Oxytocin-induced prostaglandin $F_{2\alpha}$ release and endometrial oxytocin receptor concentrations throughout pregnancy in ewes

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Endometrial oxytocin receptor concentrations and oxytocin-induced plasma concentrations of 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ were investigated on days 14 and 17 of the oestrous cycle and on days 14, 17, 25, 65, 85 and 145 of gestation in ewes. Total 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ release in response to a bolus injection of oxytocin was significantly ($P < 0.05$) higher at luteolysis (day 17 of the oestrous cycle) than at any other stage of the oestrous cycle or in early gestation. On days 65, 85 and 145 of gestation, total prostaglandin release was significantly ($P < 0.05$) increased compared with earlier in gestation. Maximum concentrations of 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ in response to oxytocin followed a similar pattern. Oxytocin receptor concentrations reflected total oxytocin-induced 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ release, with increased oxytocin receptor concentrations occurring on day 17 of the oestrous cycle, compared with those observed on day 14 of the oestrous cycle and on days 14, 17 and 25 of gestation. By day 65 of gestation, oxytocin receptor concentrations were again increased. However, on days 85 and 145 of gestation, oxytocin receptor concentrations had decreased to concentrations similar to those observed in early gestation. These results indicate that oxytocin-induced 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ release during early gestation is minimal despite the presence of endometrial oxytocin receptors. In mid-gestation, oxytocin-stimulated 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ release is increased with a concomitant increase in uterine oxytocin receptor concentrations. However, by the later stages of gestation, oxytocin receptor concentrations, but not oxytocin-stimulated 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ release, were again decreased. Thus, there appears to be a dissociation between the response to oxytocin and endometrial oxytocin concentrations at specific stages of the reproductive cycle in sheep.

Introduction

In sheep, endogenous peaks of prostaglandin $F_{2\alpha}$ (PGF $_{2\alpha}$) and 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (PGFM) are associated with simultaneous pulses of ovarian oxytocin and oxytocin-neurophysin during luteolysis (Fairclough *et al.*, 1980; Flint and Sheldrick 1983; Hooper *et al.*, 1986). Bolus injections of oxytocin evoke uterine PGF $_{2\alpha}$ and PGFM release, and maximal responses occur at the end of the oestrous cycle (Roberts *et al.*, 1976; Fairclough *et al.*, 1984). Flint and Sheldrick (1983) propose that oxytocin plays an important role in the control of uterine PGF $_{2\alpha}$ release during the late luteal phase of the oestrous cycle.

During the oestrous cycle, uterine PGF $_{2\alpha}$ release is also under the control of progesterone. Silvia and Raw (1993)

showed that progesterone controls both the magnitude and frequency of the PGF $_{2\alpha}$ pulses observed in steroid-treated ovariectomized ewes, whereas withdrawal of progesterone late in the oestrous cycle results in a rapid increase in endometrial oxytocin receptors and a gradual increase in oxytocin-induced PGFM response. However, it has been clearly demonstrated that the inhibitory effect of progesterone on uterine oxytocin receptor concentrations is reduced after 10 days of exposure to progesterone (Homanics and Silvia, 1988; Vallet *et al.*, 1990; Lau *et al.*, 1992a), possibly due to downregulation of the progesterone receptor (Ott *et al.*, 1993).

In early pregnancy, the distinctive pulsatile pattern of PGF $_{2\alpha}$ release, which occurs during the late luteal phase of the cycle, is absent (Thorburn *et al.*, 1973; Hooper *et al.*, 1986). This change in the pattern of PGF $_{2\alpha}$ secretion has been attributed to the inhibitory effect of conceptus secretory proteins, in particular interferon tau (IFN- τ), which are secreted into the uterine lumen during early pregnancy (see review by Roberts *et al.*, 1990). High-affinity binding sites for IFN- τ have been observed in sheep endometrial preparations (Godkin *et al.*, 1984a; Stewart *et al.*, 1987). Furthermore,

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Received 1 June 1994.

Vallet *et al.* (1990) demonstrated inhibition of uterine oxytocin receptors *in vivo* by IFN- τ . It appears that the anti-luteolytic effect of IFN- τ also depends on the presence of progesterone and that IFN- τ stabilizes the endometrial progesterone receptor (Bazer *et al.*, 1994). Since IFN- τ can no longer be detected after day 21 of early pregnancy (Godkin *et al.*, 1984b), the role of continuing progesterone secretion throughout pregnancy in the suppression of uterine PGF_{2 α} release must be questioned.

Burgess *et al.* (1990) demonstrated that pregnant ewes could release PGF_{2 α} in response to administered oxytocin after day 21 of pregnancy as well as later in gestation. Boshier *et al.* (1991) show that prostaglandin H synthase (PGS) activity is high in uterine tissues between days 30 and 54 of gestation and in the later stages of pregnancy, while an increase in PGS synthetic capacity also occurs in mid- and late gestation (Rice *et al.*, 1990). These observations indicate that either oxytocin receptor concentrations or the transduction of the oxytocin-prostaglandin signal, or both factors, is increased, even in the presence of high progesterone concentrations, following their inhibition in early pregnancy. This release from inhibition could be responsible for the increase in PGF_{2 α} observed in late pregnancy and at parturition in this species (Evans *et al.*, 1982; Burgess *et al.*, 1990).

The concentrations of endometrial oxytocin receptors and their functionality have not been investigated throughout pregnancy. These studies were thus performed to investigate the relationship between plasma progesterone, oxytocin-induced PGFM release and endometrial oxytocin receptor concentrations in cyclic, pregnant and parturient ewes.

Materials and Methods

Animals

Forty Merino ewes were used in this study. Ewes were mated with either a vasectomized (10 ewes) or an intact (30 ewes) ram. Ewes were randomly assigned to the following groups, days 14 and 17 nonpregnant and days 14, 17, 25, 65, 85 and 145 pregnant, corresponding to the day of oxytocin challenge and determination of endometrial oxytocin receptors. The presence of embryos in the uterus at the end of the experiment was used to determine whether animals were pregnant. Only data from animals that were demonstrated to be pregnant were used in the pregnancy groups.

Experimental protocol

On the day of oxytocin challenge, each ewe was given a bolus i.v. injection of 10 iu oxytocin (Heriot Agvet, Melbourne). Blood samples were collected via venepuncture at -10, -5, 5, 10, 20, 30, 45 and 60 min after oxytocin injection for PGFM analysis. At the completion of the oxytocin challenge, all ewes were hysterectomized under general anaesthesia (300 mg sodium pentobarbitone with 800 mg sodium thiopentone, i.v., Boehringer and Ingelheim, Melbourne). Uteri were excised and collected onto ice. Microsome fractions were prepared from the uterine endometrium (combined caruncular

and intercaruncular) and assayed for oxytocin receptors as described below.

Hormonal analysis

Progesterone. Plasma progesterone concentrations were measured using a Direct Progesterone [¹²⁵I]radioimmunoassay kit from Farnos Diagnostica (Turku, Finland). The anti-serum used was a bovine serum albumin-11 α -progesterone conjugate raised in rabbits, which was reconstituted in 11 ml of assay buffer supplied with the kit. The crossreactivity of the antisera for progesterone, 11 β -hydroxyprogesterone, 5-dihydroprogesterone, 5 β -dihydroprogesterone, 17-hydroprogesterone, 20-hydroprogesterone, 20 β -hydroprogesterone, pregnenolone, pregnenolone-3-sulfate (Na-salt), pregnenolone-3-glucuronide, corticosterone, 11-deoxycorticosterone, cortisol, cortisone, testosterone, oestrone, oestradiol and oestriol was 100%, 75%, 8.8%, 7.1%, 0.23%, 0.14%, 0.08%, 0.23%, 0.26%, 0.29%, 0.93%, <0.01%, <0.01%, <0.01%, <0.01%, <0.01%, <0.01% and <0.01%, respectively. The intra- and interassay coefficients of variation were 6.4% and 12.6%, respectively (calculated from 10 assays), with a sensitivity of 0.5 nmol l⁻¹.

PGFM. Plasma PGFM concentrations were measured by a direct radioimmunoassay as described by Burgess *et al.* (1990). The 200 μ l sample was incubated overnight at 4°C with 100 μ l [³H]PGFM (13,14 dihydro-15-keto-5,6,8,9,11,12,14-[³H(n)] PGF_{2 α}) purchased from Amersham (Amersham), and 100 μ l PGFM antisera (final dilution 1/7250). The crossreactivity of the antisera with 6,15-diketo-13,14-dihydro-PGF_{2 α} was 4.6%, that with 13,14-dihydro-15-keto-PGE₂ was 1.9%, 15-keto-PGF_{2 α} was 0.94%, 15-keto-PGE₂ was 0.33%, PGA₂ was 0.02%, 6-keto-PGF_{1 α} was <0.01%, 6-keto-PGE₁ was <0.01%, PGG was <0.01%, PGD₂ was <0.01%, PGB₂ was <0.01%, PGF₁ was <0.01%, PGF_{2 α} was <0.01% and PGE₂ was <0.01%. The sensitivity of the assay was 0.4 nmol l⁻¹, and the intra- and interassay coefficients of variation were 4.5% and 7.7%, respectively.

Oxytocin receptor binding assay. The endometrial microsomal fractions containing the oxytocin receptors were prepared by the method described by Sheldrick and Flint (1985). The abundance of oxytocin receptors was measured by a receptor binding radioimmunoassay as described by Lau *et al.* (1992a, b). Endometrial microsomes (100 μ l) containing approximately 400–800 μ g protein were incubated for 2 h at room temperature (25°C) with 100 μ l [³H]oxytocin ([tyrosyl-2,6-³H]oxytocin, 39.3 Ci mmol⁻¹, NEN; approximately 11 500 c.p.m.) and 100 μ l of 50 mmol Tris-HCl buffer l⁻¹ containing various amounts of oxytocin standard (Calbiochem, La Jolla) ranging from 0 to 4 ng. The intra- and interassay coefficients of variation for the receptor binding radioimmunoassay were 4.0% and 12.5%, respectively.

Uterine oxytocin receptor concentrations were measured using Scatchard Analysis (Scatchard, 1949), using an EBDA computer program (McPherson, Cambridge, 1985, V2.0). The oxytocin receptor concentrations were expressed as fmol mg⁻¹ protein. The dissociation constant (K_d), expressed as nmol l⁻¹, was also calculated using this program.

Protein concentrations

Protein concentrations were measured using a BioRad Protein Kit II (BioRad, Richmond) using a BSA (Fraction V) standard (Sigma; Bradford, 1976).

Data analysis and statistics

The responsiveness of the uterus to oxytocin (total PGFM release in response to oxytocin) was measured by calculating the area under the curve of PGFM release from 0 to 60 min after oxytocin injection after subtraction of baseline concentrations. The area under the curve was calculated by the trapezoidal rule (Abramowitz and Stegun, 1968), and was expressed as $\text{nmol l}^{-1} t$, where t represents the 60 min after oxytocin injection. Maximum (peak) PGFM concentrations reached during the 60 min after oxytocin injection were also used as a measure of uterine PGF_{2a} release in response to oxytocin. The relationship between oxytocin receptor concentrations and PGF_{2a} release in response to oxytocin was expressed as the ratio of total PGFM release to oxytocin receptor concentrations.

Statistical analysis was carried out via one-way analysis of variance (ANOVA) using a general purpose statistical package, Microstat (Ecosoft Inc., Manville, 1984) and by SPSSX on a VAX computer, and Student–Newman–Keuls' procedure was carried out where necessary. Data were appropriately transformed to produce homogeneous data. Statistical significance was tested to the 95% level ($P < 0.05$). Results, are expressed as means, with variance expressed as standard error of the mean ($\text{mean} \pm \text{SEM}$).

Results

Plasma progesterone

Mean plasma progesterone concentrations, in pre-oxytocin injection samples, during the oestrous cycle decreased from $12.1 \pm 1.3 \text{ nmol l}^{-1}$ on day 14 of the oestrous cycle to $6.4 \pm 1.3 \text{ nmol l}^{-1}$ on day 17 of the oestrous cycle. By day 65 of gestation, progesterone concentration had increased to $29.9 \pm 3.4 \text{ nmol l}^{-1}$. High progesterone concentrations were maintained for the remainder of gestation (see Table 1).

Basal plasma PGFM

Basal PGFM concentrations were calculated as the mean of the two pre-oxytocin injection bleeds. Basal PGFM concentrations increased as pregnancy progressed from $0.8 \pm 0.1 \text{ nmol l}^{-1}$ on day 14 of gestation to $1.8 \pm 0.4 \text{ nmol l}^{-1}$ on day 145 of gestation. Basal PGFM concentrations were similar during the oestrous cycle and the equivalent stages of pregnancy.

Oxytocin challenge

Administration of 10 iu oxytocin caused an increase in peripheral plasma PGFM concentrations in all of the groups studied. The maximum concentration of PGFM observed in response to oxytocin over the 60 min sampling period on days 14, 17 and 25 of gestation was similar to the maximum

Table 1. Progesterone concentrations in peripheral plasma throughout gestation and on days 14 and 17 of the oestrous cycle in ewes

Days of gestation (P)/ oestrous cycle (NP)	Progesterone concentration ($\text{nmol l}^{-1} \pm \text{SEM}$)*
14P	$7.3 \pm 0.6^{\text{a,b}}$
17P	$13.7 \pm 1.0^{\text{c}}$
25P	$10.5 \pm 1.3^{\text{b,c}}$
65P	$29.9 \pm 3.8^{\text{d}}$
85P	$31.5 \pm 2.9^{\text{d}}$
145P	$55.7 \pm 3.8^{\text{e}}$
14NP	$12.1 \pm 1.3^{\text{c}}$
17NP	$6.4 \pm 1.3^{\text{a}}$

*Groups with different superscripts are significantly different ($P < 0.05$). P: pregnant; NP: nonpregnant.

Table 2. Oxytocin-induced peak 13,14, dihydro-15-keto prostaglandin F_{2a} (PGFM) concentrations in peripheral plasma throughout gestation and on days 14 and 17 of the oestrous cycle in ewes

Days of gestation (P)/ oestrous cycle (NP)	Peak PGFM ($\text{nmol l}^{-1} \pm \text{SEM}$)*
14P	$1.28 \pm 0.38^{\text{a,b,c}}$
17P	$1.90 \pm 0.49^{\text{b,c,d}}$
25P	$0.63 \pm 0.23^{\text{a}}$
65P	$4.30 \pm 0.60^{\text{e,f}}$
85P	$5.64 \pm 0.89^{\text{f,g}}$
145P	$11.72 \pm 4.84^{\text{h}}$
14NP	$1.32 \pm 0.69^{\text{a,b}}$
17NP	$2.32 \pm 0.14^{\text{c,d,e}}$

*Groups with different superscripts are significantly different ($P < 0.05$). P: pregnant; NP: nonpregnant.

observed on day 14 and day 17 of the oestrous cycle. However, increases in maximum PGFM concentrations were observed on days 65, 85 and 145 of gestation compared with days 14, 17 and 25 of gestation. During the oestrous cycle, maximum PGFM concentrations were similar to those observed at the equivalent stages of gestation (Table 2).

Total PGFM release in response to oxytocin, as measured by the area under the curve, on day 17 of the oestrous cycle was increased significantly ($P < 0.05$) compared with that observed at day 14 of the oestrous cycle and days 14, 17 and 25 of gestation. However, the response to oxytocin was increased on days 65, 85 and 145 of gestation compared with that observed during earlier stages of gestation (day 14, 17 and 25). The maximum response to oxytocin was observed on day 145 of gestation (Fig. 1).

Endometrial oxytocin receptor concentrations

Oxytocin receptor concentrations were higher on day 17 of the oestrous cycle than on days 14 of the cycle or on day 14,

Fig. 1. Mean ($\pm$ SEM, $n = 4$ or 5) total 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (PGFM) released, calculated as the area under the curve, over the 60 min subsequent to i.v. oxytocin (10 iu), during day 14 and 17 of the oestrous cycle (14NP and 17NP) and throughout gestation (on days 14, 17, 25, 65, 85 and 145). Groups with different letters above bars are significantly different ($P < 0.05$).

Fig. 2. Mean ($\pm$ SEM, $n = 4$ or 5) concentrations of oxytocin receptors in uterine endometrium on days 14 and 17 of the oestrous cycle (14NP and 17NP) and throughout gestation (days 14, 17, 25, 65, 85 and 145). Groups with different letters above bars are significantly different ($P < 0.05$).

17 and 25 of gestation. Although oxytocin receptor concentrations increased in mid-gestation (day 65) to very high concentrations equivalent to those observed on day 17 of the cycle, they had again decreased by day 85 of gestation and remained low to day 145 of gestation (Fig. 2). The dissociation constant (K_d) was examined for receptors in each group studied. There was no difference between the K_d values in any of the groups studied (mean $K_d = 3.2 \pm 0.5$ nmol l^{-1}).

On day 17 of the oestrous cycle the ratio between oxytocin-induced PGFM release and oxytocin receptor concentrations was low (0.1 ± 0.01). Throughout early and mid-pregnancy this ratio showed no significant variation, with values ranging from 0.2 ± 0.04 to 0.7 ± 0.1 between days 14 and 65 of gestation.

Fig. 3. Mean ($\pm$ SEM, $n = 4$ or 5) of the ratio between the 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (PGFM) response to an oxytocin bolus and oxytocin receptor concentrations, during the oestrous cycle on days 14 and 17 (14NP and 17NP) and throughout gestation (on days 14, 17, 25, 65, 85 and 145). Groups with different letters above bars are significantly different ($P < 0.05$).

The ratio increased by day 85 of gestation to 1.02 ± 0.2 , and was increased in late pregnancy (day 145), to 3.3 ± 0.5 (Fig. 3).

Discussion

The results of this study have confirmed previous reports that in early pregnancy (preimplantation, day 14) the release of uterine PGFM in response to exogenous oxytocin is minimal and similar to that observed on day 14 of the oestrous cycle (Sheldrick and Flint, 1985; Flint and Sheldrick, 1986; Zarco *et al.*, 1988; Stewart *et al.*, 1993). The marked increase in oxytocin receptor concentration by day 17 of the oestrous cycle, together with its inhibition at an equivalent stage of gestation, also supports previous reports (McCracken, 1980; McCracken *et al.*, 1984; Sheldrick and Flint, 1985; Flint and Sheldrick, 1986). Thus, this work has confirmed that an inhibition of endometrial oxytocin receptors occurs during early pregnancy and that this inhibition is an important mechanism whereby oxytocin-induced uterine PGFM release is inhibited at this time (McCracken, 1980; Flint *et al.*, 1986; Hooper *et al.*, 1986; Burgess *et al.*, 1990). An interaction between progesterone and IFN- τ has been implicated in the inhibition of the endometrial oxytocin receptors and PGFM release (Ott *et al.* 1993). Thus, it would be expected that oxytocin receptors are inhibited to day 21 of gestation, when IFN- τ is secreted and not after this time when IFN- τ is no longer present (Godkin *et al.*, 1984b) and, presumably, down-regulation of the progesterone receptor would ensue.

Synthesis of PGF $_{2\alpha}$ in the uterus is increased in the later stages of gestation (Burgess *et al.*, 1990; Rice *et al.*, 1990; Boshier *et al.*, 1991), as is the activity of the prostaglandin synthase enzyme and its localization in maternal placenta (Boshier *et al.*, 1991), as well as tissue PGF $_{2\alpha}$ content (Rice *et al.*, 1990; Boshier *et al.*, 1991; Evans *et al.*, 1992). Thus, the increase in release of PGF $_{2\alpha}$ in response to oxytocin observed at day 65 of gestation is complementary to our observation that endometrial oxytocin receptor concentrations are increased at this

time. The availability of free arachidonic acid, the precursor for prostaglandin synthesis, has been shown to be a limiting factor in prostaglandin synthesis (Bergström *et al.*, 1964; Van Dorp *et al.*, 1964; Lands and Samuelsson, 1968; Vonkeman and Van Dorp, 1968). Changes in availability of arachidonic acid in the uterus may therefore also be important in the control of prostaglandin synthesis during gestation (Meier *et al.*, 1994).

The release of PGF_{2α} in response to oxytocin has been closely associated with endometrial oxytocin receptor concentrations under a range of physiological situations (Sheldrick and Flint, 1985). The results of this study indicate that this association is changed in late pregnancy. When uterine responsiveness to oxytocin was measured as a ratio between PGFM release and oxytocin receptor concentration, it was found that the ratio was less than 1 until day 65 of gestation but that, by day 85 and day 145, this ratio was significantly ($P < 0.05$) increased. This indicates that during the latter stages of pregnancy, and particularly close to term (day 145), the release of PGFM is dissociated from the number of endometrial oxytocin receptors. It has been suggested that only a small number of oxytocin receptors need to be occupied to elicit a response (Lopez Bernal *et al.*, 1989). Alternatively, it is possible that high oxytocin receptor concentrations, in uterine tissues other than the endometrium (Wathes *et al.*, 1993), can release PGF_{2α} at this time. Oxytocin receptors have been localized in both the endometrium and myometrium in sheep, with cellular localization varying with the oestrous cycle (Wathes and Hamon, 1993).

In contrast to the increased ratio of PGFM release to oxytocin receptor concentration in late gestation, late in the oestrous cycle (day 17) oxytocin receptor concentrations greatly increased compared with the small increase in PGFM in response to exogenous oxytocin, as indicated by a very small PGFM to oxytocin receptor ratio. Such a situation might occur if different isoforms of the oxytocin receptor are present in endometrial tissues at different reproductive stages. Pliska *et al.* (1986) proposed that there are at least two, if not three, types of oxytocin receptor with affinities ranging from 5×10^{-9} to 10^{-5} mol l⁻¹, while work by Chan *et al.* (1993) using oxytocin receptor antagonists also indicates that there are at least two oxytocin receptor subtypes, and that different functions are associated with each. If only one isoform of the receptor was measured by the assay used in the study reported here, a variation in receptor types present in endometrial tissues may contribute to this dissociation. Recent work, using an oxytocin receptor probe isolated from human myometrium mRNA, has confirmed the appearance of an oxytocin receptor during the oestrous cycle as measured by conventional receptor binding assay (Kimura *et al.*, 1992; Stewart *et al.*, 1993). However, there appears to be dissociation between the amount of mRNA encoding the oxytocin receptor measured using this probe and the oxytocin receptors measured with the binding assay used in this study (D. C. Wathes, personal communication). The observation of a dissociation between oxytocin receptors and uterine responsiveness to oxytocin is not unique. It has previously been described in ovariectomized ewes, in which oxytocin receptor concentrations are high at a time when release of PGF_{2α} in response to oxytocin is absent (Vallet and Lamming, 1991; Lau *et al.*, 1992b).

In this study we have documented the interaction between oxytocin-induced PGF_{2α} release in the uterus and endometrial oxytocin receptors during the oestrous cycle and in pregnancy. It has been demonstrated that, under certain conditions, the association between oxytocin receptor concentrations and PGF_{2α} release changes. The mechanisms underlying this dissociation are still unknown and require further research.

This work was performed in part, at the Victorian Institute of Animal Science (VIAS), Werribee and we acknowledge the assistance of VIAS and their staff. The work was undertaken with the assistance of an NHMRC grant to G. Jenkin, an Australian Meat Research Corporation grant to R. J. Fairclough and the financial assistance of Ferring Pharmaceuticals.

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