Effects of exogenous oxytocin and progesterone on GnRH-induced short luteal phases in anoestrous ewes

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Two experiments investigate the effects of oxytocin and progesterone on premature luteolysis in ewes. In Expt 1, 20 anoestrous ewes were induced to ovulate by multiple injections of GnRH (250 ng i.v. every 2 h for 24 h) followed by a bolus injection of GnRH (125 μg, i.v.). Ten ewes received a continuous infusion of oxytocin from the day after the GnRH bolus injection and the other ten ewes were infused with saline. Oxytocin infusion had no significant effect on the proportion of ewes with short luteal phases (P > 0.05). All ewes that had luteal phases of normal duration from either group (n = 9) exhibited a transient increase in plasma concentrations of progesterone 2 h after insertion of the pump. In Expt 2, 25 anoestrous ewes were treated with GnRH as in Expt 1. Five ewes were pretreated with progestagen for 11 days and ten ewes received progesterone (12 mg, i.m.) 24 h after the bolus injection of GnRH. All animals received an oxytocin injection (1 μg, i.v.) on day 4 after the GnRH bolus. All five ewes that were pretreated with progestagen had normal luteal function and none exhibited a 13,14-dihydro-15-keto PGF2α (PGFM) response to oxytocin. None of the ten ewes injected with progesterone had a normal luteal phase and six ewes exhibited a PGFM response to oxytocin. Four ewes in the control group had normal luteal function and three had short luteal phases. It is concluded that (1) administration of oxytocin from about the time of ovulation does not prevent premature luteal regression; (2) a transient increase in progesterone at about the time of ovulation is associated with luteal phases of normal duration; (3) a more extended exposure to progesterone at about the time of ovulation prevents normal luteal function and may inhibit luteinization and (4) pretreatment with progesterone prevents luteolysis by reducing the uterine response to oxytocin early in the luteal phase.

Introduction

Seasonally anoestrous ewes induced to ovulate with multiple injections of GnRH, without prior treatment with progestosterone, frequently form a corpus luteum that undergoes premature regression 4 days later (Southey et al., 1988a). These corpora lutea with a short lifespan provide a suitable model for the study of premature luteolysis which often occurs after the first ovulation following a period of ovarian quiescence (for example, at puberty, or the transition from seasonal or lactational anoestrous) (Yuthasastrakosol et al., 1975; Lamming et al., 1981).

The premature regression of the GnRH-induced corpus luteum has been shown to be a result of the precocious activation of the normal luteolytic mechanism, involving the coincident secretion of oxytocin from the corpus luteum and PGF2α from the uterus (Hunter et al., 1989; Hunter, 1991). As in the control of luteolysis at the end of a normal luteal phase, oxytocin receptors play a pivotal role in the initiation of premature luteolysis. Ewes which exhibit abnormal luteal function have higher concentrations of uterine oxytocin receptors present 5 days after GnRH treatment than do ewes displaying normal luteal function (Hunter et al., 1989). Pretreatment with progesterone before GnRH therapy prevents premature luteolysis. Ewes, pretreated with progesterone, have similar numbers of oxytocin receptors at oestrus as ewes not pretreated with progesterone. The increased numbers of receptors on day 5 in ewes not pretreated appear to result from a slower decline in receptor concentration during the early luteal phase compared with the decline in ewes pretreated with progesterone (Hunter, 1991; Beard and Hunter, 1994a). Progesterone has a negative influence on oxytocin receptors during the luteal phase and in steroid hormone treated, ovariectomized ewes (Homanics and Silvia, 1988; Lau et al., 1992; Beard et al., 1994).

Flint and Sheldrick (1985) showed that continuous infusion of oxytocin in ewes from day 13 to day 21 of the oestrous cycle delayed the return to oestrus by 7 days, by inhibiting the production of uterine PGF2α by downregulating the uterine oxytocin receptor (Sheldrick and Flint, 1990). It is not known

*Reprint requests.
Received 19 July 1995.
whether oxytocin can block premature luteolysis in a similar manner.

The aims of the present study were to investigate whether premature luteolysis in anoestrous ewes induced to ovulate by GnRH treatment could be prevented either by a continuous infusion of oxytocin beginning 24 h after the final GnRH treatment or by a single injection of progesterone administered around the time of ovulation. In the latter experiment, an oxytocin challenge was also used to investigate the role of functional oxytocin receptors.

Materials and Methods

Animals

Mature, Romney Marsh ewes (n = 45) were housed under natural conditions of daylength and temperature and both experiments were performed during April–July.

Treatment and blood sampling

Experiment 1. All animals (n = 20) were given 250 ng GnRH i.v. (Lutal; Fabwerke Hoechst AG, Frankfurt) in 2 ml sterile saline every 2 h for 24 h, followed by a bolus injection of 125 μg GnRH i.v., a regimen known to synchronize the time of the LH surge (Hunter et al., 1988; Beard and Hunter, 1994b). Time zero was the time of the bolus GnRH injection. Ten ewes (oxytocin-treated group) were implanted with an osmotic mini pump (Alzet, Model 2ML1: Alza Corp., Palo Alto, CA; flow rate = 2.08 μl h⁻¹) filled with oxytocin (1.5 mg ml⁻¹) (Fabwerke Hoechst) diluted in saline plus 0.1% (v/v) acetic acid. 24 h after the bolus GnRH injection. The other ten ewes (control group) were implanted with osmotic pumps filled with saline plus 0.1% (v/v) acetic acid. The prefilled pumps were incubated in saline at 37°C overnight and then inserted via a 3 cm incision in the axilla region under local anaesthetic (5 ml, Lignavet Plus: C-Vet Ltd, Bury St Edmunds, Suffolk). The jugular vein of all ewes was cannulated 2 days before the GnRH bolus injection. Blood samples for assay of progesterone (5 ml) and oxytocin (7 ml) were taken every 12 h from the beginning of GnRH injection and then daily from day 6 until death (12 or 13 days after insertion of the pump). A further sample was also taken 2 h after insertion of the pump. Samples for analysis of oxytocin and 13,14-dihydro-14-keto-PGF₂α (PGFM) were taken at intervals of 1 h for periods of 12 h on days 4 and 5 after the GnRH bolus injection.

Experiment 2. All animals (n = 25) were given GnRH as described for Expt 1. The control group (n = 10) received no other treatment. The progesterone pretreatment group (n = 5) were pretreated with an intravaginal prostestagen (fluoroogestone acetate) sponge for 11 days and the third group received an i.m. injection of progesterone (1 ml 12 mg ml⁻¹, in 90% corn oil) 24 h after the bolus injection of GnRH (progesterone-injected group, n = 10). Time zero was the time of the bolus GnRH injection. All groups received an oxytocin injection (1 ml of 1 μg oxytocin ml⁻¹ in saline, i.v.) on day 4. The jugular veins of all ewes were cannulated 6 days before the bolus injection of GnRH. Samples for progesterone assay (5 ml) were collected once a day from day 6 to day 1, every hour on day 1, twice a day from days 2–4, once a day on days 5–7, and then on alternate days until death (day 15). Plasma samples (5 ml) were collected for assay of PGFM at 20 min intervals in the hour before the oxytocin injection and at 10 min intervals in the following hour (post-treatment period).

Radioimmunoassays

Samples were assayed for progesterone as described by Hunter et al. (1986). In Expts 1 and 2, the mean extraction efficiencies were 81% and 81%; the limits of sensitivity were 0.13 and 0.18 ng ml⁻¹; and the intra- and interassay coefficients of variation were 9.5, 10.3 and 11.7, 11.9%, respectively.

Plasma concentrations of PGFM were measured as described by Kaker et al. (1984). In Expts 1 and 2, the mean extraction efficiencies were 88 and 85%, the limits of sensitivity were 27 and 34 pg ml⁻¹ and intra- and interassay coefficients of variation were 16, 13.4 and 20.2, 19.4%, respectively.

Plasma concentrations of oxytocin were measured in a single assay as described by Wathes et al. (1986) and the limit of sensitivity was 0.5 pg ml⁻¹ and the intra-assay coefficient of variation was 19%.

Statistical analyses

Ewes were classified as displaying either normal or abnormal luteal function using progesterone profiles and gross examination of the ovaries. Normal luteal function resulted in an increase in progesterone concentrations from day 2 to reach a concentration > 1 ng ml⁻¹ by day 6. Abnormal luteal function was defined as a transient rise in plasma concentrations of progesterone above baseline values to > 0.3 ng ml⁻¹ by day 3 followed by a decline to basal values by day 5 (Southee et al., 1988a; Beard and Hunter, 1994b). The effects of treatment on the frequency of normal/abnormal luteal function in both experiments were tested by chi-squared analysis. The effects of treatments on the progesterone profiles (area under the curve) and on masses of corpora lutea (Expt 1) were examined by Student’s t test.

The PGFM data in Expt 2 were examined by ANOVA, the residuals were normally distributed and the magnitude of the residuals remained approximately constant over the range of values recorded. Effects of treatment on the post-treatment PGFM concentration were determined by split-spot ANOVA with individual sheep used as plots and samples as subplots. Differences in group means were evaluated by orthogonal contrasts (Lowry, 1992) (the control group was divided into two subgroups of ewes displaying abnormal and normal luteal function). A PGFM response was defined as an increase in at least two points during the post-treatment period that exceeded the pretreatment mean + 2 SD.

Results

Experiment 1

Ovarian activity. Two animals, one in each group, had high progesterone profiles at the start of GnRH treatment and a
All phases values further defined groups (P > 0.05), are shown (Fig. 1b). In both groups, progesterone values had started to decline before day 5. None of the ewes showed an increase in progesterone around the time of pump insertion.

**Oxytocin profiles.** In the oxytocin-treated animals, oxytocin concentrations had reached at least 20 pg ml⁻¹ 24 h after insertion of the pump and remained continuously increased at between 20 and 35 pg ml⁻¹ in all animals until slaughter. Oxytocin concentrations were measured in two ewes randomly selected from the control group and were below 10 pg ml⁻¹ throughout the treatment period.

**Frequent sampling.** Sampling every hour revealed surges of oxytocin reaching > 20 pg ml⁻¹ in two of the three ewes in the control group displaying short luteal phases. In both of these ewes there was a simultaneous release of PGFM. This coincident release pattern was not observed in any of the ewes with normal luteal phases (results not shown).

**Experiment 2**

**Ovarian activity.** All ewes in the progesterone-pretreated group had apparently normal corpora lutea, as did four ewes in the control group; the remaining three ewes failed to form corpora lutea and thus progesterone concentrations remained at basal values. The ovaries of one ewe in the progesterone-injected group had only follicles. All other ewes had corpora albicantia < 0.01 g, with the exception of one ewe in the progesterone-injected group which had a corpus albicans of 0.12 g.

**Progesterone profiles.** Progesterone profiles indicate that all ewes in the progesterone-pretreated group displayed normal luteal function, as did four ewes in the control group (Fig. 2a). These profiles were not different between the groups (P > 0.05). Three ewes in the control group displayed a progesterone profile typical of a short luteal phase (Fig. 2b). The proportion of ewes exhibiting abnormal luteal function was greater in the control group than in the progesterone-pretreated group (P < 0.05). No ewes in the progesterone-injected group displayed normal luteal function (Fig. 3), and it is probable that all ewes in this group failed to form corpus luteum capable of secreting measurable amounts of progesterone. Progesterone concentrations increased rapidly to between 1.5 and 2.0 ng ml⁻¹, and then declined exponentially; however, concentrations were still above basal values 72 h after progesterone administration. A transient increase on days 3 and 4, indicative of the formation of a corpus luteum of short lifespan, was not evident.

**PGFM.** In the progesterone-pretreated group, no ewes exhibited a defined PGFM response to the administered oxytocin. In the control group, one ewe in each of the categories displaying normal or abnormal luteal function responded to oxytocin (25 and 33% of ewes, respectively), whereas six ewes in the progesterone-injected group demonstrated a defined response to oxytocin (60% of ewes). The mean PGFM response pattern for each group is shown (Fig. 4).
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The only characterized every (Southee and control, oxytocin increase PGFM (a; and i.v.). induced Fig. 2. Plasma progesterone profiles (mean ± SEM) of anoestrous ewes induced to ovulate with multiple injections of GnRH (250 ng i.v. every 2 h for 24 h) followed by a bolus injection of GnRH (125 µg, i.v.). One group was pretreated with progestagen for 11 days before GnRH administration; the control group received no other treatment. (a) Normal luteal function in the progesterone-pretreated (■; n = 3) and control (○; n = 4) groups and (b) short luteal phases in the control (▲; n = 3) group. Time 0 is the time of the GnRH bolus injection.

There was an effect of treatment on mean PGFM concentrations following oxytocin administration (P < 0.05). Ewes pretreated with progesterone had lower concentrations of PGFM following oxytocin administration than did ewes not pretreated with progesterone (P < 0.05). Despite an apparent increase in the mean PGFM concentration, in response to oxytocin in the control, short-lifespan group, this was not significantly different to the concentration measured in the control, normal group. However, there was a large inter-animal variation within groups.

Discussion

Experiment 1 confirmed that the abnormal luteal function that occurs following GnRH therapy in some anoestrous ewes is characterized by a corpus luteum of short lifespan, producing only a transient increase in plasma progesterone concentrations (Southee et al., 1988a; Hunter, 1991; Beard and Hunter, 1994b). The proportion of corpora lutea having a short lifespan was not affected by continuous oxytocin infusion. This result may appear initially to contradict that obtained by Flint and Sheldrick (1985) who showed that continuous infusion of a similar amount of oxytocin, beginning on day 13, blocked luteolysis and extended the luteal phase in cyclic ewes. They demonstrated that oxytocin treatment prevented the development of oxytocin receptors, which normally precedes oestrus, and thereby disrupted the functioning of the feedback loop linking the uterus and ovary that is vital to the luteolytic mechanism. In the present study, the premature regression occurred via the normal luteolytic mechanism since synchronous pulses of oxytocin and PGFM were detected. Thus, it appears that the oxytocin infusion was unable to downregulate the existing oxytocin receptors present when the infusion began on day 1 (Hunter, 1991). Sheldrick and Flint (1986) showed that administration of oxytocin to ewes with large numbers of uterine oxytocin receptors already present had no effect on numbers of receptors and this provides an explanation for the difference in the results obtained.

In Expt 1, the incidence of abnormal luteal function was lower in the control group of ewes than that reported by McLeod et al. (1982) and Southee et al. (1988a,b). It appears likely that this increase in the proportion of normal luteal phases is related to the short-lived discharge of progesterone that occurred at the time of pump insertion, since this release only occurred in ewes that subsequently had normal luteal phases. The temporarily increased progesterone concentrations may cause subsequent normal luteal function by decreasing the amount of functional oxytocin receptors in the uterus. The increase in plasma progesterone may have been due to a stress release of progesterone similar to that reported previously in fallow deer (Asher et al., 1989) and calves (Cooper et al., 1995). It is unknown whether the short increase in progesterone and the normal luteal function were the result of an unknown independent mechanism, or, whether the progesterone rise was the cause of subsequent normal luteal function. Expt 2 was designed to investigate this further.

The progesterone injection administered in Expt 2 was designed to simulate the rise measured in Expt 1. The release/metabolism of progesterone administered on day 1 was considerably slower than expected from previous work (Beard

Fig. 2. Plasma progesterone profiles (mean ± SEM) of anoestrous ewes induced to ovulate with multiple injections of GnRH (250 ng i.v. every 2 h for 24 h), followed by a bolus injection of GnRH (125 µg, i.v.) and then 12 mg progesterone (i.m.) 24 h after the GnRH bolus (n = 10). Time 0 is the time of the GnRH bolus injection.

Fig. 3. Plasma progesterone profiles (mean ± SEM) of ewes that had received multiple injections of GnRH (250 ng, i.v. every 2 h for 24 h), followed by a bolus injection of GnRH (125 µg, i.v.) and then 12 mg progesterone (i.m.) 24 h after the GnRH bolus (n = 10). Time 0 is the time of the GnRH bolus injection.
and Hunter, 1994a), as progesterone concentrations were still above pretreatment concentrations 72 h after treatment. The reasons for the slower release/metabolism were not established, although differences in the site of injection, or release from the injection site may account for the differences between studies. The slow decline in progesterone concentrations following the injections made it difficult to determine whether any endogenous progesterone secretion had occurred. However, progesterone concentrations were basal on days 4 and 5, and the formation of corpora lutea of short lifespan is usually characterized by increased progesterone concentrations (to approximately 0.5 ng ml⁻¹) at this time. It was, therefore, concluded that ewes treated with progesterone failed to form functional corpora lutea of any description, that is, neither normal, nor short lifespan corpora lutea were formed.

It was not possible to determine conclusively whether the lack of progesterone secretion in the progesterone-injected ewes was due to the disruption of ovulation or to luteinization. However, studies have shown that the bolus injection of GnRH (at time zero) immediately stimulates the LH surge (McLeod and Haresign, 1984), and this is thought to stimulate ovulation 21–26 h later (Cumming et al., 1973). Therefore, it is unlikely
that the progesterone injection (administered 24 h after the GnRH bolus) inhibited ovulation, which may have already occurred in a number of ewes. It is proposed that the progesterone injection inhibited the normal luteinization mechanism in an unknown manner. In previous studies in sheep, progesterone administration early in the luteal phase allowed luteal function to continue as normal until luteolysis, which was induced a number of days early (Bray et al., 1976; Otobore et al., 1986). A large single injection of progesterone administered to ewes on the day of oestrus, or on the following day, did not appear to inhibit luteinization (Dixon and Thwaites, 1973). Therefore, the inhibition of luteal development by progesterone treatment 24 h after the LH surge is a novel and unexpected observation.

In Expt 2, pretreatment with progesterone inhibited the premature regression of the induced corpus luteum. This study is the first to show that progesterone treatment inhibits the PGFM response to oxytocin administered to ewes at the time of premature luteolysis. This result supports the contention that premature luteolysis is the result of the precocious activation of the normal luteolytic mechanism involving the secretion of luteal oxytocin and uterine PGFM. Furthermore, it indicates that pretreatment with progesterone prevents premature luteolysis by inactivating the luteolytic positive-feedback mechanism at the point of the uterine response to oxytocin. Progesterone pretreatment has been shown to prevent premature luteolysis in corpora lutea induced by GnRH treatment (McLeod et al., 1982; Hunter et al., 1986; Southee et al., 1988a). Pretreatment with progesterone reduces the endogenous pulsatile secretion of PGFM in GnRH-treated ewes (Hunter et al., 1989), and the oxytocin-induced PGFM release in post partum cows (Zollers et al., 1989). The lack of a response to oxytocin in the group pretreated with progesterone in this study confirms this result in ewes. The ability of progesterone pretreatment to decrease the number of oxytocin receptors during the early luteal phase in ewes (Hunter et al., 1989) and cows (Zollers et al., 1993) is probably the cause of the attenuation in the secretion of PGFM and, therefore, the prevention of premature luteolysis.

In Expt 2, a physiological dose of oxytocin administered on day 4 evoked a PGFM response in 29% of the ewes not pretreated with progesterone. In ewes that displayed abnormal luteal function in the present study, the PGFM concentrations measured following oxytocin administration were approximately 130% of baseline concentrations. Zollers et al. (1989) used a large dose of oxytocin (100 iu) to investigate the uterine PGFM response in post partum cows exhibiting normal and abnormal luteal function. Oxytocin caused an increase in PGFM concentrations in cows not pretreated with progesterone (abnormal) on day 5 which was equal in magnitude to that seen in progesterone pretreated (normal) cows on day 16. Therefore, in both ewes and cows an oxytocin challenge administered during the early luteal phase of a short oestrous cycle evokes an increase in PGFM concentrations similar to that seen following oxytocin administration during the late luteal phase of a normal cycle. Furthermore, in all cases, the PGFM response to oxytocin was apparent before a decrease in progesterone was detected (see results above and Zollers et al., 1989; Silvia et al., 1992). This indicates that the attainment of the prostaglandin response to oxytocin is a critical regulatory event which initiates the pulsatile secretion of PGFM during premature and normal luteolysis.

In conclusion, continuous oxytocin administration at about the time of ovulation does not prevent premature luteal regression, probably because oxytocin fails to downregulate existing oxytocin receptors. A transient increase in progesterone concentrations around the time of ovulation is associated with the prevention of premature luteal regression. The luteal response to an extended exposure to progesterone around the same period was unclear as luteinization did not appear to take place. This study has demonstrated that pretreatment with progesterone is associated with a decrease in the uterine PGFM response to oxytocin, and provides further evidence that premature luteolysis occurs owing to an increased number of functional oxytocin receptors during the early luteal phase.

A. P. Beard was in receipt of a MAFF postgraduate scholarship. We thank the staff of JABU for their help with the animal work and J. Craigon for statistical advice.

References


Oxytocin, progesterone and short luteal phases in sheep


