmunoassay (RIA). Blood flow (ml min⁻¹) was calculated by measuring the time taken to collect a known volume of ovarian venous blood. The packed cell volume (PCV) was determined at 1 h intervals and the plasma flow (ml min⁻¹) was calculated by multiplying the blood flow by 100-PCV divided by 100. The secretion rate of oxytocin and progesterone (ng min⁻¹) was obtained by multiplying the plasma flow (ml min⁻¹) by the concentration of hormone in the ovarian venous plasma (ng ml⁻¹).

Hormone analysis

PGFM assay. Plasma PGFM concentrations were measured by RIA (Burgess *et al.*, 1990) with a sensitivity of 8 pg ml⁻¹. The intra- and interassay coefficients of variation were 8 and 11%, respectively.

Oestradiol assay. The concentration of oestradiol was measured in peripheral blood plasma by RIA (Burgess *et al.*, 1990) with a sensitivity of 7 pg ml⁻¹. The samples were measured in a single assay and the intra-assay coefficient of variation was 4.7%.

Progesterone assay. Progesterone was assayed in 100 µl ovarian plasma extracted with 2 ml *n*-hexane (Crown Scientific, Victoria) according to the method of Rice *et al.* (1986). The sensitivity of the assay was 0.25 ng ml⁻¹. The samples were measured in a single assay and the intra-assay coefficient of variation was 7%.

Oxytocin assay. Plasma oxytocin concentrations were measured by RIA as described by Al-Matubsi *et al.* (1997). The sensitivity of the assay was 16 pg ml⁻¹, and the intra- and interassay coefficients of variation were 6 and 11.9%, respectively.

Statistical analysis

Statistically significant pulses of ovarian vein oxytocin and jugular vein PGFM were determined using a Pulsar program (Merriam and Wachter, 1982). Assay variability

Fig. 1. Oxytocin (●) and progesterone (△) secretion rates into ovarian venous plasma and concentrations of peripheral 13,14-dihydro-15-keto PGF_{2α} (PGFM; ○) from individual ewes (a–e)

was estimated by regression analysis of the standard deviation for duplicate determinations and the mean at each point. Baseline was calculated representing the contribution of long-term trends (15 h) but not fluctuations of shorter duration (30 min). The amplitudes of the ovarian oxytocin and peripheral PGFM pulses were calculated by subtracting baseline values. The resulting values were then rescaled in terms of standard deviation units by dividing the rescaled values by an estimate of assay variability. The amplitude of the rescaled pulses was identified by applying height and duration criteria specified by user-defined cut-off points $G(n)$ for pulses. These calculations were repeated until two iterations produced the same values for pulses or until the preset limit of six iterations was completed. The quadratic (a), linear (b), and constant (c) terms for Pulsar were as follows: for oxytocin: $a = 0.00$, $b = 11.91$ and $c = 0.00$; and for PGFM: $a = 0.00$, $b = 11.17$ and $c = 0.00$. The following $G(n)$ values were selected for both oxytocin and PGFM pulses: $G(1) = 6.5$, $G(2) = 4.45$, $G(3) = 3.25$, $G(4) = 2.57$ and $G(5) = 2.05$. Coincident episodes in the secretion of oxytocin and PGFM were defined as those that showed an increase in the value of the PGFM pulse at the same time as a defined oxytocin pulse. The plasma secretion rates of oxytocin and concentrations of PGFM pulses were expressed in ng min^{-1} and pg ml^{-1} , respectively, and the duration of that pulse was designated as τ being the number of minutes between the last time point before and the first time point after a significant increase in hormone concentration as detected by the Pulsar program. The area under the significant ovarian oxytocin and peripheral PGFM pulses was then calculated for each ewe and was expressed as $(\text{ng min}^{-1}) \tau$ and $(\text{pg ml}^{-1}) \tau$, respectively. The overall mean concentration, pulse amplitude and duration of the pulse, and the area under the pulse were obtained using the Pulsar analysis program. The values were expressed as mean $\pm$ SEM. Individual characteristics of these responses and the differences in concentrations of oestrogen and progesterone were compared using a Student's unpaired *t* test. The number of ewes that showed pulses of oxytocin and PGFM after oestradiol or oestradiol plus finadyne injections was compared using a chi-squared test.

Results

The progesterone secretion rate of oestradiol-treated ewes ($679.04 \pm 87.98 \text{ ng min}^{-1}$) was not significantly different from that in the oestradiol–finadyne-treated ewes ($762.77 \pm 141.76 \text{ ng min}^{-1}$). Progesterone secretion remained high during the sampling period, indicating the presence of a functional corpus luteum in both groups (Figs 1 and 2). In both treated groups, circulating concentrations

treated with oestradiol only on day 15 after oestrus. ^a and ^b indicate significant episodes in secretion of ovarian oxytocin and PGFM, respectively. ∇: Identifies synchronous episodes of secretion of both compounds. ↓: Indicates time of oestradiol injection and ⇓ indicates times of injection of finadyne vehicle (control).

Fig. 2. Oxytocin (●) and progesterone (△) secretion rates into ovarian venous plasma and concentrations of peripheral 13,14-dihydro-15-keto PGF_{2α} (PGFM; ○) from individual ewes (a–d) treated with oestradiol–finadyne on day 15 after oestrus. a and b indicate significant episodes in secretion of ovarian oxytocin and PGFM, respectively. ∇: Identifies synchronous episodes of secretion of both compounds. ↓ and ↓↓: Indicate times of oestradiol and finadyne injections, respectively.

of oestradiol were significantly ($P < 0.001$) higher during the 9 h after oestradiol injection compared with the 6 h period before oestradiol injection (21.48 ± 1.14 versus 9.99 ± 0.89 and 18.66 ± 1.29 versus 10.66 ± 1.0 pg ml⁻¹, respectively).

The effect of intramuscular injections of oestradiol only and oestradiol plus finadyne on peripheral PGFM concentrations and ovarian oxytocin secretion are shown (Figs 1 and 2, respectively). The mean basal ovarian oxytocin secretion rate for oestradiol–finadyne-treated ewes (0.47 ± 0.09 ng min⁻¹) was not significantly different from that in oestradiol-treated ewes (0.50 ± 0.16 ng min⁻¹). During the first 6 h of the sampling period, before the oestrogen and finadyne injections, the mean amplitude (6.57 ± 1.39 ng min⁻¹) and the mean area under the curve (2.33 ± 0.88 ng min⁻¹) τ for the ovarian oxytocin pulses in oestradiol–finadyne-treated ewes were not significantly different from those in oestradiol-treated ewes (10.17 ± 3.23 ng min⁻¹ and 10.68 ± 3.31 ng min⁻¹ τ , respectively).

Administration of oestradiol plus finadyne to ovarian autotransplanted ewes on day 15 of the oestrous cycle significantly ($P < 0.05$) reduced the number of ewes showing pulses of oxytocin ($n = 0$ versus $n = 5$) and PGFM ($n = 0$ versus $n = 5$) when compared with ewes treated with oestradiol only. None of the oestradiol–finadyne-treated ewes showed significant pulses in ovarian oxytocin secretion after injection. In oestradiol-treated ewes, at least one detectable pulse of ovarian oxytocin was observed after oestrogen injection. The mean amplitude (17.7 ± 7.29 ng min⁻¹) of these pulses was not significantly different from those measured before oestrogen injection (10.17 ± 3.23 ng min⁻¹). However, a significant ($P < 0.05$) increase in the area under the curve for ovarian oxytocin secretion pulses (30.57 ± 7.3 ng min⁻¹) τ was observed after oestrogen injection when compared with samples collected before injection (10.68 ± 3.31 ng min⁻¹) τ . In these ewes, the ovarian oxytocin pulses were detected at a mean of 5.05 ± 0.37 h and the mean inter-pulse interval was 3.36 ± 0.45 h. In oestradiol-treated ewes, administration of oestrogen significantly ($P < 0.05$) increased the duration of ovarian oxytocin pulses (54 ± 5.5 versus 26.25 ± 7.2 min) compared with corresponding values measured before oestrogen injection.

None of the oestradiol–finadyne-treated ewes and all of the ewes treated with oestradiol only showed significant pulses of PGFM in peripheral plasma after oestradiol or finadyne injections. Mean basal circulating concentrations of PGFM were significantly ($P < 0.05$) different in oestradiol–finadyne-treated ewes (14.55 ± 3.0 pg ml⁻¹) compared with ewes that received oestrogen only (28.45 ± 2.10 pg ml⁻¹) over the sampling period.

In oestradiol-treated ewes, at least one detectable pulse in plasma PGFM concentration was observed after injection. The mean amplitude of these pulses (237.18 ± 43.13 pg ml⁻¹) was not significantly different from those measured before oestrogen injection (176.16 ± 68.37 pg ml⁻¹). However, there was a significant ($P < 0.05$) increase in the area under the curve (1062.11 ± 309.67 versus 302.65 ± 128.91 pg ml⁻¹) τ and duration of the pulse (109.5 ± 16.65 versus 36 ± 6 min) of the PGFM response measured in peripheral plasma

collected after oestrogen injection compared with samples collected before injection. Plasma PGFM pulses were observed in all ewes treated with oestradiol only at > 4 h after injection.

During the sampling period, 62.5% of ovarian oxytocin pulses were associated with, or preceded, the increase in peripheral PGFM concentrations. In contrast, 46.15% of the plasma PGFM pulses occurred immediately before or coincided with a significant increase in the ovarian oxytocin pulses.

Discussion

In this study, ovarian autotransplanted ewes were used as a model to determine whether oestrogen acts to stimulate release of ovarian oxytocin directly or indirectly via release of PGF_{2α}, which in turn stimulates ovarian oxytocin. The concentrations of oxytocin in ovarian venous plasma were 20–1403 pg ml⁻¹ in the present study, which are similar to those detected by Hooper *et al.* (1986) in the utero–ovarian vein (50–1499 pg ml⁻¹) and were much higher than those in peripheral plasma (20–220 pg ml⁻¹; Hooper *et al.*, 1986). Together, these observations indicate that, in the present study, oxytocin in ovarian venous plasma represents luteal rather than posterior pituitary secretion.

As would be expected in ovarian autotransplanted ewes (Goding *et al.*, 1967), the secretion of progesterone remained high in both groups, indicating that the corpus luteum of the transplanted ovary is maintained despite intermittent surges of peripheral plasma PGFM in all ewes before oestradiol injection and in ewes treated with oestradiol only after injection. Our observation that the administration of oestradiol can induce the simultaneous release of ovarian oxytocin and uterine PGF_{2α} in ovarian autotransplanted ewes after a latency period of 4 h in all ewes treated with oestradiol only is in agreement with the study of Al-Matubsi *et al.* (1997).

In the present study, synchronous pulses of ovarian oxytocin and uterine PGF_{2α} were observed during the first 6 h of the sampling period before oestradiol treatment. However, this did not affect subsequent synchronous secretion of these hormones after oestradiol treatment. Thus, the uterine refractoriness to ovarian oxytocin release and uterine PGF_{2α} secretion can be eliminated as a reason for variability in timing of the response. The results from the present study and other studies (Hooper *et al.*, 1986; Al-Matubsi *et al.*, 1998) demonstrate that oxytocin pulses in utero–ovarian or ovarian venous plasma frequently occur in the absence of any significant increase in utero–ovarian PGF_{2α} or peripheral PGFM concentrations and indicate that ovarian oxytocin can occur independently of uterine PGF_{2α}. In contrast, Lamsa *et al.* (1989) observed that uterine PGF_{2α} secretion into the utero–ovarian vein begins to increase before the discharge of luteal oxytocin. Mann (1999) demonstrated that normal frequency of episodes of PGF_{2α} release, with lower amplitude and of longer duration, can occur at the anticipated time of luteolysis in

the absence of luteal oxytocin release. Hooper *et al.* (1986) reported that, in ewes, 56% of oxytocin pulses were coincident with pulses in uterine PGF_{2α} and 97% of all pulses of uterine PGF_{2α} release were accompanied or followed by pulses of oxytocin in the ovarian vein. In the present study, the percentage of PGFM pulses that occurred immediately before or coincided with a significant increase in ovarian oxytocin pulses was decreased (46.15%) by administration of oestradiol. Thus, the present study reaffirms the findings of Zhang *et al.* (1991), who reported similar effects of oestradiol administered to ewes treated with either sham or X-irradiated ovarian follicles.

The mechanism by which oestrogen stimulates ovarian oxytocin and uterine PGF_{2α} release is not fully understood. Oestrogen may act indirectly, perhaps via the uterus, to release PGF_{2α}, which, in turn, could stimulate ovarian oxytocin release. Such a mechanism of action is unlikely to have occurred in the present study as PGF_{2α} would need to act through the systemic circulation to stimulate ovarian oxytocin and it has been shown that 99% of PGF_{2α} is cleared from blood after one passage through the lungs (Piper *et al.*, 1970). However, using ewes with ovarian autotransplants does not necessarily preclude the possibility that the effects of oestrogen and finadyne are mediated through uterine release of PGF_{2α}, as PGFM is known to stimulate luteal oxytocin–neurophysin secretion (Watkins and Moore, 1987). Another possibility is that the corpus luteum of ewes bearing ovarian autotransplants becomes hypersensitive to low concentrations of PGF_{2α} in the absence of normal basal concentrations from the adjacent uterine horn. Such hypersensitivity may allow the corpus luteum to release luteal oxytocin in response to even low concentrations of PGF_{2α} that escape degradation by the lungs. An alternative site of oestradiol action may be directly on the ovary to induce ovarian oxytocin release. Oestradiol (Glass *et al.*, 1984) and PGF_{2α} (Fitz *et al.*, 1982) receptors have been reported in large luteal cells, which are the sites of oxytocin synthesis (Rodgers *et al.*, 1983). Infusion of oestrogen into the corpus luteum causes luteal regression (Cook *et al.*, 1974) and luteal cells from sheep (Tsai and Wiltbank, 1997) and cows (Milvae and Hansel, 1983; Tsai *et al.*, 1996) can produce prostaglandins, such as PGF_{2α}, PGE₂ and PGI₂. On the basis of these findings it is possible that oestrogen stimulates release of ovarian oxytocin through luteal prostaglandins (or some other metabolite of arachidonic acid) (Cooke and Ahmed, 1998). However, the physiological role of luteal prostaglandins during the oestrous cycle and the mechanisms controlling its production remain to be elucidated.

Thus, in intact ewes, the initiation of the arachidonic acid cascade is of importance for the secretion of oxytocin after oestrogen treatment.

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