Regulation of pulsatile secretion of prostaglandin $F_{2\alpha}$ from the ovine uterus by ovarian steroids

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Two experiments were conducted to determine how progesterone and oestradiol regulate pulsatile secretion of $PGF_{2\alpha}$ from the ovine uterus. In Expt 1, ovariectomized ewes received: (1) no treatment, (2) oestradiol, (3) progesterone, or (4) oestradiol and progesterone ($n = 5$ ewes per treatment group) to approximate the changes in steroids that occur during the oestrous cycle. Jugular venous blood samples were collected at 30 min intervals for 48 h beginning at 08:00 on day 14 of steroid replacement. Blood samples were collected from five intact ewes at a comparable time of the oestrous cycle for comparison. The number and magnitude of pulses in 13,14-dihydro-15-keto- $PGF_{2\alpha}$ (PGFM) in jugular venous blood samples were used to assess uterine secretion of $PGF_{2\alpha}$. Experiment 2 was conducted as Expt 1, except that the progesterone replacement protocol was modified to duplicate more closely the temporal pattern of progesterone observed in intact ewes. Results were similar in both experiments. Intact ewes averaged 4.4 ± 0.6 pulses per 48 h blood sampling period. The frequency of pulses was less in ovariectomized ewes ($P < 0.05$). The number of pulses was increased by progesterone treatment ($P < 0.01$); the number of pulses in ovariectomized ewes receiving progesterone replacement was similar to that observed in intact ewes. There was a tendency for oestradiol to have a positive effect on the number of pulses ($P = 0.12$). The magnitude of pulses in intact ewes averaged 419 ± 38 pg ml⁻¹ and was much less in ovariectomized ewes ($P < 0.05$) than in intact ewes. The magnitude of pulses was only partially restored by progesterone replacement ($P = 0.03$). No effect of oestradiol on pulse magnitude was observed. In conclusion, progesterone plays an important role in regulating the number and magnitude of pulsatile secretion of $PGF_{2\alpha}$ from the ovine uterus. The ability of progesterone to completely restore the frequency of pulses but not the magnitude of $PGF_{2\alpha}$ release in ovariectomized ewes indicates that ovarian secretory products other than progesterone (for example, luteal oxytocin) are not required to initiate pulsatile secretion of $PGF_{2\alpha}$ but may be required to achieve full pulse magnitude.

Introduction

In nonpregnant ewes, luteal regression is caused by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) secreted from the uterus (McCracken *et al.*, 1972; Inskeep and Murdoch, 1980). Prostaglandin $F_{2\alpha}$ is released from the uterus in a series of five to eight discrete pulses (Thorburn *et al.*, 1973; Fairclough *et al.*, 1980; Webb *et al.*, 1981; Flint and Sheldrick, 1983; Hooper *et al.*, 1986). Pulses occur typically at 6–8 h intervals. Pulses appear just before the onset of luteal regression (i.e. before any significant fall in peripheral concentrations of progesterone) (Zarco *et al.*, 1988). The corpus luteum appears to be particularly sensitive to the luteolytic effects of $PGF_{2\alpha}$ when it is administered in a pulsatile fashion (Schramm *et al.*, 1983). The onset of pulsatile secretion of $PGF_{2\alpha}$ therefore appears to initiate luteal regression. Prostaglandin $F_{2\alpha}$ can be secreted in a pulsatile fashion only if there is an intermittent signal to initiate each episode of secretion (a pulse generator) and if the uterus can respond to the pulse generator signal by releasing $PGF_{2\alpha}$. The endocrine factors that regulate the timing

and pattern of pulsatile secretion of $PGF_{2\alpha}$ from the ovine uterus have not been completely determined.

In sheep, pulses of $PGF_{2\alpha}$ occur concurrently with pulses of oxytocin (or its associated neurophysin) during luteolysis (Fairclough *et al.*, 1980; Webb *et al.*, 1981; Flint and Sheldrick, 1983). Most of this oxytocin appears to be secreted from the corpus luteum (Hooper *et al.*, 1986; Moore *et al.*, 1986). The role of luteal oxytocin in uterine secretion of $PGF_{2\alpha}$ and luteolysis has been reviewed by Schams (1989) and Flint *et al.* (1990). Briefly, uterine $PGF_{2\alpha}$ and luteal oxytocin comprise a positive feedback loop. Oxytocin can stimulate uterine secretion of $PGF_{2\alpha}$ (Sharma and Fitzpatrick, 1974; Roberts *et al.*, 1975; Roberts and McCracken, 1976). Prostaglandin $F_{2\alpha}$ can stimulate secretion of oxytocin from the corpus luteum (Flint and Sheldrick, 1982; Watkins and Moore, 1987; Heap *et al.*, 1989; Lamsa *et al.*, 1989). Flint *et al.* (1986) proposed that the very high concentrations of each hormone achieved within pulses are the result of activation of this feedback loop. However, the factors that initiate and terminate the loop have not been determined.

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During spontaneous episodes of $\text{PGF}_{2\alpha}$ secretion, concentrations of $\text{PGF}_{2\alpha}$ in the utero-ovarian venous effluent increase before any detectable increase in concentrations of oxytocin (Moore *et al.*, 1986). This implies that activation of the feedback loop begins on the uterine side of the loop. However, the stimulus that initiates secretion of $\text{PGF}_{2\alpha}$ is not known. McCracken *et al.* (1991) proposed that neurohypophyseal oxytocin, released in a pulsatile fashion, serves as the pulse generator signal, initiating secretion of $\text{PGF}_{2\alpha}$ from the uterus. In sheep, pulses of $\text{PGF}_{2\alpha}$ are frequently associated with increases in both neurohypophyseal and luteal secretion of oxytocin (Hooper *et al.*, 1986).

Changes in the ability of oxytocin to stimulate uterine secretion of $\text{PGF}_{2\alpha}$ help determine when pulsatile secretion of $\text{PGF}_{2\alpha}$ occurs during the oestrous cycle. Uterine secretory responsiveness to oxytocin develops late in the oestrous cycle, at about the same time that pulsatile secretion of $\text{PGF}_{2\alpha}$ and luteolysis begin (Roberts and McCracken, 1976; Roberts *et al.*, 1976; Fairclough *et al.*, 1984; Silvia *et al.*, 1992). One of the proposed components of the uterine:luteal positive feedback loop is therefore not functional for much of the oestrous cycle.

Ovarian steroid hormones also contribute to the regulation of uterine $\text{PGF}_{2\alpha}$ secretion. Changes in uterine secretory responsiveness to oxytocin during the oestrous cycle appear to be regulated by progesterone and oestradiol (Sharma and Fitzpatrick, 1974; McCracken, 1980; Homanics and Silvia, 1988; Vallet *et al.*, 1990). Progesterone and oestradiol may also exert effects at the level of the pulse generator. Infusions of relatively low doses of oestradiol into ovariectomized ewes result in a pulsatile pattern of neurohypophyseal oxytocin similar to that observed in intact ewes during the follicular phase (McCracken *et al.*, 1984a, b). Pulses occurred at intervals of about 20 min. Increasing the dose of oestradiol resulted in 1–2 h episodes during which the frequency of oxytocin release increased dramatically (McCracken *et al.*, 1991). These episodes occurred at 3 h intervals.

The objectives of the experiments presented in this report were to determine whether progesterone and oestradiol exert an effect on the pulsatile pattern of uterine $\text{PGF}_{2\alpha}$ secretion in ovariectomized ewes and whether pulsatile secretion of $\text{PGF}_{2\alpha}$ can occur in the absence of the ovary (i.e. in the absence of luteal oxytocin).

Materials and Methods

Experiment 1

Twenty-five crossbred ewes were used in this experiment. Twenty ewes were ovariectomized via midventral laparotomy under sodium pentobarbital anaesthesia (20 mg kg⁻¹ body weight). Ovariectomies were performed at least 30 days before the start of the experiment to allow sufficient time for endogenous steroids to be cleared from the blood.

All 20 ovariectomized ewes underwent 7 days of steroid pretreatment (days -6 to 0) during which progesterone and oestradiol were administered as described by Homanics and Silvia (1988). This injection protocol has been shown to induce oestrous behaviour and a preovulatory-like surge in LH on day -1 or 0 (Homanics and Silvia, 1988). Five intact ewes were

checked for oestrus twice a day with a vasectomized ram. They received an injection of $\text{PGF}_{2\alpha}$ (15 mg, i.m.; Lutalyse: Upjohn Co., Kalamazoo, MI), during the mid-luteal phase of the oestrous cycle (days 6–12 post-oestrus), 48 h before the expected onset of oestrous behaviour in the ovariectomized animals, to induce oestrus synchronously with the ovariectomized ewes.

On day 0, ovariectomized ewes were randomly assigned to one of four treatment groups: (1) control, (2) oestradiol, (3) progesterone and (4) progesterone and oestradiol ($n = 5$ per treatment group). Starting at 08:00 h on day 1, ewes assigned to groups 3 and 4 received intramuscular injections of progesterone twice a day according to the increasing dose protocol developed by Miller *et al.* (1977) as described by Raw and Silvia (1991; see Expt 1). Ewes assigned to groups 1 and 2 received the same volume of corn oil vehicle. At 09:00 h on day 1, ewes in groups 2 and 4 received a subcutaneous, Silastic implant containing oestradiol (2 cm long, 3.35 mm i.d. and 4.65 mm o.d.). Similar implants have been shown to maintain circulating concentrations of oestradiol at 3–4 pg ml⁻¹ (Karsch *et al.*, 1980; Homanics and Silvia, 1988). Ewes in groups 1 and 3 received empty implants. All steroid pretreatments and treatments were administered during the breeding season (September to December). Jugular venous blood samples (10 ml) for progesterone measurement were collected by venepuncture, immediately before the morning injection of progesterone or vehicle, on days 1–16. Samples were allowed to clot for 8–12 h at 4°C. Serum was decanted from the clot after centrifugation (2000 g, 30 min). Serum was stored at -20°C until assay. At 07:00 h on day 14 after oestrus, an indwelling cannula was inserted into the jugular vein. Starting at 08:00 h, jugular venous blood samples (5 ml) were collected at intervals of 30 min for 48 h. Samples were processed and stored as described above. Concentrations of progesterone were determined by radioimmunoassay as described by Silvia *et al.* (1992). Samples were run in three assays. Intra- and interassay coefficients of variation were both 5%. Concentrations of 13,14-dihydro-15-keto- $\text{PGF}_{2\alpha}$ (PGFM) in the samples were determined by radioimmunoassay as described by Homanics and Silvia (1988). Samples were run in 25 assays. Intra- and interassay coefficients of variation were 7 and 17%, respectively.

Experiment 2

Twenty-three ewes were used in this experiment. Nineteen ewes were ovariectomized. All of these ewes received steroid pretreatment on days -6 to 0 as previously described. Ewes were then randomly assigned to one of four treatment groups: (1) control ($n = 4$), (2) oestradiol ($n = 5$), (3) progesterone ($n = 5$) and (4) progesterone and oestradiol ($n = 5$). Oestradiol was administered as described in Expt 1. The injection protocol for progesterone was altered slightly to hasten the increase in peripheral concentrations of progesterone by about 24 h (Raw and Silvia, 1991; see Expt 2). This was performed to duplicate more accurately the changes in progesterone that occur during the oestrous cycle in the intact animals. As in Expt 1, oestrus was synchronized in four intact ewes so that they could be sampled simultaneously with the ovariectomized ewes at a comparable time after oestrus. Jugular venous blood samples were collected, processed and assayed for progesterone and PGFM as described in Expt 1. Samples for progesterone were

run in three assays. Intra- and interassay coefficients of variation were 5 and 15%, respectively. Samples for PGFM were run in 23 assays. Intra- and interassay coefficients of variation were 4 and 13%, respectively.

Statistical analysis

Pulses of PGFM were identified using the Pulsar program of Merriam and Wachter (1982) as modified for the personal computer by Gitzen and Ramirez (1986). Quadratic, linear and constant terms for calculating assay SD in Pulsar were 0.00, 10.0 and 1.5, respectively. The $G(n)$ values for defining pulses were as follows: $G(1) = 10.0$, $G(2) = 5.0$, $G(3) = 3.0$, $G(4) = 1.5$, $G(5) = 1.13$. These values were selected empirically by running several test cases through the program and are similar to the values reported by Zhang *et al.* (1991) for the identification of PGFM pulses in ewes. Since basal concentrations of PGFM were often at or below the sensitivity of the radioimmunoassay (40.6 pg ml^{-1}), the PC Pulsar program frequently identified very small increases above the baseline as pulses. To compensate for these small fluctuations, we stipulated that pulses had to have a magnitude of at least 40.6 pg ml^{-1} above baseline to be classified as such. Once pulses were identified, pulse magnitude and duration were determined for each pulse and average values calculated for each animal. Effects of progesterone and oestradiol on number of pulses, pulse magnitude and pulse duration were determined by analysis of variance using the GLM procedure of SAS (1985). Since the designs of the two experiments were essentially identical, data from both experiments were included in a single analysis. Effects of experiment and interaction of experiments with treatments were also tested for. The interval between pulses within each ewe was also calculated when two or more pulses occurred. This occurrence was rare in ovariectomized ewes receiving no progesterone replacement; therefore, data from both experiments were considered in a single analysis. Owing to the limited number of observations in some treatment groups, the effect of experiment and interactions of treatments with experiment on pulse interval could not be tested for. Comparisons between each ovariectomized, steroid-treatment group and the intact group for each response variable were performed using Tukey's mean comparison procedure (SAS, 1985).

Results

Concentrations of progesterone in the intact group were similar to those reported previously by others (Stabenfeldt *et al.*, 1969; Thorburn *et al.*, 1969) (Fig. 1). A similar temporal pattern of concentrations was observed in the ovariectomized ewes receiving progesterone replacement. In Expt 1, concentrations of progesterone during the period from day 4 to day 8 tended to be lower than in the intact ewes. In Expt 2, the doses of progesterone administered during that period were adjusted to compensate for this difference. As a result, concentrations of progesterone in intact and ovariectomized ewes receiving progesterone replacement were essentially identical throughout the blood sampling period in Expt 2. Concentrations of progesterone were maintained at low (follicular phase) values through-

Fig. 1. Concentrations of progesterone in jugular venous serum of intact and ovariectomized ewes receiving progesterone or oestradiol replacement or both treatments. Intact (●); ovariectomized (◆); ovariectomized plus oestradiol (■); ovariectomized plus progesterone (◇); ovariectomized plus oestradiol and progesterone (□). (a) Expt 1; (b) Expt 2.

out the experiment in the ovariectomized ewes receiving the vehicle injection. The difference in progesterone injection protocols between the two experiments had no effect on pulsatile release of PGFM. There was no effect of experiment ($P > 0.20$) or interactions involving the effect of experiment ($P > 0.40$) on the number, magnitude or duration of PGFM pulses.

The number of PGFM pulses observed during the 48 h blood sampling period was low in ovariectomized ewes (Figs 2 and 3). The number of pulses was increased by progesterone replacement ($P < 0.01$) to a value comparable to that observed in intact ewes. Oestradiol also tended to have a positive effect on the number of pulses ($P = 0.10$). No interaction between steroids was observed ($P = 0.40$).

The magnitude of PGFM pulses was very low in ovariectomized ewes (Fig. 4), but was increased by treatment with progesterone ($P = 0.04$). However, progesterone replacement did not restore pulse magnitude to that observed in intact animals. Oestradiol replacement had no effect on pulse magnitude ($P = 0.41$). No interaction between steroids was observed ($P = 0.48$).

The mean duration of PGFM pulses ranged from 2.5 to 3.3 h in the four ovariectomized steroid-replacement groups. Neither oestradiol nor progesterone had any effect on the duration of pulses ($P = 0.39$, $P = 0.33$, respectively). No interaction between steroids was observed ($P = 0.43$). The duration of pulses in the ovariectomized, steroid-treated ewes was not different from that observed in the intact ewes (3.0 h).

Fig. 2. Concentrations of 13,14-dihydro-15-keto-PGF_{2α} (PGFM) in jugular venous serum of individual ewes from each treatment group; (a) intact; (b) ovariectomized; (c) ovariectomized plus oestradiol; (d) ovariectomized plus progesterone; (e) ovariectomized plus progesterone and oestradiol. *Pulses defined using the PULSAR algorithm.

The mean interval between pulses of PGFM ranged from 8 to 14.4 h in the four ovariectomized, steroid-treated groups. Neither oestradiol nor progesterone had any effect on the inter-pulse interval ($P = 0.89$, $P = 0.29$, respectively). No interaction between steroids was observed ($P = 0.58$). The interval between pulses of PGFM was similar in the ovariectomized, steroid-treated and the intact ewes (9.5 h).

Discussion

Pulsatile secretion of PGF_{2α} is greatly diminished in the absence of ovarian steroids. Scaramuzzi *et al.* (1977) observed a similar reduction in pulsatile secretion of PGF_{2α} following ovariectomy. Progesterone appears to play an extremely important role in regulating pulsatile secretion of PGF_{2α} from the ovine uterus. It can completely restore the number of pulses and partially restore pulse magnitude when administered to ovariectomized

Fig. 3. Effects of progesterone and oestradiol replacement on the number of pulses of 13,14-dihydro-15-keto-PGF_{2α} observed during the 48 h sampling period in ovariectomized ewes. Lines above bars represent SEM. Numbers at the base of each bar indicate the number of animals in that treatment group. *Significantly different from intact animals ($P < 0.05$).

Fig. 4. Effects of progesterone and oestradiol replacement on the magnitude of pulsatile 13,14-dihydro-15-keto-PGF_{2α} release in ovariectomized ewes. Lines above bars represent SEM. Numbers at the base of each bar indicate the number of animals in that treatment group. *Significantly different from intact animals ($P < 0.05$).

ewes. These effects may be due in part to actions at the level of the uterus. Progesterone is required for the uterus to develop secretory responsiveness to oxytocin (McCracken, 1980; Homanics and Silvia, 1988; Vallet *et al.*, 1990). Progesterone may exert this effect through several mechanisms; it has been shown to increase the number of receptors for oxytocin (Vallet *et al.*, 1990), the ability of oxytocin to stimulate activity of phospholipase C (Vallet and Bazer, 1989; Raw and Silvia, 1991), the synthesis and activity of prostaglandin H endoperoxide synthase (Raw *et al.*, 1988; Eggleston *et al.*, 1990) and the accumulation of lipid droplets, a potential precursor pool for PGF_{2α} synthesis (Brinsfield and Hawk, 1973). It is possible that the ability of the uterus to synthesize PGF_{2α} is so limited in

the absence of progesterone that the uterus cannot synthesize PGF_{2α} in response to any pulse generator signal. Thus, effects of progesterone at the level of the uterus could account for apparent changes in the number of pulses. If pulsatile secretion of PGF_{2α} is dependent upon oxytocin, then it is not surprising that pulses are difficult to detect in ovariectomized ewes that receive no progesterone replacement. In fact our ability to detect pulses in these ewes may imply that a stimulus other than oxytocin can trigger secretion of PGF_{2α} from the uterus. Alternatively, the uterus may possess an endogenous mechanism for pulsatile release of PGF_{2α} that is independent of any external signal.

The ability of progesterone to increase the number of pulses could be due to a direct effect at the level of the pulse generator. However, progesterone appears to reduce the frequency of oxytocin release from the posterior pituitary gland (McCracken *et al.*, 1991).

The ability of progesterone partially to restore pulsatile secretion of PGF_{2α} in ovariectomized ewes in the experiment reported here is in contrast to the failure of medroxyprogesterone acetate (MPA, a synthetic progestin) to restore pulsatile secretion of PGF_{2α} in ewes ovariectomized on day 10 after oestrus (Fairclough *et al.*, 1983). Supplementation with MPA suppressed the number and magnitude of PGFM pulses when administered to intact ewes. Vincent and Inskeep (1986) observed an increase in utero-ovarian venous concentrations of PGF_{2α} 24 h after ovariectomy on day 12 after oestrus. This response was blocked by replacement with progesterone. Ottobre *et al.* (1984) failed to detect any effect of ovariectomy, lutectomy or progestin (fluorogestone acetate) replacement on pulsatile release of PGF_{2α} in the utero-ovarian vein when the surgical manipulations were performed on day 9 or 14 after oestrus. Results from our experiment are difficult to compare with results from the experiments cited above because the acute effects of steroid removal and replacement were examined during the latter half of the oestrous cycle, whereas we focused on effects of steroids administered chronically throughout an artificial oestrous cycle. Differences in the sampling location, prostaglandin measured, sampling interval, sampling frequency, procedures for identification of pulses and progestins used may also contribute to the apparent differences in results observed in these studies.

A role of oestradiol in regulating pulsatile secretion of PGF_{2α} is not clearly evident. There was a tendency for oestradiol to increase the number of pulses. Perhaps oestradiol has a stimulatory effect at the level of the pulse generator. In rats and primates, oestradiol has been shown to stimulate secretion of oxytocin from the posterior pituitary gland (Robinson *et al.*, 1976; Yamaguchi *et al.*, 1979; Amico *et al.*, 1981; Shukovski *et al.*, 1989). Oestrogens affect the morphology (Rhodes *et al.*, 1981; Pfaff 1983; Jirikowski *et al.*, 1988) and electrophysiology (Negoro *et al.*, 1973; Akaishi and Sakuma, 1985) of neurones releasing oxytocin. Recently, Richard and Zingg (1990) found an oestrogen-responsive element within the 5' region of the human oxytocin gene. In sheep and goats, oestradiol enhances the release of oxytocin from the posterior pituitary gland in response to vaginal distension (Robert and Share, 1969; Roberts, 1973) and progesterone suppresses this response (Roberts, 1974). Oestradiol appears to have a stimulatory effect on the frequency of neurohypophyseal oxytocin secretion (McCracken *et al.*, 1991). Zhang *et al.* (1991) observed an

increase in the frequency of PGFM pulses in intact ewes receiving oestradiol supplementation. Yet, destruction of ovarian follicles by X-irradiation had no effect on the frequency or magnitude of PGFM pulses. Oestradiol has also been shown to increase the release of PGF_{2α} induced by oxytocin in ewes receiving progesterone replacement (McCracken, 1980; Homanics and Silvia, 1988). We could not detect any beneficial effect of oestradiol on PGF_{2α} pulse magnitude in the present experiment. This may indicate that a signal other than oxytocin is driving pulsatile secretion of PGF_{2α} in the ovariectomized ewe.

Pulsatile PGF_{2α} secretion can occur in ovariectomized ewes if the steroid environment is appropriate. Clearly, a pulse generator functions to drive the secretory system in ewes lacking a corpus luteum and luteal oxytocin. The results from this experiment provide no conclusive evidence as to the location of this pulse generator; nor do they rule out the possibility that luteal oxytocin serves as a pulse generator signal in intact ewes.

Ovariectomized ewes failed to achieve a normal pulse magnitude, regardless of steroid supplementation. One possible explanation for this is that the steroid replacement protocol used in this experiment did not duplicate the changes that occur in progesterone and oestradiol concentrations during luteolysis in intact ewes. In the steroid replacement protocol, progesterone is maintained at high concentrations up to day 16 after oestrus. A decrease in progesterone may be essential for pulses of PGF_{2α} to reach maximum magnitude (Silvia *et al.*, 1991). An increase in uterine secretory responsiveness to oxytocin occurs in ovariectomized ewes 24–48 h after cessation of progesterone replacement (Lau *et al.*, 1992; Ott *et al.*, 1992). The increase in oestradiol during luteolysis may also contribute to the maximum pulse magnitude (Silvia *et al.*, 1991). Acute administration of oestradiol to intact ewes increases concentrations of receptors for oxytocin in endometrial tissue (Hixon and Flint, 1987). Another possible explanation for the failure of ovariectomized ewes to reach normal pulse magnitude is that some non-steroidal, ovarian factor (perhaps luteal oxytocin) is required for maximum release of PGF_{2α}. The positive feedback loop between uterine PGF_{2α} and luteal oxytocin may, therefore, be an important component in the normal secretory process. It has not been determined whether the low magnitude pulses achieved in the absence of luteal oxytocin would be adequate to induce luteal regression.

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