Oxytocin- and aluminium fluoride-induced phospholipase C activity and prostaglandin F_{2α} secretion during the ovine luteolytic period

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A series of studies was conducted to characterize changes in components of the cell signalling cascade that mediates oxytocin-induced prostaglandin F_{2α} (PGF_{2α}) synthesis at the onset of luteolysis in sheep. In the first experiment, caruncular tissue was dissected from 20 ewes on days 12–15 of the oestrous cycle, and incubated for the measurement of phospholipase C (PLC) activity or secretion of PGF_{2α}. Activation of GTP-binding proteins with aluminium fluoride stimulated both inositol phosphate accumulation and PGF_{2α} secretion on all days examined. However, oxytocin did not stimulate PLC activity or PGF_{2α} accumulation until day 13. While the ability of oxytocin to stimulate PLC activity increased after day 13, oxytocin-induced PGF_{2α} secretion declined slightly from day 13 to 15, suggesting that cell signalling components downstream from PLC modulate the response to oxytocin after day 13. Oxytocin failed to stimulate PGF_{2α} synthesis on day 14 after oestrus. Secretion of endogenous luteal oxytocin may have rendered uterine tissues collected on day 14 refractory to oxytocin *in vitro*. Therefore, a second study was conducted in ovariectomized, steroid replaced ewes. Ovarian steroids were administered to mimic endogenous changes in progesterone and oestradiol. The temporal patterns of PGF_{2α} synthesis in response to oxytocin and pharmacological agents were similar to uterine tissues from cyclic ewes in the first experiment; however, the magnitude of the response was less. These data suggest that oxytocin receptors are absent or are not coupled to PLC until day 13 after oestrus.

Introduction

In sheep, luteal regression is caused by a series of short duration, high amplitude pulses of prostaglandin F_{2α} (PGF_{2α}) (McCracken *et al.*, 1972; Fairclough *et al.*, 1980; Webb *et al.*, 1981; Zarco *et al.*, 1988). Uterine PGF_{2α} stimulates oxytocin release from the corpus luteum (Flint and Sheldrick, 1982; Watkins *et al.*, 1984; Watkins and Moore, 1987; Lamsa *et al.*, 1989), while oxytocin stimulates uterine secretion of PGF_{2α} (Sharma and Fitzpatrick, 1974; Mitchell *et al.*, 1975; Roberts and McCracken, 1976; Fairclough *et al.*, 1984; Burgess *et al.*, 1990). Thus PGF_{2α} and oxytocin comprise a positive feedback loop (Flint *et al.*, 1986). The repeated activation of this loop may result in the pulsatile secretion of uterine PGF_{2α} which mediates structural and functional luteolysis (Schramm *et al.*, 1983).

Prostaglandin F_{2α} stimulates the release of luteal oxytocin well before the onset of luteal regression (Lamsa *et al.*, 1989). However, oxytocin is only capable of stimulating secretion of uterine PGF_{2α} at the time of luteal regression *in vivo* (Fairclough *et al.*, 1984; Silvia *et al.*, 1992) and *in vitro* (Roberts *et al.*, 1976; Silvia and Raw, 1993). Therefore, the maturation of the luteolytic positive feedback loop, and hence luteal regression, is determined by the onset of uterine responsiveness to oxytocin.

McCracken (1980) proposed that the onset of uterine responsiveness was dependent upon the acquisition of uterine oxytocin receptors. However, conventional binding assays have failed to demonstrate a significant increase in the number of oxytocin binding sites before a decline in peripheral progesterone concentrations at the onset of spontaneous luteal regression (Sheldrick and Flint, 1985). In ovariectomized ewes administered progesterone and oestradiol, uterine responsiveness to oxytocin develops between days 12 and 14 of a simulated oestrous cycle without a change in oxytocin binding (Lau *et al.*, 1992). In contrast, Ayad *et al.* (1991) used autoradiography to demonstrate an increase in oxytocin binding within the luminal epithelium before luteolysis in cyclic ewes. Consequently, it remains unclear whether oxytocin receptors increase before the onset of luteolysis and whether the increase in binding sites mediates uterine secretory responsiveness to oxytocin.

Alternatively, the onset of uterine responsiveness to oxytocin may be mediated by changes in second messenger-generating enzymes or effector enzymes within the oxytocin-induced PGF_{2α} synthesis cascade. Cyclic changes in prostaglandin synthase activity (Huslig *et al.*, 1979), phospholipase A₂ (PLA₂) activity (Downing and Poyser, 1983), and the accumulation of lipid droplets (Brinsfield and Hawk, 1973) have been reported in sheep and other mammals. In addition, concentrations and activity of prostaglandin synthase

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(Salamonson *et al.*, 1991; Raw *et al.*, 1995) and PLA_2 (Dey *et al.*, 1982) have been shown to be modulated by ovarian steroids in sheep and rats, respectively.

The objective of these experiments was to characterize the changes that occur at the onset of luteal regression in the ability of oxytocin and aluminium fluoride (AlF_4^-) to stimulate PLC activity and $\text{PGF}_{2\alpha}$ secretion. From these data, it can be determined whether the acquisition of endometrial responsiveness to oxytocin occurs through a change in the concentration of the oxytocin receptor and its linkage to PLC or in some other downstream component(s) of the oxytocin-induced $\text{PGF}_{2\alpha}$ synthesis signalling cascade.

Materials and Methods

Experiment 1

Animals and treatment. A flock of aged (> 2 years) cross-bred ewes was checked twice per day for oestrous behaviour (at 08:00 and 17:00 h) with a vasectomized ram. All ewes in the experiment had at least one cycle of normal duration (16–18 days) before the beginning of the study. Individuals were chosen randomly from the pool of eligible ewes on the first morning of behavioural oestrus (day 0), and assigned to be killed on day 12, 13, 14 or 15 of the oestrous cycle ($n = 5$ ewes day⁻¹). Beginning on day 0, 5 ml of blood was collected each day by jugular venepuncture. Serum was harvested and stored at -20°C until assayed for progesterone. Concentrations of progesterone were determined to assess luteal function by a solid phase radioimmunoassay (Coat-a-count, Diagnostic Products Corp., Los Angeles, CA) as described by Silvia *et al.* (1992). Two ewes were killed by captive bolt concussion and exsanguination on each morning (at 08:00 h), and no two ewes from the same day of the cycle were killed on a given morning. Uteri were immediately removed and taken to the laboratory for dissection within 10 min of collection. Caruncles from each ewe were dissected and placed in Krebs–Henseleit–bicarbonate buffer (KHB; 118 mol NaCl l^{-1} , 4.7 mmol KCl l^{-1} , 2.56 mmol $\text{CaCl}_2 \text{ l}^{-1}$, 1.13 mmol $\text{MgCl}_2 \text{ l}^{-1}$, 25 mmol $\text{NaHCO}_3 \text{ l}^{-1}$, 1.15 mmol $\text{NaH}_2\text{PO}_4 \text{ l}^{-1}$, 5.55 mmol glucose l^{-1} , 20 mmol Hepes, l^{-1} , 0.013 mmol phenol red l^{-1} , pH 7.4). Explants were selected randomly from the pool of caruncles, weighed (40–80 mg), transferred to 12 mm $\times$ 75 mm borosilicate glass culture tubes and incubated to measure PLC activity or $\text{PGF}_{2\alpha}$ secretion rate. All procedures involving animals were approved by the University of Kentucky Institutional Animal Care and Use Committee.

Phospholipase C activity. The activity of PLC was assessed by subjecting six explants from each ewe to an incubation protocol consisting of four consecutive periods of 90, 60, 5 and 30 min, respectively (Silvia and Homanics, 1988). All incubations were carried out at 37°C in a shaking incubator. During period 1, all explants were incubated in the presence of 6 μCi [^3H]myo-inositol to label inositol containing phospholipids. In period 2, explants were incubated in KHB to allow for diffusion of unincorporated labelled inositol. In period 3, 10 mmol LiCl l^{-1} was added to the medium to inhibit endogenous inositol phosphatases. Treatments were applied in period 4 in KHB plus

10 mmol LiCl l^{-1} . Explants were exposed to one of three treatments: (1) control medium, (2) oxytocin (10^{-7} mol l^{-1}) or (3) aluminium fluoride (5×10^{-2} mol $\text{NaF l}^{-1} + 10^{-5}$ mol $\text{AlCl}_3 \text{ l}^{-1}$; $n = 2$ explants per treatment). At the end of period 4, reactions were terminated, inositol phosphates extracted, separated from inositol into inositol mono-, bis- and trisphosphate fractions, and quantitated. The effects of treatments on individual inositol phosphate fractions were similar and, therefore, activity was expressed as c.p.m. of total inositol phosphates produced per gram of tissue in 30 min.

$\text{PGF}_{2\alpha}$ synthesis. Sixteen explants from each ewe were subjected to a similar four-period incubation protocol to assess synthesis and secretion of $\text{PGF}_{2\alpha}$ (Silvia and Homanics, 1988). In periods 1–3, explants were incubated in KHB. During period 4, explants were incubated in KHB and one of four treatments: (1) control medium, (2) oxytocin (10^{-7} mol l^{-1}), (3) aluminium fluoride (5×10^{-2} mol $\text{NaF l}^{-1} + 10^{-5}$ mol $\text{AlCl}_3 \text{ l}^{-1}$), or (4) arachidonic acid (6.13×10^{-6} mol l^{-1} ; $n = 4$ explants per treatment). At the end of period 4, media were collected, diluted 1:10 in PBS and stored at -20°C until assayed for content of $\text{PGF}_{2\alpha}$. Concentrations of $\text{PGF}_{2\alpha}$ in the media were determined by radioimmunoassay. Samples were run in four assays, and inter- and intra-assay coefficients of variation were 21% and 12%, respectively.

Statistical analyses. Both $\text{PGF}_{2\alpha}$ and PLC data were log transformed to reduce heterogeneity of variance among treatment groups. Least squares analysis of variance was used to test for effects of day, treatment and their interaction after transformation using the General Linear Models procedures of the Statistical Analysis System (SAS, 1985). The mean square for ewe nested within day was used as the error term to test the main effect of day on PLC activity and $\text{PGF}_{2\alpha}$ secretion. The main effect of treatment and the treatment by day interaction were tested using the mean square for treatment by ewe nested within day as the error term. When appropriate, the effect of day within a given treatment was analysed for linear, quadratic and cubic effects using orthogonal contrasts and the mean square for ewe nested within day as the error term. When appropriate, effects of treatments within a given day were compared with basal secretion using the pdiff option of the LSMEANS procedure of SAS (1985).

Experiment 2

Unexpectedly, oxytocin did not stimulate $\text{PGF}_{2\alpha}$ synthesis *in vitro* on day 14 of the cycle in Expt 1. It is possible that uterine tissues were collected during active pulsatile secretion of oxytocin and $\text{PGF}_{2\alpha}$ and were, therefore, refractory to oxytocin *in vitro*. A second study was planned in ovariectomized, steroid replaced ewes to eliminate luteal pulses of oxytocin. Therefore, it was necessary to characterize the temporal development of uterine responsiveness to oxytocin *in vivo* in this model.

Experiment 2a: animals and treatments. Four aged ewes of mixed breeding were ovariectomized by midventral laparotomy under sodium pentobarbital anaesthesia. Ovariectomies

Table 1. Progesterone treatment regimen doses designed to mimic luteal phase concentrations of peripheral progesterone in ovariectomized ewes

Day of treatment	Progesterone (mg)	
	08:00 h	20:00 h
1	0.50	0.63
2	0.75	1.00
3	1.50	2.00
4	3.00	4.00
5	5.00	6.00
6–7	8.00	8.00
8–15	12.00	12.00

were performed at least 30 days before the beginning of the experiment. Ewes were pretreated with progesterone and oestradiol as described by Homanics and Silvia (1988). After pretreatment was completed, progesterone was administered twice a day in doses designed to mimic luteal phase concentrations of peripheral progesterone (Table 1). Oestradiol was given in the form of a subcutaneous Silastic implant. This regimen has previously been shown to mimic progesterone profiles of cyclic ewes and to render uterine tissues responsive to oxytocin *in vitro* (Raw and Silvia, 1991). As in Expt 1, ewes were checked with a vasectomized ram to verify the efficacy of steroid pretreatment and, beginning on day 0, blood samples were taken each day to verify peripheral progesterone concentrations. On days 11–15 of the simulated oestrous cycle, ewes were challenged daily with oxytocin (10 iu in 1 ml sterile saline) as described by Homanics and Silvia (1988). Briefly, samples were collected by jugular venepuncture 30, 15 and 0 min before oxytocin administration to determine basal production of 13,14-dihydro-15-keto PGF_{2a} (PGFM). At time 0, oxytocin was administered into the jugular vein. Blood samples were collected at 5, 10, 15, 30, 45 and 60 min after injection. Serum was harvested and stored at -20°C until assayed for PGFM. Serum concentrations of PGFM were determined by radioimmunoassay as described by Homanics and Silvia (1988). Samples were run in three assays, and inter- and intra-assay coefficients of variation were 19% and 5%, respectively.

Statistical analyses. Least squares analysis of variance was used to detect differences in baseline, peak response and area under the curve using the General Linear Models procedures of the SAS system (1985). Ewes were considered as blocks, and the effect of day was tested using the residual mean square error. Baseline was calculated as the mean of the three samples before injection, and area under the curve was calculated as described by Homanics and Silvia (1988). Peak response is defined as the greatest concentration achieved during the post-injection period above baseline. Ewes were classified as responsive to oxytocin if peak concentration was more than two standard errors above baseline PGFM concentrations.

Experiment 2b. Twenty aged, crossbred ewes were ovariectomized, pretreated and treated with ovarian steroids as described in Expt 2a. Five ewes on each of 4 days of the

Fig. 1. Concentrations of progesterone in jugular venous serum of intact (Expt 1) and ovariectomized ewes receiving progesterone and oestradiol replacement (Expt 2a). Intact (●; days 0–12, $n = 20$; day 13, $n = 15$; day 14, $n = 10$; day 15, $n = 5$); ovariectomized (○). Vertical bars represent standard errors of the LS means.

simulated oestrous cycle (days 11–14) were killed, uteri were collected and caruncular tissue was dissected and subjected to the four-period incubation protocol for the measurement of PGF_{2a} synthesis as described in Expt 1. These days were selected based on the *in vivo* response to oxytocin observed in Expt 2a and corresponded to days 12–15 in intact, cyclic ewes. Treatments were the same as in Expt 1, with the exception of arachidonic acid, which was increased from $6.33 \times 10^{-6} \text{ mol l}^{-1}$ to $10^{-5} \text{ mol l}^{-1}$ owing to the relatively modest, but significant induction of PGF_{2a} synthesis observed in Expt 1. Data were analysed as described for Expt 1.

Results

Experiment 1

Progesterone concentrations followed temporal patterns that were typical of cyclic ewes (Fig. 1). On the basis of these concentrations of progesterone, three of five ewes killed on day 15 of the cycle appeared to be actively undergoing functional luteolysis. Responses to treatment were similar among the five animals; therefore, all five were included in the statistical analyses of PGF_{2a} and PLC.

The overall rate of release of PGF_{2a} was similar on all 4 days of the oestrous cycle ($P > 0.2$). Analysis indicated that synthesis of PGF_{2a} was affected by treatment *in vitro* ($P < 0.01$). A treatment by day interaction was not observed in the initial analysis of variance ($P > 0.2$). However, variation among explants treated with aluminium fluoride was greater than that observed in the remaining treatment groups (for example, variances were heterogeneous even after log transformation, $P < 0.05$). Therefore, three analyses were performed in which explants treated with control media were compared with the remaining three treatments (oxytocin, aluminium fluoride or

Fig. 2. Rate of release of $\text{PGF}_{2\alpha}$ by ovine caruncular endometrial tissue incubated *in vitro*, in the presence of control medium (■), oxytocin (▨), AlF_4^- (□), or arachidonic acid (▤). Tissue was collected from intact ewes on days 12, 13, 14 and 15 of the oestrous cycle ($n = 5 \text{ day}^{-1}$). Bars represent anti-log values of the least-squares means of logarithmically transformed data. Vertical lines represent anti-log values of standard errors of the least squares means. *Significant difference from control within day ($P < 0.05$).

arachidonic acid) independently. When explants treated with oxytocin or control media were compared, a main effect of day was not detected ($P > 0.2$). The rate of $\text{PGF}_{2\alpha}$ synthesis was affected by *in vitro* treatment ($P < 0.05$). Analysis of $\text{PGF}_{2\alpha}$ secretion on each day indicated that oxytocin increased $\text{PGF}_{2\alpha}$ secretion above basal concentrations on days 13 and 15 ($P < 0.05$), but only tended to increase $\text{PGF}_{2\alpha}$ secretion on day 14 ($P = 0.18$). Among explants treated with oxytocin, secretion of $\text{PGF}_{2\alpha}$ was dependent on day of the oestrous cycle ($P < 0.05$, quadratic). Synthesis of $\text{PGF}_{2\alpha}$ increased from day 12 to day 13 and declined slightly from day 13 to day 15 (Fig. 2). When explants treated with aluminium fluoride or control media alone were compared, secretion of $\text{PGF}_{2\alpha}$ was not affected by day ($P > 0.2$), but was affected by *in vitro* treatment ($P < 0.05$). Treatment of explants with aluminium fluoride increased $\text{PGF}_{2\alpha}$ synthesis irrespective of day of the oestrous cycle ($P < 0.01$). Similar results were observed among explants treated with arachidonic acid. Secretion of $\text{PGF}_{2\alpha}$ was not affected by day ($P > 0.2$), but was affected by *in vitro* treatment ($P < 0.05$). Arachidonic acid stimulated $\text{PGF}_{2\alpha}$ secretion regardless of day of oestrous cycle ($P < 0.05$).

Overall, the accumulation of inositol phosphates was not affected by day of the oestrous cycle ($P > 0.2$). A main effect of treatment and a treatment by day interaction were detected at $P < 0.05$. Among explants treated with oxytocin or media alone, the main effect of treatment and treatment by day interaction were significant ($P < 0.05$). The effect of day among oxytocin treated explants was detected and increased linearly ($P < 0.05$) from day 12 to day 15 (Fig. 3). Within day, oxytocin increased PLC activity above basal concentrations on days 13 and 15, and tended to increase accumulation on day 14 ($P = 0.17$). A main effect of treatment was detected among

Fig. 3. Activity of phospholipase C, expressed as total inositol phosphates (c.p.m. g^{-1} tissue), in ovine caruncular endometrial tissue incubated *in vitro*, in the presence of control medium (■), oxytocin (▨) or AlF_4^- (□). Tissue was collected from intact ewes on days 12, 13, 14 and 15 of the oestrous cycle ($n = 5 \text{ day}^{-1}$). Bars represent anti-log values of the least-squares means of logarithmically transformed data. Vertical bars represent anti-log values of standard errors of the least squares means. *Significant difference from control within day ($P < 0.05$).

explants treated with aluminium fluoride or media alone ($P < 0.01$). Aluminium fluoride increased accumulation of inositol phosphates irrespective of day. The main effect of day and the treatment by day interaction were not significant ($P > 0.2$).

Experiment 2a

The temporal pattern of progesterone concentrations in ovariectomized, steroid replaced ewes was similar to that observed for intact ewes in Expt 1 (Fig. 1); however, absolute concentrations of progesterone after day 5 were approximately 65% of intact values. By day 9 of the simulated cycle, progesterone concentrations were approximately 3.5 ng ml^{-1} and remained near this concentration until day 15.

Baseline concentrations of PGFM were not different among days of the simulated cycle ($P > 0.2$; Fig. 4). Peak responses to oxytocin were relatively low on days 11, 12 and 13, increased to a maximum on day 14, and declined on day 15. Results of area under the curve analysis indicated that ewes failed to respond on days 11 or 12, but did respond on days 13–15 ($P < 0.05$).

Experiment 2b

A main effect of treatment ($P < 0.01$), but no effect of day or treatment by day interaction were detected in the overall analysis of variance ($P > 0.2$). Therefore, comparisons between basal secretion of $\text{PGF}_{2\alpha}$ and other treatment groups were made irrespective of day (Fig. 5). Oxytocin tended to stimulate

Fig. 4. Concentrations of 13,14-dihydro-15-keto-PGF_{2α} (PGFM) in ovariectomized ewes before and after an intravenous injection of 10 iu oxytocin at time 0 on days 11 (●), 12 (▲), 13 (□), 14 (■) and 15 (○) of a simulated oestrous cycle.

Fig. 5. Rate of release of PGF_{2α} by ovine caruncular endometrial tissue incubated *in vitro*, in the presence of control medium (■), oxytocin (▨), AlF₄⁻ (□), or arachidonic acid (▩). Tissue was collected from ovariectomized ewes on days 11, 12, 13 and 14 of a simulated oestrous cycle ($n = 5 \text{ day}^{-1}$). Bars represent anti-log values of the least-squares means of logarithmically transformed data. Vertical lines represent anti-log values of standard errors of the least squares means.

PGF_{2α} synthesis above basal secretion across all days ($P < 0.07$). As in Expt 1, aluminium fluoride and arachidonic acid stimulated secretion of PGF_{2α} above baseline across all days.

Discussion

In Expt 1, both aluminium fluoride and arachidonic acid stimulated production of PGF_{2α} on all days examined.

Aluminium fluoride also stimulated inositol phosphate accumulation throughout the experimental period. These data indicate that all components of the oxytocin-induced PGF_{2α} synthesis cascade are functional by day 12 of the oestrous cycle. In contrast, oxytocin failed to stimulate inositol phosphate accumulation or PGF_{2α} synthesis before day 13. Therefore, the onset of oxytocin-induced PGF_{2α} synthesis is mediated by upregulation in the capacity of oxytocin to induce PLC activity in uterine tissues between days 12 and 13 of the oestrous cycle. The increase in oxytocin-induced inositol phosphate accumulation from day 12 to day 15 may be due to an increase in the number of oxytocin receptors or in the capacity of the receptor to couple to PLC. These findings are consistent with the observed increase in oxytocin binding sites within the luminal epithelium between days 12 and 14 of the oestrous cycle reported by Wathes and Hamon (1993). The linear increase in inositol phosphate accumulation in response to oxytocin was not accompanied by a similar increase in PGF_{2α} synthesis. However, oxytocin-induced PGF_{2α} synthesis declined after day 13. Divergence between oxytocin-induced inositol phosphate accumulation and PGF_{2α} synthesis suggests that other components of this cell signalling cascade are modulated downstream from PLC. A divergence between inositol phosphate accumulation and PGF_{2α} synthesis was also observed in the response to aluminium fluoride. Although aluminium fluoride was the most potent stimulus of PGF_{2α} synthesis in the present study, this is not the case for inositol phosphate accumulation. One possibility is that aluminium fluoride stimulates the production of PGF_{2α} through a PLC-independent pathway. AlF₄⁻ is a nonspecific stimulus for most G protein subclasses, including those that are linked to adenylate cyclase (Gs, Gi) and ion channels (Go; Simon *et al.*, 1991). It is possible that activation of one or more of these G proteins may stimulate PGF_{2α} synthesis through a PLC-independent pathway. Alternatively, G protein $\beta\gamma$ subunit complexes freed by AlF₄⁻ activity may increase PLA₂ activity as has been demonstrated in bovine rod outer segments (Jelsema and Axelrod, 1987).

Clearly, there is no correlation between the amount of inositol phosphates generated and the quantity of PGF_{2α} synthesized. However, in no case did oxytocin or aluminium fluoride stimulate PGF_{2α} without increasing the concentration of inositol phosphates. Thus, while the PLC activity appears to be necessary for oxytocin-induced PGF_{2α} synthesis, the quantity of PGF_{2α} generated is dependent upon some other component of this cell signalling cascade. The availability of arachidonic acid appears to be one factor limiting PGF_{2α} synthesis in the guinea-pig uterus (Poyser, 1995).

The failure to detect significant stimulation of PLC activity or PGF_{2α} synthesis on day 14 after oestrus is difficult to explain, particularly when stimulation was detected on days 13 and 15 in the present study and has been observed on day 14 in a previous study (Silvia and Raw, 1993). One possibility is that some ewes killed on days 14 and 15 were undergoing spontaneous pulsatile secretion of oxytocin and PGF_{2α} and, therefore, may have been refractory to oxytocin. Uterine tissues are refractory for up to 6 h after exposure to oxytocin *in vivo* (Sheldrick and Flint, 1986). Uterine responsiveness to oxytocin was examined in an ovariectomized, steroid-replaced ewe model in Expt 2, to control for the possible contribution of

endogenous luteal oxytocin in diminishing the capacity of the uterus to synthesize $\text{PGF}_{2\alpha}$ *in vitro* after day 13. This model has been shown to mimic luteal phase concentrations of progesterone and to restore normal uterine secretory responsiveness to exogenous oxytocin *in vivo* (Homanics and Silvia, 1988). However, the amplitude of spontaneous pulses of PGFM observed during the 'luteolytic' period *in vivo* were severely diminished in this model, presumably as a result of the lack of luteal oxytocin (Silvia and Raw, 1993). In the present study, the progesterone replacement protocol effectively increased progesterone concentrations in a temporal pattern similar to that observed in the cyclic ewes of Expt 1. However, the absolute magnitude of the progesterone appeared to be only 65% of luteal concentrations in intact ewes (Fig. 1), although this discrepancy did not adversely affect the capacity of oxytocin to stimulate $\text{PGF}_{2\alpha}$ synthesis *in vivo*. Concentrations of PGFM observed after injection of oxytocin are comparable with those reported in cyclic ewes (Silvia *et al.*, 1992). In fact, the ability of the uterus to respond to oxytocin developed 1 day earlier. The timing of the development of uterine responsiveness to oxytocin in ovariectomized ewes appears to be normal when the progesterone injection protocol is modified to reach plateau concentrations 24 h later than in the present study (Battye *et al.*, 1992). The ability to stimulate normal $\text{PGF}_{2\alpha}$ secretion *in vivo* in the face of what appear to be significantly reduced progesterone concentrations is interesting. It is possible that the lower concentrations of progesterone observed are adequate to stimulate normal uterine function. This appears to be the case for other aspects of uterine function. For example, a similar progesterone replacement protocol can support embryo growth and pregnancy in ovariectomized ewes (Miller *et al.*, 1977).

In contrast to the response *in vivo*, $\text{PGF}_{2\alpha}$ synthesis was less in tissue from steroid-replaced ewes (Expt 2) when compared with cyclic ewes (Expt 1). As in Expt 1, the effects of arachidonic acid and aluminium fluoride on secretion of $\text{PGF}_{2\alpha}$ were detectable on all days examined despite the diminished secretion. The response to oxytocin was severely attenuated in the present study. Although not statistically significant, the temporal pattern of uterine responsiveness to oxytocin appears to be similar to that observed in Expt 1. Synthesis of $\text{PGF}_{2\alpha}$ in response to oxytocin is low on the first day examined (day 11), increases to a maximum on the second day (day 12) and then declines on the third and fourth days (days 13 and 14). However, the response to oxytocin *in vitro* did not parallel the response *in vivo*. The mechanism for this decline in responsiveness to oxytocin *in vitro* (day 14 in Expt 1 and days 13–14 in Expt 2b) is not known. However, it does not appear to be caused by desensitization of the uterus due to endogenous pulses of luteal oxytocin, since the phenomenon was observed in both intact and ovariectomized, steroid-replaced ewes.

In conclusion, the acquisition of functional oxytocin receptors or their linkage to PLC, or both, mediates the onset of uterine responsiveness to oxytocin and hence luteal regression. This is most likely due to an increase in oxytocin binding sites within the luminal epithelium over this period.

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