Activity of phospholipase C and release of prostaglandin $F_{2\alpha}$ by endometrial tissue from ewes during the oestrous cycle and early pregnancy

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Oxytocin appears to play an important role in regulating uterine secretion of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in sheep. Changes in uterine secretion of $PGF_{2\alpha}$ throughout the oestrous cycle and early pregnancy may be due to changes in the intracellular regulatory pathways that control synthesis of $PGF_{2\alpha}$ in response to oxytocin. In this experiment, caruncular endometrial tissue was collected from ewes throughout the oestrous cycle and early pregnancy. Endometrial tissue was incubated *in vitro* to assess release of $PGF_{2\alpha}$ and activity of phospholipase C (PLC) in response to oxytocin. Release of $PGF_{2\alpha}$ in the presence of arachidonic acid was used to assess the activity of prostaglandin H_2 endoperoxide synthase (PGS). In non-pregnant ewes, oxytocin stimulated release of $PGF_{2\alpha}$ from endometrial tissue collected on days 14 and 16, but not on days 4–7, 10 or 12 after oestrus. This coincided with times when oxytocin stimulated the activity of PLC. Release of $PGF_{2\alpha}$ was enhanced by the addition of arachidonic acid to tissues collected on days 12, 14 and 16 after oestrus. As with tissue from nonpregnant ewes, oxytocin could stimulate release of $PGF_{2\alpha}$ on days 14 and 16 of early pregnancy. Yet, oxytocin had no effect on activity of PLC in tissue from pregnant ewes. Release of $PGF_{2\alpha}$ in the presence of arachidonic acid by tissue from pregnant ewes was similar to that in nonpregnant ewes at comparable times after oestrus. On the basis of these results, it was concluded that (1) an increase in the ability of oxytocin to stimulate activity of PLC occurs coincidentally with the increase in its ability to stimulate release of $PGF_{2\alpha}$ from ovine endometrial tissue; (2) activity of PGS in endometrial tissue increases at least 2 days before an increase in secretory responsiveness to oxytocin; (3) the ability of the conceptus to inhibit release of $PGF_{2\alpha}$ in response to oxytocin *in vivo*, as demonstrated in several previous reports, is lost during *in vitro* incubation in the absence of the conceptus; and (4) oxytocin stimulates release of $PGF_{2\alpha}$ by endometrial tissue from pregnant ewes despite the fact that oxytocin does not stimulate activity of PLC in this same tissue. Thus, the role of PLC in mediating the stimulatory effect of oxytocin on endometrial secretion of $PGF_{2\alpha}$ appears questionable.

Introduction

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), from the uterus, causes luteal regression in ewes (McCracken *et al.*, 1972; Inskeep and Murdoch, 1980). During luteal regression, $PGF_{2\alpha}$ is secreted in a series of high amplitude, short duration pulses (Thorburn *et al.*, 1973; Barcikowski *et al.*, 1974; Fairclough *et al.*, 1980; Webb *et al.*, 1981; Flint and Sheldrick, 1983; Zarco *et al.*, 1988a). Oxytocin may play an important role in generating this pulsatile pattern of secretion (Flint *et al.*, 1990). Uterine $PGF_{2\alpha}$ and luteal oxytocin exist in a positive feedback loop (Flint *et al.*, 1986). Each pulse of $PGF_{2\alpha}$ may result from the activation of this feedback loop.

Maturation of the positive feedback loop for uterine $PGF_{2\alpha}$: luteal oxytocin may determine when pulsatile secretion of $PGF_{2\alpha}$ occurs during the oestrous cycle. The ability of the uterus

to secrete $PGF_{2\alpha}$ in response to oxytocin does not develop until day 13–14 after oestrus (Roberts *et al.*, 1975; Roberts and McCracken, 1976; Fairclough *et al.*, 1984; Silvia *et al.*, 1992). This coincides with the onset of endogenous pulsatile secretion of $PGF_{2\alpha}$ from the uterus (Thorburn *et al.*, 1973; Flint and Sheldrick, 1983; Zarco *et al.*, 1988a). Similar differences in secretory responsiveness to oxytocin were observed, *in vitro*, when endometrial tissue was collected from ewes at various stages of the oestrous cycle (Roberts *et al.*, 1976). Changes in uterine secretory responsiveness to oxytocin may be the result of changes in the concentration of receptors for oxytocin which occur during the oestrous cycle (Sheldrick and Flint, 1985). Whether the increase in receptors for oxytocin occurs prior to, or following, the onset of luteal regression remains to be determined conclusively.

It is possible that changes in uterine secretory responsiveness to oxytocin during the oestrous cycle are the result of changes

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occurring in the intracellular regulatory systems that link the receptor to the synthesis and secretion of $\text{PGF}_{2\alpha}$ (i.e. at the post-receptor level). Oxytocin stimulates activity of an endometrial phospholipase C (PLC) that can cleave phosphatidylinositol bisphosphate into diacylglycerol and inositol triphosphate (Flint *et al.*, 1986; Silvia and Homanics, 1988). Agonists of second messengers generated by activation of PLC stimulate release of $\text{PGF}_{2\alpha}$ from endometrial tissue *in vitro* (Silvia and Homanics, 1988; Raw and Silvia, 1991). Stimulation of synthesis and secretion of $\text{PGF}_{2\alpha}$ by oxytocin, therefore, appears to be mediated through the activation of PLC. Changes in the ability of oxytocin to activate PLC could account for the changes in uterine secretory responsiveness to oxytocin observed during the oestrous cycle. Mirando *et al.* (1990a) showed that the ability of oxytocin to stimulate activity of PLC is much greater on day 16 than on day 12 after oestrus. However, it has not been determined whether this change in responsiveness of PLC precedes the onset of luteal regression. It is also possible that changes may occur, during the oestrous cycle, in the ability of the second messengers produced by PLC to activate synthesis of $\text{PGF}_{2\alpha}$.

Prostaglandins are synthesized from the fatty acid precursor, arachidonic acid. In many cell types, agonists that stimulate synthesis of prostaglandins do so by promoting the mobilization of arachidonic acid from phospholipid storage pools (Irvine, 1982). Oxytocin may have a similar effect in uterine tissue. The first step in prostaglandin synthesis is the peroxidation of arachidonic acid to PGH_2 by prostaglandin H endoperoxide synthase (PGS; Smith, 1989). Changes in the ability of oxytocin to stimulate uterine secretion of $\text{PGF}_{2\alpha}$ during the oestrous cycle may be due to changes in the concentration or activity of PGS.

During early pregnancy, the pulsatile pattern of uterine $\text{PGF}_{2\alpha}$ secretion is suppressed (Thorburn *et al.*, 1973; Barcikowski *et al.*, 1974; Nett *et al.*, 1976; Peterson *et al.*, 1976; Webb *et al.*, 1981; Moore *et al.*, 1982; Hooper *et al.*, 1986; Zarco *et al.*, 1988b). One factor that may contribute to this suppression is a disruption of the uterine $\text{PGF}_{2\alpha}$ luteal oxytocin positive feedback loop. The ability of oxytocin to stimulate secretion of $\text{PGF}_{2\alpha}$ on days 13–16 of pregnancy is much less than its ability to do so on corresponding days of the oestrous cycle (Fairclough *et al.*, 1984; McCracken *et al.*, 1984; Burgess *et al.*, 1990; Silvia *et al.*, 1992). The increase in concentration of endometrial receptors for oxytocin associated with luteolysis in nonpregnant ewes does not occur in pregnant ewes (McCracken *et al.*, 1984; Sheldrick and Flint, 1985). However, changes in endometrial tissue occurring at the post-receptor level may also contribute to the suppression of endometrial secretory responsiveness to oxytocin during early pregnancy. The ability of oxytocin to stimulate activity of PLC on day 16 after oestrus was much lower in pregnant than in nonpregnant ewes (Mirando *et al.*, 1990a).

The objectives of this experiment were to characterize the changes that occur during the oestrous cycle and early pregnancy in (1) the ability of oxytocin to stimulate activity of PLC, (2) the ability of caruncular endometrial tissue to release $\text{PGF}_{2\alpha}$ in response to agonists of second messengers produced by PLC and (3) the capacity of endometrial tissue to convert arachidonic acid to $\text{PGF}_{2\alpha}$. Changes in these variables were then related to changes in the ability of oxytocin

to stimulate release of $\text{PGF}_{2\alpha}$ during the oestrous cycle and early pregnancy.

Materials and Methods

Forty-three ewes were checked for oestrous behaviour twice daily (07:00 h and 17:00 h) with a vasectomized ram. After at least one oestrous cycle of normal duration (15.5–18.0 days), ewes were randomly assigned to one of two treatment groups: nonpregnant ($n = 24$) or bred ($n = 19$). Ewes assigned to the bred group were mated with an intact ram at oestrus (day 0). Beginning on day 0, blood samples from the jugular vein were collected by venepuncture daily at 08:00 h until tissue collection. Blood samples were allowed to clot for 12–24 h at 4°C. Serum was harvested and stored at –20°C until assayed. Concentrations of progesterone were determined in these samples, to assess luteal function at the time of tissue collection, using a commercial, solid-phase radioimmunoassay kit (Coat-a-Count, Diagnostic Products Corp., Los Angeles, CA) as described by Silvia *et al.* (1992). Samples were run in three assays. Intra- and interassay coefficients of variation were 8 and 5%, respectively.

Uteri were collected immediately after death from nonpregnant ewes on days 4–7 ($n = 4$), 10 ($n = 4$), 12 ($n = 6$), 14 ($n = 5$) or 16 ($n = 5$) and from bred ewes on days 12 ($n = 8$), 14 ($n = 4$) and 16 ($n = 7$) after oestrus. Ewes were killed by exsanguination after captive bolt concussion. For bred ewes, each uterine horn was flushed with 20 ml of Krebs–Henseleit–bicarbonate buffer (KHB; 118 mmol NaCl l⁻¹, 4.7 mmol KCl l⁻¹, 2.56 mmol CaCl₂ l⁻¹, 1.13 mmol MgCl₂ l⁻¹, 25 mmol NaHCO₃ l⁻¹, 1.15 mmol NaH₂PO₄ l⁻¹, 5.55 mmol glucose l⁻¹, 20 mmol Hepes l⁻¹, 0.013 mmol phenol red l⁻¹, pH 7.4) to recover embryos and confirm pregnancy. Thirty-six caruncular endometrial explants (40–100 mg) were dissected from the uterus and placed in KHB. Explants were weighed and transferred to 12 × 75 mm borosilicate glass culture tubes containing KHB and then incubated to measure activity of PLC or release of $\text{PGF}_{2\alpha}$.

Assay for phospholipase C

Four explants were used to measure the activity of phospholipase C as described by Silvia and Homanics (1988). Briefly, explants were incubated in four successive periods lasting 90, 60, 5 and 30 min, respectively. During periods 1–3, explants were prepared for measurement of phospholipase C activity. This consisted of labelling phosphatidylinositol bisphosphate by incubation of explants with [³H]myo-inositol and inactivation of endogenous inositol phosphate phosphatase by incubation with LiCl. All explants were treated identically during periods 1–3. In period 4, two explants from each ewe were incubated in 0.5 ml KHB, including 10 mmol LiCl l⁻¹ and 1 μmol l⁻¹ oxytocin. The remaining two explants were incubated in LiCl-supplemented KHB to serve as controls. Incubations were terminated and [³H]inositol phosphates (inositol tris-, bis- and monophosphates) extracted and measured as described by Silvia and Homanics (1988). Activity of PLC was expressed as total inositol phosphates (c.p.m.) per gram of tissue.

Release of prostaglandin F_{2α}

Thirty-two explants from the same ewes were subjected to a similar, four-period, *in vitro* incubation protocol. Explants were incubated in KHB during periods 1–3. In period 4, four explants of each tissue type were incubated in 0.5 ml KHB with the following additions: (1) no addition (control), (2) oxytocin (1 μmol l⁻¹), (3) arachidonic acid (20 mg l⁻¹), (4) oxytocin plus arachidonic acid, (5) phorbol 12-myristate 13-acetate (PMA, 0.1 μmol l⁻¹), (6) A23187 (1 μmol l⁻¹), (7) PMA plus A23187 or (8) indomethacin (10 mg l⁻¹). Incubations were terminated and concentrations of PGF_{2α} in incubation medium were measured by radioimmunoassay as described by Silvia and Homanics (1988). The samples were run in 12 assays. The intra- and inter-assay coefficients of variation were 13 and 19%, respectively. The rate of release of PGF_{2α} was expressed as ng min⁻¹ g⁻¹ wet tissue.

Pregnancy rate

Embryos were recovered from four of four bred ewes killed on day 14 and from four of seven ewes killed on day 16 after mating. A definitive embryo was recovered from only one of eight bred ewes killed on day 12. Tissue fragments that could have been from embryos were recovered from another three ewes. No embryos or fragments were recovered from the remaining four ewes. This low recovery rate on day 12 was not unusual or unexpected, particularly when dealing with ewes that had not been superovulated (Silvia and Niswender, 1986). We assumed that the pregnancy rate in the group killed on day 12 would be similar to that in those killed on days 14 and 16 (8 pregnant/11 bred; 73%) when embryos are more easily recovered. In a study conducted earlier in the breeding season (Silvia *et al.*, 1992), using the same flock of ewes and the same rams under similar breeding conditions, the pregnancy rate was also 73% (33 pregnant/45 bred). Only six of the eight bred ewes killed on day 12 were therefore likely to have been pregnant. Since we had no conclusive evidence with which to identify the pregnant ewes, we considered all eight ewes to be pregnant and analysed the data accordingly.

Statistical analysis

For those ewes killed on day 14 or 16, the mean and standard deviation of the concentrations of progesterone in the five daily blood samples collected during the midluteal phase (days 8–12 after oestrus) were calculated. Ewes were defined as undergoing luteal regression if the concentration of progesterone in the sample collected immediately prior to death was more than two standard deviations below the mean of the midluteal phase. Luteal regression was defined as being completed if concentrations of progesterone had fallen below 1 ng ml⁻¹.

Release of PGF_{2α} in the presence of indomethacin is presumed to be due to the release of PGF_{2α} synthesized prior to the onset of the final treatment incubation period. Indomethacin-suppressible release of prostaglandins (i.e. the difference between release from control explants and release from indomethacin-treated explants) is assumed to represent release of PGF_{2α} that depends on synthesis during the final incubation period. Since one objective of this investigation was to relate synthesis of

PGF_{2α} during the final incubation period to activity of PLC during the same period, values for release of prostaglandins from all other *in vitro* treatments have been adjusted by subtracting the release of PGF_{2α} from the indomethacin-treated explants for that ewe. On average, indomethacin suppressed release of PGF_{2α} from explants by 44%.

Effects of day after oestrus, pregnancy and oxytocin on activity of phospholipase C were determined by analysis of variance for a split-plot design. Effects of day after oestrus and pregnancy were considered main plots and oxytocin considered a subplot within ewe. Effects of day after oestrus, pregnancy and *in vitro* treatments on release of PGF_{2α} were determined using analysis of variance for a split-plot design. Effects of day after oestrus and pregnancy were considered main plots and *in vitro* treatments (oxytocin and arachidonic acid, or PMA and A23187) considered subplots within ewe applied in a 2 × 2 factorial arrangement. When the initial analysis of variance indicated that it was appropriate, effects of *in vitro* treatment and day after oestrus were determined in pregnant and nonpregnant ewes, separately. All data were transformed logarithmically prior to analysis to reduce heterogeneity of variance among treatment groups. All analyses of variance were conducted using the General Linear Models procedure of SAS (1985).

Results

Concentrations of progesterone up to the time of death were typical for ewes experiencing normal oestrous cycles. All ewes appeared to reach a normal level of luteal function (2–6 ng progesterone ml⁻¹) during the oestrous cycle of tissue collection. On the basis of the statistical criteria described above, luteal regression had been completed in one of five nonpregnant ewes killed on day 14. The remaining four ewes were killed prior to the onset of luteolysis. Luteal regression had been completed in four of five ewes killed on day 16. The remaining ewe was killed during luteolysis.

When overall release of PGF_{2α} is considered across *in vitro* treatment groups, an effect of day after oestrus was observed ($P < 0.01$, Fig. 1). Release of PGF_{2α} was relatively low on days 5 and 10. It increased successively on days 12 and 14, then decreased to an intermediate level by day 16. Release of PGF_{2α} was enhanced by addition of arachidonic acid ($P < 0.01$) irrespective of the day on which tissue was collected. The increase was relatively small on days 5 and 10. Release in the presence of arachidonic acid was much higher on days 12, 14 and 16 after oestrus. Release of PGF_{2α} was stimulated by oxytocin on days 14 and 16 after oestrus ($P < 0.01$), but not at any earlier time points. The effects of arachidonic acid and oxytocin were additive (i.e. there was no oxytocin × arachidonic acid interaction).

A similar temporal pattern of release over the oestrous cycle was observed when release estimates were pooled across explants receiving PMA or A23187 (Fig. 2). Release of PGF_{2α} was enhanced by PMA ($P < 0.01$) and the response was consistent across days of the oestrous cycle (i.e. there were no interactions involving PMA and day after oestrus). A stimulatory effect of A23187 was also observed ($P = 0.01$); however, the response was affected by the day after oestrus ($P = 0.02$). A23187 could induce an increase in release of PGF_{2α} only from tissues collected on days 12 and 16 after oestrus ($P < 0.09$ and

Fig. 1. Rates of release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) by ovine caruncular endometrial tissue incubated *in vitro*, in the presence of control medium ($\square$), $1 \mu\text{mol}$ oxytocin l^{-1} ($\blacksquare$), 20 mg arachidonic acid l^{-1} ($\square$) or oxytocin plus arachidonic acid ($\boxtimes$). Tissue was collected from non-pregnant ewes on days 4–7 ($n = 4$), 10 ($n = 4$), 12 ($n = 6$), 14 ($n = 5$) or 16 ($n = 5$) after oestrus. Bars represent antilog values of the least-squares means of logarithmically transformed data. Lines above bars represent anti-log values of standard errors of the least-squares means. Different superscript letters denote significant differences in release due to day of tissue collection considered across *in vitro* treatment groups ($P < 0.05$).

Fig. 2. Rates of release of $PGF_{2\alpha}$ by ovine caruncular endometrial tissue incubated *in vitro*, in the presence of control medium ($\square$), $0.1 \mu\text{mol}$ phorbol 12-myristate 13-acetate l^{-1} (PMA) ($\blacksquare$), $1 \mu\text{mol}$ A23187 l^{-1} ($\square$) or PMA plus A23187 ($\boxtimes$). Tissue was collected from nonpregnant ewes on days 4–7 ($n = 4$), 10 ($n = 4$), 12 ($n = 6$), 14 ($n = 5$) or 16 ($n = 5$) after oestrus. Bars represent antilog values of the least-squares means of logarithmically transformed data. Lines above bars represent anti-log values of standard errors of the least-squares means. Different superscripts denote significant differences in release due to day of tissue collection considered across *in vitro* treatment groups ($P < 0.05$).

$P = 0.01$, respectively). Tissues collected at other times in the oestrous cycle did not respond to A23187 ($P > 0.32$). No interactions of PMA with A23187 were observed.

Basal activity of PLC was low in tissues collected at all five points in the oestrous cycle (Fig. 3). Oxytocin had no effect on

Fig. 3. Activity of phospholipase C, expressed as total inositol phosphates (c.p.m.) per gram of tissue, in ovine caruncular endometrial tissue incubated *in vitro*, in the presence of control medium ($\square$) or oxytocin ($\blacksquare$). Tissue was collected from nonpregnant ewes on days 4–7 ($n = 4$), 10 ($n = 4$), 12 ($n = 6$), 14 ($n = 5$) and 16 ($n = 5$) after oestrus. Bars represent antilog values of the least-squares means of logarithmically transformed data. Lines above bars represent anti-log values of standard errors of the least-squares means. Asterisks indicate stimulation of activity by oxytocin ($P < 0.05$).

Fig. 4. Rates of release of $PGF_{2\alpha}$ by caruncular endometrial tissue incubated *in vitro*, in the presence of control medium ($\square$), oxytocin ($\blacksquare$), arachidonic acid ($\square$) or oxytocin plus arachidonic acid ($\boxtimes$). Tissue was collected from pregnant ewes on days 12 ($n = 8$), 14 ($n = 4$) and 16 ($n = 4$) after oestrus. Bars represent antilog values of the least-squares means of logarithmically transformed data. Lines above bars represent anti-log values of standard errors of the least-squares means.

activity of PLC in tissues collected on days 5, 10 or 12 after oestrus. Oxytocin stimulated activity of PLC on days 14 and 16 after oestrus ($P = 0.06$ and $P < 0.01$, respectively). Stimulation was much greater on day 16 than on day 14 ($P = 0.02$).

A relatively high level of $PGF_{2\alpha}$ release was sustained from day 12 through day 16 of early pregnancy (Fig. 4). Release was comparable to that observed for tissues collected from non-pregnant ewes on day 12 after oestrus and much greater than release from tissues collected from nonpregnant ewes either on day 5 or 10 after oestrus. Release of $PGF_{2\alpha}$ was enhanced

Fig. 5. Rates of release of $\text{PGF}_{2\alpha}$ by caruncular endometrial tissue incubated *in vitro*, in the presence of control medium (□), PMA (■), A23187 (▨) or PMA plus A23187 (▩). Tissue was collected from pregnant ewes on days 12 ($n = 8$), 14 ($n = 4$) and 16 ($n = 4$) after oestrus. Bars represent anti-log values of the least-squares means of logarithmically transformed data. Lines above bars represent anti-log values of standard errors of the least-squares means.

Fig. 6. Activity of phospholipase C in caruncular endometrial tissue incubated *in vitro*, in the presence of control medium (□) or oxytocin (■). Tissue was collected from nonpregnant ewes on days 12 ($n = 8$), 14 ($n = 4$) and 16 ($n = 4$) after oestrus. Bars represent anti-log values of the least-squares means of logarithmically transformed data. Lines above bars represent anti-log values of standard errors of the least-squares means.

by oxytocin ($P < 0.01$). Effects of pregnancy or interactions involving pregnancy and oxytocin were not observed implying that responses to oxytocin were similar in tissues from pregnant and nonpregnant ewes. Within the pregnant ewes, a slight but not significant oxytocin by day interaction was observed ($P = 0.14$) owing to the lower response to oxytocin observed in tissues collected on day 12 compared with days 14 or 16.

Addition of arachidonic acid to the incubation resulted in an increase in $\text{PGF}_{2\alpha}$ release ($P < 0.04$) at all three times after oestrus. An interaction of arachidonic acid with pregnancy status was observed ($P < 0.01$). This appears to be due to a greater conversion of arachidonic acid to $\text{PGF}_{2\alpha}$ by tissues from pregnant ewes, particularly when collected on days 14 and 16 after mating. An interaction of oxytocin with arachidonic acid was also observed ($P < 0.01$). This was probably due to the failure of oxytocin to consistently induce further stimulation of $\text{PGF}_{2\alpha}$ release in the presence of arachidonic acid.

Within pregnant ewes, release of $\text{PGF}_{2\alpha}$ was slightly but not significantly enhanced by both PMA and A23187 ($P = 0.10$ and $P = 0.06$, respectively; Fig. 5). There were no interactions involving day after oestrus, PMA or A23187, implying that responses to PMA and A23187 were consistent across days. When release of $\text{PGF}_{2\alpha}$ was considered across *in vitro* treatment groups, no difference in overall release was observed among days. When compared with nonpregnant ewes at comparable stages of the oestrous cycle, no effects of pregnancy or interactions involving pregnancy status were observed.

Basal activity of PLC was low in tissues collected at all three stages of early pregnancy (Fig. 6). Oxytocin was not able to stimulate activity of PLC on any of the days examined.

Discussion

Release of $\text{PGF}_{2\alpha}$ from caruncular endometrial explants collected on days 5 or 10 of the oestrous cycle was very low. There was a

dramatic increase in release from tissue collected on day 12 after oestrus. Maximum release was observed from tissues collected on day 14; there was then a decline in release from tissues collected on day 16. The temporal pattern of release of $\text{PGF}_{2\alpha}$ from ovine caruncular observed in this study is similar to that described by Roberts *et al.* (1976). The ability of oxytocin to stimulate uterine release of $\text{PGF}_{2\alpha}$ was evident on days 14 and 16 after oestrus. Roberts *et al.* (1976) observed a weak secretory response to oxytocin from tissue collected on day 13 after oestrus. A much more robust response was observed from tissues collected on day 15. Release was somewhat less from tissues collected on the day of oestrus. Very minimal responses to oxytocin were observed from tissues collected on days 3 or 5 after oestrus. The temporal pattern of responsiveness to oxytocin described in the experiment reported here and in the report of Roberts *et al.* (1976) is in accord with previous reports describing changes in the ability of oxytocin to stimulate uterine secretion of $\text{PGF}_{2\alpha}$ when administered *in vivo* at various points in the oestrous cycle (Roberts *et al.*, 1975; Roberts and McCracken, 1976; Fairclough *et al.*, 1984; Silvia *et al.*, 1992).

The ability of endometrial tissue to convert added arachidonic acid to $\text{PGF}_{2\alpha}$ was used as an indirect measure of PGS activity. This interpretation depends on the assumption that the relative conversion of PGH_2 (the immediate end-product of PGS activity) to $\text{PGF}_{2\alpha}$ rather than other prostaglandins is not altered by stage of the oestrous cycle or by pregnancy status. Endometrial tissue can convert arachidonic acid into several prostaglandins including PGE_2 and PGI_2 . Uterine secretion of PGE_2 is greater during early pregnancy than during the corresponding stages of the oestrous cycle (Silvia *et al.*, 1984; Vincent and Inskeep, 1986). This is reflected in the release of PGE_2 by endometrial tissue *in vitro* (Ellinwood *et al.*, 1979; Lacroix and Kann, 1982). However, even in tissue from pregnant ewes, $\text{PGF}_{2\alpha}$ release exceeds that of PGE_2 by 4–5 times (Ellinwood *et al.*, 1979; Hyland *et al.*, 1982; Lewis and Waterman, 1985). The rate of secretion of PGI_2 from endometrial tissue is similar to that for $\text{PGF}_{2\alpha}$ (Silvia

et al., 1984; Lewis and Waterman, 1985); however, secretion does not appear to be affected by pregnancy status. It is therefore unlikely that the dramatic changes in $\text{PGF}_{2\alpha}$ release in the presence of arachidonic acid reported here are due to increases in the formation of PGE_2 or PGI_2 at the expense of $\text{PGF}_{2\alpha}$.

In this experiment, conversion of PGH_2 to $\text{PGF}_{2\alpha}$ was minimal on days 5 and 10 after oestrus. Conversion increased substantially on day 12 and was maintained up to day 14, and then fell on day 16. Huslig *et al.* (1979) measured PGS activity in homogenates of ovine endometrial tissue collected at various stages of the oestrous cycle. They observed an increase in total PGS activity beginning on day 13 after oestrus. This appeared to be due to an increase in the concentration of the enzyme rather than any change in its specific activity. More recently, Salamonsen and Findlay (1990) used an immunohistochemical procedure to demonstrate that the concentration of the enzyme increased on day 10 after oestrus and remained high until day 15. Taken together, these observations indicate that the synthesis of the enzyme increases prior to any change in enzymatic activity. An endogenous inhibitor of PGS activity may be present in endometrial tissue prior to day 12 after oestrus. The concentration of this inhibitor may decline after day 12 to allow endometrial synthesis of PGS. An inhibitor of PGS activity has been demonstrated in homogenates of bovine endometrial tissue and the changes in activity of this inhibitor throughout the oestrous cycle are compatible with such a regulatory role (Basu and Kindahl, 1987; Gross *et al.*, 1988). Clearly, increases in the concentration and activity of PGS occur well in advance of the increase in uterine secretory responsiveness to oxytocin that occurs on day 14.

Release of $\text{PGF}_{2\alpha}$ from caruncular endometrial tissue in the presence of PMA or A23187 or both was used as a means of assessing the integrity of the intracellular regulatory mechanisms controlling $\text{PGF}_{2\alpha}$ synthesis at various points in the oestrous cycle. The phorbol ester, PMA, induced a small but consistent increase in $\text{PGF}_{2\alpha}$ release from endometrial tissues collected throughout the oestrous cycle. The Ca^{2+} ionophore A23187 could stimulate release of $\text{PGF}_{2\alpha}$ only from tissues collected late in the oestrous cycle (days 12 and 16) and the response was again rather low. Its failure to stimulate release from tissues collected on day 14 is difficult to explain, particularly since oxytocin and PMA were both effective at this time. In a previous experiment with ovariectomized ewes receiving steroid replacement therapy (Raw and Silvia, 1991), PMA and A23187 stimulated release of $\text{PGF}_{2\alpha}$ but the response was also very small. The steroid-replacement protocol used in that experiment was designed to simulate steroid conditions during the latter portion of the luteal phase. In contrast, responses to PMA and A23187 were greater in tissues collected from ovariectomized ewes subjected to a steroid replacement protocol that was more representative of the late follicular phase or oestrus (Silvia and Homanics, 1988). PMA (but not A23187) stimulated release of $\text{PGF}_{2\alpha}$ from bovine endometrial tissue collected on the day of oestrus (Lafrance and Goff, 1990). Thus, responsiveness to PMA and A23187 appears to be characteristic of a uterus under oestrogenic dominance. Responses to these compounds may have limited relevance to the secretion of $\text{PGF}_{2\alpha}$ that occurs prior to and during luteolysis, when the uterus is under the influence of progesterone.

The ability of oxytocin to stimulate activity of PLC was not observed until day 14 after oestrus. This coincides with the earliest time point at which oxytocin can stimulate release of $\text{PGF}_{2\alpha}$. This agrees with the report of Mirando *et al.* (1990a) that oxytocin could stimulate activity of PLC in endometrial tissue collected from ewes on day 16 but not on day 12 after oestrus. In the study reported here, maximum activity of PLC in response to oxytocin was observed in tissues collected on day 16. Even though a much higher level of PLC activity was observed on day 16 after oestrus, the amount of $\text{PGF}_{2\alpha}$ released in response to oxytocin (in excess of basal release) was similar on days 14 and 16. Clearly, the activity of PLC is not the sole factor limiting the amount of $\text{PGF}_{2\alpha}$ produced in response to oxytocin. A similar conclusion was reached in an experiment with ovariectomized ewes receiving steroid replacement (Raw and Silvia, 1991).

The increase in the ability of oxytocin to stimulate activity of PLC appears to be associated with the completion of luteal regression. As noted above, responsiveness to oxytocin is much greater in tissues collected on day 16 than on day 14. Only one of five nonpregnant ewes providing tissue on day 14 after oestrus had completed luteal regression. The increase in activity of PLC induced by oxytocin was six-fold greater in tissue from this ewe ($30.7 \times 10^3 \text{ c.p.m. g}^{-1}$) than from the remaining four that had not yet initiated luteolysis ($4.9 \pm 1.3 \times 10^3 \text{ c.p.m. g}^{-1}$ (mean $\pm$ SEM)). Only one of five ewes killed on day 16 had not completed luteal regression. The increase in activity of PLC induced by oxytocin was much less in tissue from this ewe ($13.8 \times 10^3 \text{ c.p.m. g}^{-1}$) than in tissue from the remaining four ewes that had completed luteal regression ($53.0 \pm 17.5 \times 10^3 \text{ c.p.m. g}^{-1}$). On days 14 and 16, release of $\text{PGF}_{2\alpha}$ induced by oxytocin tended to be greater from tissue collected before the completion of luteal regression. Thus, the lack of an association between activity of PLC and release of $\text{PGF}_{2\alpha}$ is also observed within treatment groups. Responsiveness of endometrial PLC to oxytocin may develop as a consequence of luteolysis. The precise mechanism through which the corpus luteum inhibits endometrial PLC is not clear. Responsiveness of endometrial PLC to oxytocin can develop in ovariectomized ewes in the presence of continuous high concentrations of progesterone (Vallet and Bazer, 1989; Raw and Silvia, 1991).

The ability of oxytocin to stimulate activity of PLC appears to correspond to changes in endometrial concentrations of receptors for oxytocin observed during the oestrous cycle (Sheldrick and Flint, 1985). Concentrations of oxytocin receptors are relatively low throughout most of the oestrous cycle. The first reliably detectable increase in concentration occurs on day 14 after oestrus and concentrations continue to increase up to the day of oestrus. In contrast, the ability of oxytocin to stimulate release of $\text{PGF}_{2\alpha}$ from endometrial tissue peaks on day 15 and then declines (Roberts *et al.*, 1976; Silvia *et al.*, 1992). As with the activity of PLC, the relationship between the ability of oxytocin to stimulate release of $\text{PGF}_{2\alpha}$ (both *in vivo* and *in vitro*) and the concentration of receptors for oxytocin appears to be weak. The apparent separation of PLC activity and release of $\text{PGF}_{2\alpha}$ may be due to differences in cellular localization. The highest concentrations of PGS and its mRNA are found in epithelial cells (Raw *et al.*, 1988; Eggleston *et al.*, 1990; Salamonsen and Findlay, 1990; Salamonsen *et al.*, 1991), suggesting that synthesis of PGF occurs primarily within this layer. Receptors

for oxytocin are present in the epithelial cells and in the underlying caruncular stroma (Ayad *et al.*, 1991; Wallace *et al.*, 1991). The increase in oxytocin receptors observed between days 14 and 16 after oestrus occurs mainly in the stroma. Perhaps the oxytocin-responsive PLC is also found in the stroma.

The release of PGF_{2α} from caruncular endometrial explants collected on days 12, 14 and 16 of early pregnancy was quite comparable to but more variable than that observed in non-pregnant ewes. The variability in secretion of PGF_{2α} after administration of oxytocin *in vivo* is also greater in pregnant than in nonpregnant ewes (Burgess *et al.*, 1990; Silvia *et al.*, 1992). Oxytocin was equally effective in stimulating release of PGF_{2α} from endometrial tissue collected from pregnant and nonpregnant ewes. This is in sharp contrast to the inability of oxytocin to stimulate secretion of PGF_{2α} from the uterus when administered *in vivo* to ewes on days 12 to 16 of pregnancy (Fairclough *et al.*, 1984; McCracken *et al.*, 1984; Silvia *et al.*, 1992). The reason why oxytocin can stimulate release of PGF_{2α} from endometrial tissue *in vitro* but not *in vivo* is not clear. Apparently, the continuous presence of the conceptus is required for this suppression. In the present study, incubation of uterine tissue, in the absence of the conceptus for just 2.5 h, appears to be sufficient time for the suppressive effect of the conceptus to wear off. This implies that some extremely labile factor is involved in mediating the suppressive effect. The conceptus may suppress uterine secretion of PGF_{2α} through its secretion of ovine trophoblast protein-1 (oTP-1). Ovine TP-1 is an interferon-like protein that has been shown to delay luteal regression when infused into the uterus (Godkin *et al.*, 1984; Vallet *et al.*, 1988). It may delay luteolysis through its ability to suppress oxytocin-induced secretion of PGF_{2α} from the uterus (Vallet *et al.*, 1988; Mirando *et al.*, 1990b). Acute exposure of dispersed, ovine endometrial cells to oTP-1 or recombinant human interferon-α *in vitro* suppressed spontaneous release of PGF_{2α} (Salamonsen *et al.*, 1988, 1989). Perhaps oTP-1 or some other conceptus-secreted product promotes the synthesis of a labile factor which exerts an inhibitory action at some point in the intracellular regulatory pathways controlling synthesis of PGF_{2α}. In the absence of oTP-1, the rate of synthesis of the factor declines such that biologically active concentrations cannot be maintained.

The ability of endometrial tissue from pregnant ewes to convert arachidonic acid to PGF_{2α} was not impaired during early pregnancy. Salamonsen and Findlay (1990) found no differences in immunohistochemically detectable PGS in endometrial tissue collected from pregnant and nonpregnant ewes on day 15 after oestrus. Thus, the conceptus does not appear to suppress secretion of PGF_{2α} by reducing the concentration or activity of PGS in endometrial tissue.

The stimulatory effects of PMA and A23187 on release of PGF_{2α} were relatively small in magnitude and similar to the responses observed in nonpregnant ewes at comparable stages after oestrus. The biological significance of these effects is not clear. As reported by Mirando *et al.* (1990a, b), the ability of oxytocin to stimulate activity of PLC was totally suppressed in pregnant ewes. The mechanism by which the conceptus suppresses activation of PLC by oxytocin is unclear. Chronic administration of oTP-1 to nonpregnant ewes reduced the ability of oxytocin to stimulate activity of PLC (Mirando *et al.*, 1990a, b). Yet, acute exposure of ovine endometrial tissue to

oTP-1 *in vitro* had very little effect on the ability of oxytocin to stimulate activity of PLC (Vallet and Bazer, 1989). Since oTP-1 was effective in suppressing release of PGF_{2α} under similar conditions *in vitro*, it seems unlikely that the inhibitory effect of the conceptus on PLC is exerted by oTP-1 directly.

The failure of oxytocin to stimulate activity of PLC in tissue from pregnant ewes is surprising, particularly in light of the fact that oxytocin could stimulate release of PGF_{2α} very effectively. Taken together, these data (the very limited stimulatory effects of PMA and A23187 and the discrepancies between activity of PLC and release of PGF_{2α} in tissue from pregnant and non-pregnant ewes) indicate that the stimulatory effects of oxytocin on secretion of PGF_{2α} are mediated through a mechanism other than one involving an increase in activity of phosphatidylinositol-specific PLC.

A relationship between activity of PLC and receptors for oxytocin is very apparent in pregnant ewes. Concentrations of receptors for oxytocin are maintained at low, luteal-phase levels up to day 19 of early pregnancy (McCracken *et al.*, 1984; Sheldrick and Flint, 1985), as is the ability of oxytocin to stimulate activity of PLC. These animals have low concentrations of receptors for oxytocin, show no increase in activity of PLC in response to oxytocin, yet release PGF_{2α} in response to an oxytocin challenge *in vitro*. It seems unlikely that a substantial increase in the concentration of receptors for oxytocin could have occurred during the 2.5 h preincubation period. Recently, a dissociation between the concentration of oxytocin receptors and the ability of oxytocin to stimulate uterine secretion of PGF_{2α} has been demonstrated in ewes (Vallet and Lamming, 1990; Vallet *et al.*, 1990). We therefore suggest that the ability of oxytocin to stimulate release of PGF_{2α} from endometrial tissue probably requires a relatively low, luteal-phase concentration of receptors for oxytocin. The amount of PGF_{2α} released by endometrial tissue in response to oxytocin is probably determined by factors at the post-receptor level.

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