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Effect of Oxytocin on Plasma Concentrations of 13,14-Dihydro-15-Keto Prostaglandin F and the Oxytocin-Associated Neurophysin During the Estrous Cycle and Early Pregnancy in the Ewe

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ABSTRACT

This study was undertaken to determine the effect of exogenous oxytocin on plasma concentrations of the prostaglandin (PG) F metabolite 13,14-dihydro-15-keto-PGF (PGFM) and the oxytocin-associated neurophysin (OT-N) during the estrous cycle and early pregnancy in the ewe. Ewes were given oxytocin (250 mU, i.v.) on Days 3 (n=4), 8 (n=5), 13 (n=4) or 14 (n=5) of the estrous cycle, and a further 6 ewes were injected on Days 13 (n=2) and 14 (n=4) of pregnancy. No significant rises in plasma concentrations of PGFM were observed on Days 3 and 8 of the estrous cycle and on Days 13 and 14 of pregnancy. A marked increase in plasma PGFM concentrations occurred on Day 14 of the estrous cycle with the PGFM levels rising from a mean basal value of 120 pg/ml to a mean maximum value of 415 pg/ml within 2–10 min of administering oxytocin ($P<0.001$). No increases in plasma OT-N concentrations were found in early pregnancy and only 1 of 4 ewes at Day 14 of the cycle showed any significant increase in OT-N concentrations. It is concluded that there is an increase in the responsiveness of the uterine-PGF secretory system to oxytocin during the latter stages of the estrous cycle. During early pregnancy this response was blocked by the presence of the embryo. The lack of a consistent rise in plasma OT-N concentrations in ewes during the time of elevated plasma PGFM concentrations does not support the view that oxytocin from the corpus luteum is stimulated by an increase in uterine PGF secretion.

INTRODUCTION

It is now well established that prostaglandin (PG) $F_{2\alpha}$ is the uterine luteolytic factor in the ewe responsible for the regression of the corpus luteum (CL) over Days 13–15 of the estrous cycle (Goding, 1974; Horton and Poyser, 1977). Pulsatile surges of PGF in utero-ovarian plasma, or the PGF metabolite 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (PGFM) in peripheral plasma, have been observed in ewes at luteolysis (Thorburn et al., 1973; Barcikowski et al., 1974; Peterson et al., 1976; Webb et al., 1981), while the administration of a prostaglandin synthetase inhibitor, indomethacin, (Lewis and Warren, 1977) or passive immunization against PGF (Fairclough et al., 1981) prolongs the duration of the luteal phase of the estrous cycle.

More recent studies have demonstrated simultaneous release of PGFM and oxytocin or its associated neurophysin (OT-N) in ewes during the time of luteal regression and the fall in plasma progesterone concentrations (Fairclough et al., 1980, 1983; Flint and Sheldrick, 1983a). These data, when combined with previous studies showing uterine-PGF release following an oxytocin stimulus (Roberts et al., 1976) and prolongation of the estrous cycle in ewes actively immunized against oxytocin (Sheldrick et al., 1980), have provided good evidence that oxytocin may play a role in regulating uterine PGF release in vivo.

In the early pregnant ewe it has been reported that there is a diminution in the magnitude of PGF surges in utero-ovarian plasma over Days 13–15 of pregnancy (Thorburn et al., 1973; Barcikowski et al., 1974). Other investigations,

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however, have found similar or higher levels of PGF in uterine plasma of pregnant, compared with nonpregnant ewes (Wilson et al., 1972; Pexton et al., 1975; Lewis et al., 1977). In view of these conflicting reports on PGF production by the gravid uterus and the recent observations implicating oxytocin in luteolysis, we have measured PGFM concentrations in both the pregnant and nonpregnant ewe using an oxytocin stimulus to assess the uterine PGF secretory capacity. We also determined plasma concentrations of OT-N in the oxytocin-treated ewes to test the proposal of Flint and Sheldrick (1983a) that a rise in uterine PGF output may stimulate oxytocin release from the CL of ewes on Days 13–14 of the estrous cycle. Our earlier proposal that changes in peripheral OT-N concentrations may reflect ovarian oxytocin release (Fairclough et al., 1980, 1983), has been strengthened by more recent results showing that the concentrations of oxytocin and OT-N in ovarian or utero-ovarian venous plasma in ewes on Day 11 of the estrous cycle were severalfold higher than the corresponding concentrations in femoral arterial plasma, both before and after administering the $PGF_{2\alpha}$ analogue, cloprostenol (Watkins et al., 1984).

MATERIALS AND METHODS

Animals and Treatments

The Romney ewes used for the study all showed regular estrous cycles of 16–18 days before commencing the experiment. Intravaginal sponges containing 60 mg medroxyprogesterone acetate were used to synchronize estrus as described previously (Peterson et al., 1976). At the first estrus after sponge removal, 12 ewes were mated with intact rams while 19 other ewes were run with a teaser ram fitted with sine-sire harness to detect estrus. On Days 3, 8, 13 and 14 of the estrous cycle the nonmated ewes (n=4, 5, 5 and 5, respectively), were given 250 mU oxytocin (Koch Light) by intravenous injection into the right jugular vein. The mated ewes were also given similar injections of oxytocin on Days 13 (n=5) and 14 (n=7) postestrus. Blood samples were collected by venipuncture from the left jugular vein into 10-ml heparinized Vacutainer tubes. Samples were withdrawn at −60, −30, 0, 2, 5, 10, 20, 30 and 60 min around the time of the oxytocin injection, immediately placed on ice and centrifuged within 15 min of collection. Those mated ewes which did not return to estrus were necropsied 45 days after joining with the ram to confirm pregnancy status.

Hormone Analyses

Plasma concentrations of progesterone were assayed in duplicate by radioimmunoassay using the method of Fairclough et al. (1975). In brief, samples (0.2 ml) were extracted with hexane (4 vol), the extract taken to dryness and the dried film redissolved in 0.1 ml phosphate-buffered saline (PBS) containing antisera produced in rabbits against a progesterone-11-bovine serum albumin (BSA) complex. This antisera was relatively specific for progesterone, cross-reacting 4.2% and 1% with 20α-hydroxyprogesterone and 17α-hydroxyprogesterone, respectively, and <0.1% with all other steroids tested. The intra- and interassay coefficients of variation for a bulk sample containing 2.67 ng/ml progesterone were 9.6% and 11.8%, respectively. The limit of sensitivity of the assay was 0.2 ng/ml plasma.

The radioimmunoassay for determining PGFM concentrations in plasma has been described previously (Fairclough and Payne, 1975). The samples (0.5 ml) were acidified to pH 3, extracted with 4 vol diethyl ether and the extract assayed using antisera produced against a PGFM-BSA conjugate. This antisera, which was a gift from Dr. K. Kirton of The Upjohn Co., cross-reacted 20% and 0.5% with 15-keto $PGF_{2\alpha}$ and 13,14-dihydro-$PGF_{2\alpha}$, respectively, and <1% with other prostaglandins tested. Since the antisera cross-reacted 50% with $PGF_{1\alpha}$, the results are given as PGF equivalents. A bulk sample pool assayed with each assay gave intra- and interassay coefficients of variation of 9.7% and 8.5%, respectively. This assay gave a limit of sensitivity of 25 pg/ml plasma.

The assay used to measure plasma concentrations of OT-N has been described recently (Moore and Watkins, 1983). The antisera used for the assay was produced against the oxytocin-associated neurophysin I which cross-reacted 2.4% with the vasopressin-associated neurophysin and <0.07% with all other proteins tested. The intra- and interassay coefficients of variation for this assay were 8.9% and 7.9%, respectively, and the limit of sensitivity was 500 pg/ml.

Statistical Analysis

Significance was tested using log-transformed data by analysis of variance and by Student's *t* test. For the analysis we tested the difference between the three basal and first three postinjection concentrations. However, in two animals sampled on Day 14 of the estrous cycle, the −60 and −30-min PGFM values were removed from the analysis since the high level recorded for these samples probably reflected an endogenous surge of PGFM concentrations (Fairclough et al., 1983). Differences in basal levels between pregnant and nonpregnant ewes were tested using analysis of variance. For the presentation of data, arithmetic means were used rather than back-transformed log means.

RESULTS

Animals

All of the nonmated ewes had estrous cycles of 15–18 days. However, only 2 of the Day 13 mated ewes and 4 of the Day 14 mated ewes were pregnant 45 days after joining with the ram. Only those ewes found pregnant at this time are reported in this study.

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Hormonal Patterns

Individual hormone patterns for PGFM, OT-N and progesterone concentrations for the 4 nonpregnant ewes on Day 13 and the 5 ewes on Day 14 of the estrous cycle, are depicted in Figs. 1 and 2. For the Day 13 ewes (Fig. 1) there was a small but significant ($P<0.05$) increase observed in the plasma PGFM response to the oxytocin stimulus, but no significant increase in plasma OT-N concentration was recorded. All ewes on Day 14 of the estrous cycle gave a marked PGFM response to the oxytocin stimulus ($P<0.001$). Plasma concentrations of OT-N showed no change after the oxytocin injection in 4 of the 5 ewes; in the 5th ewe, plasma OT-N concentrations increased from around 2 ng/ml to 4 ng/ml within 30 min of the oxytocin injection. Plasma progesterone levels at the time of injection ranged from 0.5–2.8 ng/ml, indicating that the CL of the ewe with the lowest plasma progesterone level

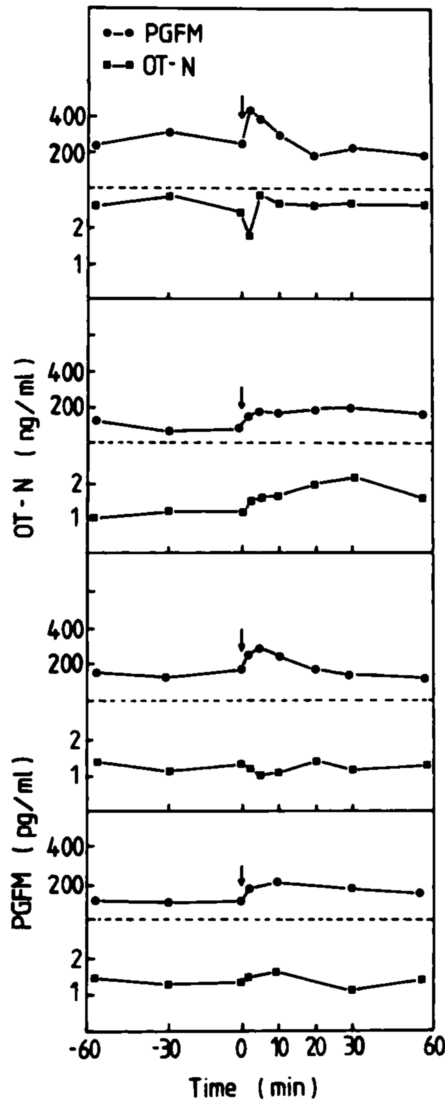

FIG. 1. Plasma concentrations of 13,14-dihydro-15-keto-PGF (*PGFM*) and the oxytocin-associated neurophysin (*OT-N*), in 4 ewes given oxytocin (250 mU, i.v.) on Day 13 of the estrous cycle. The *arrows* indicate injection of oxytocin.

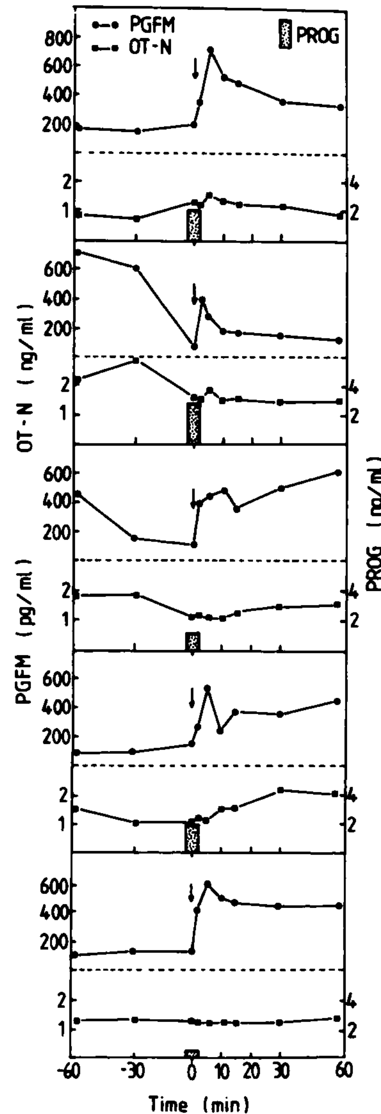

FIG. 2. Plasma concentrations of PGFM, OT-N and progesterone in 5 ewes given oxytocin (250 mU, i.v.) on Day 14 of the estrous cycle. The *arrows* indicate the injection of oxytocin.

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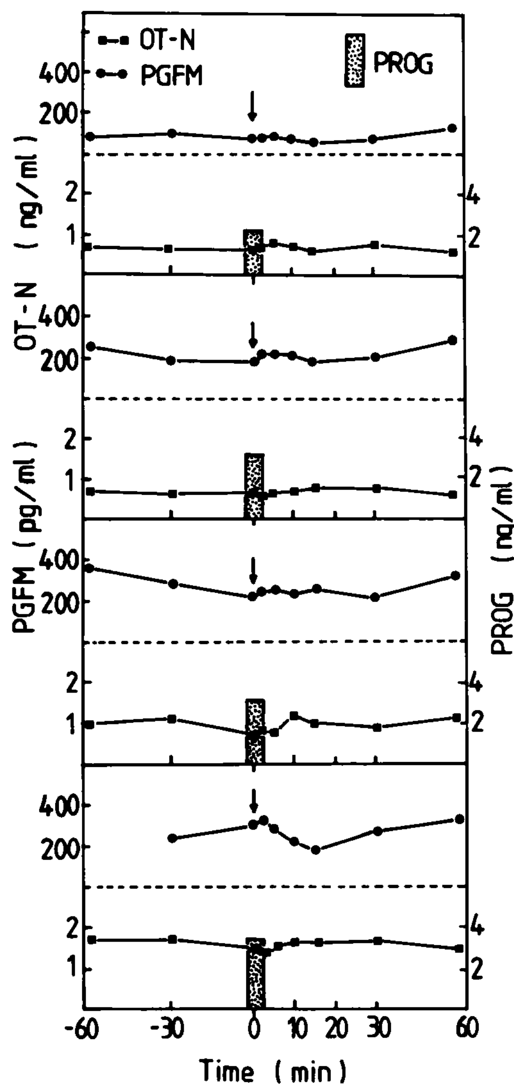

FIG. 3. Plasma concentrations of PGFM, OT-N and progesterone (*PROG*) in 4 ewes given oxytocin (250 mU, i.v.) on Day 14 of pregnancy. *Arrows* indicate the injection of oxytocin.

of 0.5 ng/ml was regressing before the oxytocin stimulus was given. This animal returned to estrus after 15 days, whereas the other 4 animals had estrous cycle lengths of 16–18 days.

Plasma concentrations of PGFM, OT-N and progesterone for the 4 ewes treated on Day 14 of pregnancy are shown in Fig. 3. In all of these ewes no significant increases in plasma concentrations of either PGFM or OT-N were observed. Plasma progesterone levels of 2–3.6 ng/ml were consistent with the postmortem data which indicated that these ewes were pregnant at 45 days after mating. Data on individual mated ewes on Day 13 of pregnancy (not shown) also showed no significant increases in PGFM and OT-N concentrations in response to the oxytocin stimulus.

The mean basal and oxytocin-stimulated PGFM levels in the Days 13 and 14 pregnant ewes and the ewes on Days 3, 8, 13 and 14 of the cycle are shown in Fig. 4. On Day 13 of the estrous cycle there was a small but significant increase ($P<0.05$) in plasma PGFM concentrations. By Day 14 there was a rapid and highly significant ($P<0.001$) PGFM response to an oxytocin stimulus, with the basal PGFM concentrations increasing from a mean value of 120 pg/ml to a mean maximum of 415 pg/ml within 2–10 min of administering exogenous oxytocin. No significant increases in PGFM concentrations in peripheral plasma were observed in ewes on Days 3 and 8 of the estrous cycle and in the pregnant ewes on Days 13–14 postmating. Analysis of basal PGFM concentrations indicated that the levels were higher in pregnant compared with the nonpregnant ewes ($P<0.05$).

DISCUSSION

The finding in this study of a dramatic increase in the oxytocin-induced PGFM response over Days 13–14 of the estrous cycle agree closely with an earlier report of Roberts and McCracken (1976) who demonstrated a marked increase in PGF secretion from the autotransplanted ovine uterus following the infusion of oxytocin into the uterine artery over the late luteal phase of the estrous cycle. These data, which also indicate that peripheral PGFM concentrations can reflect uterine PGF secretion, are given support by an earlier study of Louis et al. (1977) showing a high correlation between utero-ovarian PGF and peripheral PGFM concentrations in ovariectomized ewes treated with progesterone and /or estradiol. Contributing factors which may account for the increase in the responsiveness of the endometrial PGF secretory system to an oxytocin stimulus are a marked rise in concentrations of oxytocin receptor sites (Roberts et al., 1976) and to a twofold increase in PGF cyclooxygenase activity over Days 5–14 of the cycle (Huslig et al., 1979). In addition, there may be an increase in substrate availability for $PGF_{2\alpha}$ synthesis since Brinsfield and Hawk (1973) have shown

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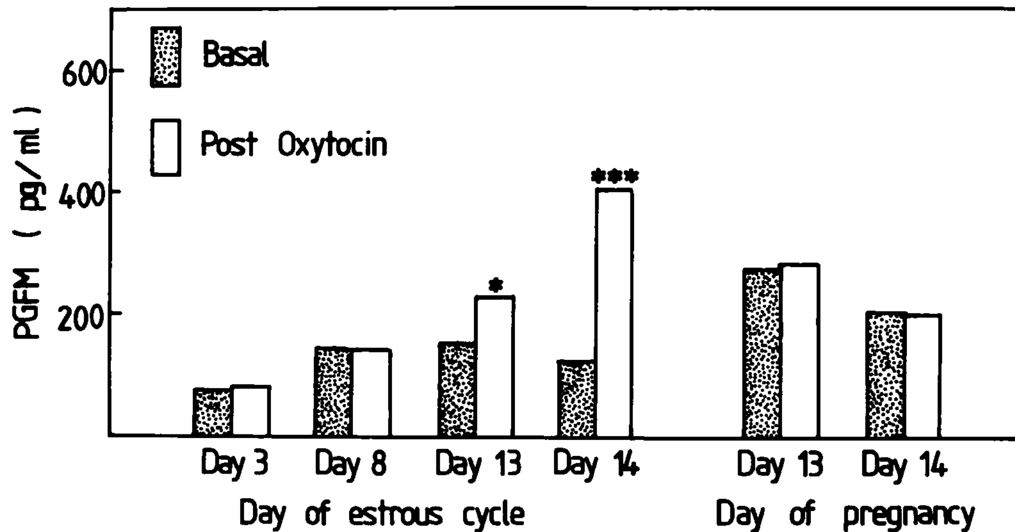

FIG. 4. Mean basal and mean peak concentrations of PGFM in ewes injected with oxytocin (250 mU, i.v.) on Days 3, 8, 13 and 14 of the estrous cycle and Days 13 and 14 of pregnancy. There were 5 ewes on Days 8 and 14 of the estrous cycle, 2 on Day 13 of pregnancy and 4 for all other treatment groups. *Different from mean basal level, $P<0.05$. ***Different from mean basal level, $P<0.001$.

increased lipid droplet formation in the ovine endometrial cell associated with the development of the CL. These factors may, in turn, be regulated by the ovarian steroids, estrogen and progesterone. Sharma and Fitzpatrick (1974) showed that the oxytocin-induced uterine PGF response in the ovariectomized ewe was enhanced if the ewes were first primed with estrogen. More recently, McCracken (1980) has shown that exogenous progesterone initially inhibits the oxytocin-induced PGF response in the estrogen-primed ewe, but that after administering progesterone for a period of around 10 days there is a dramatic increase in the responsiveness of the uterine PGF secretory system to oxytocin.

Evidence that ovarian oxytocin does play an important role in luteal regression in the ewe has been accumulating over the last few years. Previous studies have demonstrated that uterine $PGF_{2\alpha}$ secretion can be stimulated by exogenous oxytocin with the concentrations of $PGF_{2\alpha}$ in utero-ovarian venous plasma rising to a maximum within 5 min of starting the oxytocin infusion (Roberts and McCracken, 1976), while active immunization against oxytocin has been shown to prolong the luteal phase of the estrous cycle with the length of the cycle being related to the antibody titer (Sheldrick et al., 1980; Schams et al., 1982). It has also been reported that PGFM and oxytocin or OT-N are released simultaneously during the time of declining plasma progesterone concentrations on Days 13–14 of the estrous cycle (Fairclough et al., 1980, 1983; Flint and Sheldrick, 1983a). Moreover, active secretion of oxytocin or OT-N by the ovary has been demonstrated in ewes under basal conditions and after administering cloprostenol to stimulate ovarian oxytocin production (Flint and Sheldrick, 1982, 1983; Watkins et al., 1984).

The data obtained in this and an earlier paper of Roberts and McCracken (1976) have indicated that the uterine $PGF_{2\alpha}$ secretory system is relatively refractory to an oxytocin stimulus on Days 12–13 of the estrous cycle. These observations may offer some explanation for our earlier results which showed that during the early stages of luteolysis (Days 12–13) major surges in plasma OT-N concentrations were observed at times when there was little or no detectable increases in plasma PGFM concentrations (Fairclough et al., 1980, 1983). However by Day 14, uterine $PGF_{2\alpha}$ release can be stimulated with exogenous oxytocin (Roberts and McCracken, 1976; this study) and it is during this middle stage of luteolysis that coincident peaks of OT-N and PGFM were observed (Fairclough et al., 1980, 1983). The observation showing surges in plasma PGFM

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concentrations in the absence of any marked increases in plasma OT-N concentrations on Day 15 of the estrous cycle (Fairclough et al., 1980; 1983) is compatible with previous proposals suggesting that during the latter stages of luteolysis uterine-$PGF_{2\alpha}$ release may be provoked by declining plasma progesterone concentrations (Fairclough et al., 1983) and rising plasma estrogen concentrations (Barcikowski et al., 1974). In conclusion, it would appear that the lack of a correlation between PGFM and oxytocin or OT-N concentrations in some samples collected from ewes over luteolysis (Fairclough et al., 1980, 1983; Flint and Sheldrick, 1983) may be due to changes in the sensitivity of the uterine $PGF_{2\alpha}$ release system to oxytocin and the possibility that factors other than oxytocin may induced uterine $PGF_{2\alpha}$ production on Day 15 of the estrous cycle.

In early pregnancy there is a marked reduction in basal levels of oxytocin in peripheral plasma (Webb et al., 1981) and an absence of intermittent surges in plasma OT-N concentrations (Moore et al., 1982), suggesting that there is a suppression of oxytocin release over Days 13–15 postmating. The results of this study indicate that the presence of the embryo may also cause a reduction in the oxytocin-induced PGFM response over Days 13–14 postestrus. McCracken (1980) reported similar findings when he demonstrated a marked suppression of the oxytocin-induced uterine $PGF_{2\alpha}$ response in pregnant compared to nonpregnant ewes 16 days after mating. These data therefore may offer some explanation for earlier results showing a reduction in the amplitude of $PGF_{2\alpha}$ surges in utero-ovarian venous plasma (Thorburn et al., 1973; Barcikowski et al., 1974), or a reduced frequency of $PGF_{2\alpha}$ peaks (Nett et al., 1976) in ewes on Days 14–16 of pregnancy. Not all investigators agree that uterine $PGF_{2\alpha}$ production in the ewe is reduced during early pregnancy. Some studies have shown that plasma $PGF_{2\alpha}$ concentrations in uterine venous plasma are either higher than (Wilson et al., 1972), or similar to (Pexton et al., 1975; Lewis et al., 1977, 1978) the corresponding levels found in nonpregnant ewes at luteolysis. However, in these studies blood samples were collected only once or twice daily (Wilson et al., 1972; Pexton et al., 1972) or at 30-min intervals over 1 or 5 h (Lewis et al., 1977, 1978), and these sampling times are probably not sufficient to detect surges in plasma PGF concentrations in uterine venous or PGFM concentrations in peripheral plasma which occur intermittently at intervals of 8–15 h over Days 13–15 of the cycle (Thorburn et al., 1973; Barcikowski et al., 1974; Fairclough et al., 1980, 1983). Taken together the data would suggest that when the blood samples are collected at 1- to 3-h intervals over the time of luteal regression, then a reduction in uterine PGF output in vivo can be demonstrated. Support for this view has been strengthened by two other studies showing that when blood samples were taken at 2- to 3-h intervals from the jugular vein a marked suppression of plasma PGFM concentrations can be demonstrated over Days 13–15 of pregnancy (Peterson et al., 1976; Webb et al., 1981).

Studies on the synthesis and secretion of $PGF_{2\alpha}$ by ovine endometrial tissues in vitro have shown that the embryo either had no effect on $PGF_{2\alpha}$ production (Findlay et al., 1981) or appeared to stimulate the secretion of PGE_2 and $PGF_{2\alpha}$ (Ellinwood et al., 1979). However, in view of the new role which has been proposed for oxytocin in the estrous cycle, these in vitro studies may need to be extended by measuring uterine-$PGF_{2\alpha}$ secretion before and after adding exogenous oxytocin. Roberts et al. (1976) have shown that oxytocin can cause a net release of PGF from endometrial tissue in nonpregnant ewes but to our knowledge no comparable studies have been performed using tissue from pregnant ewes. Such data are urgently required because they may offer some explanation for the apparent discrepancies between the in vitro results and the temporal findings in the intact animal.

Of interest in this regard is the finding in this study that the basal levels of PGFM were significantly higher in pregnant than in nonpregnant ewes ($P<0.05$). Thus it would appear, on the basis of these results, that endometrial tissue from pregnant ewes does secrete higher amounts of $PGF_{2\alpha}$ compared with tissue collected from nonpregnant ewes, which is in agreement with the earlier results of Ellinwood et al. (1979). At the same time, however, there is a marked reduction in the oxytocin-induced PGF response in the early pregnant ewe (McCracken, 1980; this study). Similar observations have recently been reported by Bloch et al. (1983) in rabbits. They showed that microsomes prepared from rabbit uterine tissue showed a much greater capacity to metabolize arachidonic acid when the tissue was taken

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from pregnant rather than nonpregnant animals. On the other hand, the PGE_2 response to an oxytocin stimulus using an ex vivo perfused uterine preparation was markedly reduced in early pregnancy.

Flint and Sheldrick (1982, 1983a) administered cloprostenol to nonpregnant ewes on Day 11 postestrus and reported a dramatic increase in oxytocin output from the CL. These findings led the authors to suggest that during the normal estrous cycle, the surges of $PGF_{2\alpha}$ from the uterus may provide the stimulus for ovarian oxytocin release during the late luteal phase of the cycle. However, Fairclough et al. (1980, 1983) have shown that during the early stages of luteolysis, surges in the plasma concentrations of OT-N could be detected in the absence of any significant increases in plasma PGFM concentrations. The possibility that surges of PGFM did occur but were not detected because of the dilution of PGFM concentrations in peripheral plasma or because of an inadequate sampling frequency, appears to be discounted by the results obtained in this study showing no significant increase in plasma OT-N concentrations despite a marked rise in PGFM output (Fig. 2). These data suggest that uterine $PGF_{2\alpha}$ may not play a major role in regulating luteal oxytocin release, although we cannot rule out the possibility that an extra uterine source of $PGF_{2\alpha}$ may regulate oxytocin secretion. In this respect, our results are compatible with recent findings of Flint and Sheldrick (1983b) showing that in hysterectomized ewes the levels of oxytocin in the CL declined at the time of expected luteolysis.

It is concluded that the uterine $PGF_{2\alpha}$ response to an oxytocin stimulus increases sharply over the late luteal phase of the estrous cycle in sheep but this response is blocked during early pregnancy. The lack of a significant increase in OT-N concentrations in ewes given oxytocin on Day 14 of the estrous cycle to stimulate uterine $PGF_{2\alpha}$ release does not support the view that luteal oxytocin is stimulated by an increase in uterine $PGF_{2\alpha}$ secretion.

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