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A dual role for progesterone in the control of cyclicity in ruminants

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Introduction

As the female ruminant enters a state of ovarian cyclicity either at puberty, at the start of the breeding season or after postpartum anoestrus, a luteolytic mechanism must first be initiated and then controlled to provide regular ovarian cycles until the animal becomes pregnant. The precise timing of this luteolytic mechanism is the key event in determining cycle duration, and its prevention is the key feature of the establishment of pregnancy. In ruminants, luteolysis results from the release of episodes of $PGF_{2\alpha}$ from the uterus in response to the binding of luteal oxytocin to newly developed receptors on the endometrium (McCracken *et al.*, 1984). Numerous studies in both cyclic and steroid-treated ovariectomized ewes have demonstrated that the development of the luteolytic mechanism is controlled by progesterone and oestradiol (for review see Silvia *et al.*, 1991; Flint *et al.*, 1994). However, further studies are required to determine the precise control of this mechanism in cows, in which the timing of luteolysis and thus cycle duration differs from that in sheep. We therefore undertook a series of experiments to investigate the role of progesterone in the control of luteolysis in cows.

Materials and Methods

Three studies were carried out in long-term ovariectomized shorthorn × Galloway cows treated with physiological concentrations of progesterone and oestradiol. Oxytocin receptor concentrations were measured in samples of endometrium collected using a trans-cervical biopsy technique (Mann and Lamming, 1994) using the assay of Sheldrick and Flint (1985) as modified by Jenner *et al.* (1989). The development of the luteolytic signal (i.e. the secretion of $PGF_{2\alpha}$ in response to oxytocin) was monitored by measuring plasma concentrations of 13,14 dihydro-15-keto$PGF_{2\alpha}$ (PGFM), the principal metabolite of $PGF_{2\alpha}$, in plasma samples collected for 1 h before and 1 h after exogenous challenge with 50 iu oxytocin i.v. (Lamming and Mann, 1995). Plasma concentrations of oestradiol were determined by the method of Mann *et al.* (1995) and plasma progesterone concentrations by the method of Haresign *et al.* (1975).

Experiment 1

In long-term ovariectomized cows oxytocin receptors are present on the endometrium but $PGF_{2\alpha}$ is not produced in response to oxytocin treatment. The effects of progesterone and oestradiol on responsiveness of these receptors was determined by giving cows oxytocin challenges on days 0, 6, 12 and 18 of treatment with either progesterone via an intravaginal CIDR (7.0 ± 0.8 ng ml^{-1} plasma; $n = 4$) or oestradiol via an s.c. implant (1.9 ± 0.6 pg ml^{-1} plasma; $n = 4$) (Fig. 1a). Increases in PGFM following oxytocin were analysed by repeated sample analysis of variance.

Experiment 2

Ovariectomized cows were first pretreated for 14 days with progesterone via an intra-vaginal CIDR followed by 2 days with oestradiol via six i.m. injections in corn oil (25, 50, 75, 100, 100 and 100 μg

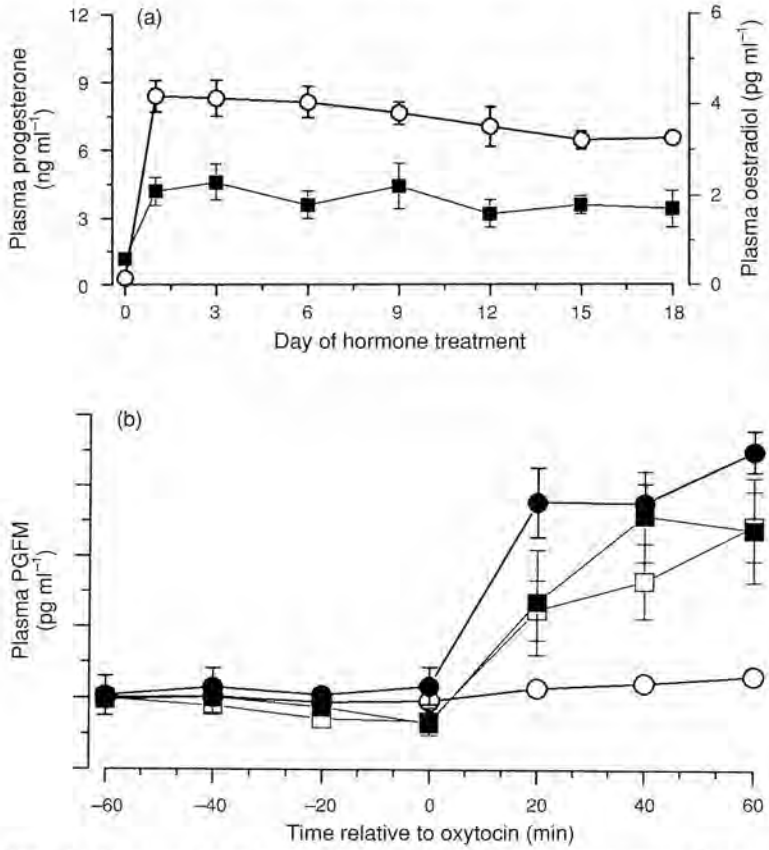

Fig. 1. (a) Mean (± SEM) plasma concentrations of progesterone (○) and oestradiol (■) in long-term ovariectomized cows treated with either progesterone ($n = 4$) or oestradiol ($n = 4$). (b) Mean (± SEM) plasma concentrations of 13,14 dihydro-15-ketoPGF$_{2\alpha}$ (PGFM) before and after oxytocin challenge in long-term ovariectomized cows ($n = 4$) treated with progesterone (○ day 0; ● day 6; □ day 12; ■ day 18).

oestradiol) at intervals of 8 h. This pretreatment induced oestrus, defined as day 0, and relative to which the timing of subsequent treatments is described. For a further 20 days cows were either administered progesterone (via a series of s.c. implants) and oestradiol (via a single s.c. implant) in a pattern designed to mimic a typical luteal phase ($n = 5$; Fig. 2a) or were given no further hormone treatment ($n = 5$). Oxytocin challenges were given and repeated endometrial biopsies collected on days 8, 12, 16 and 20 after induced oestrus to monitor development of oxytocin receptors and the luteolytic signal. Increases in plasma concentrations of PGFM following oxytocin challenges were analysed by repeated sample analysis of variance. Differences in oxytocin receptors were analysed using Student's t tests on data that had been log transformed to reduce heterogeneity of variance.

Experiment 3

In this experiment the effects of the concentration of progesterone on the development of the luteolytic signal were investigated. It has been shown that cows failing to maintain pregnancy have lower milk concentrations of progesterone from day 10 after mating (Lamming *et al.*, 1989). Cows were first pretreated with progesterone and oestradiol, as in Expt 1, and then administered oestradiol (via an

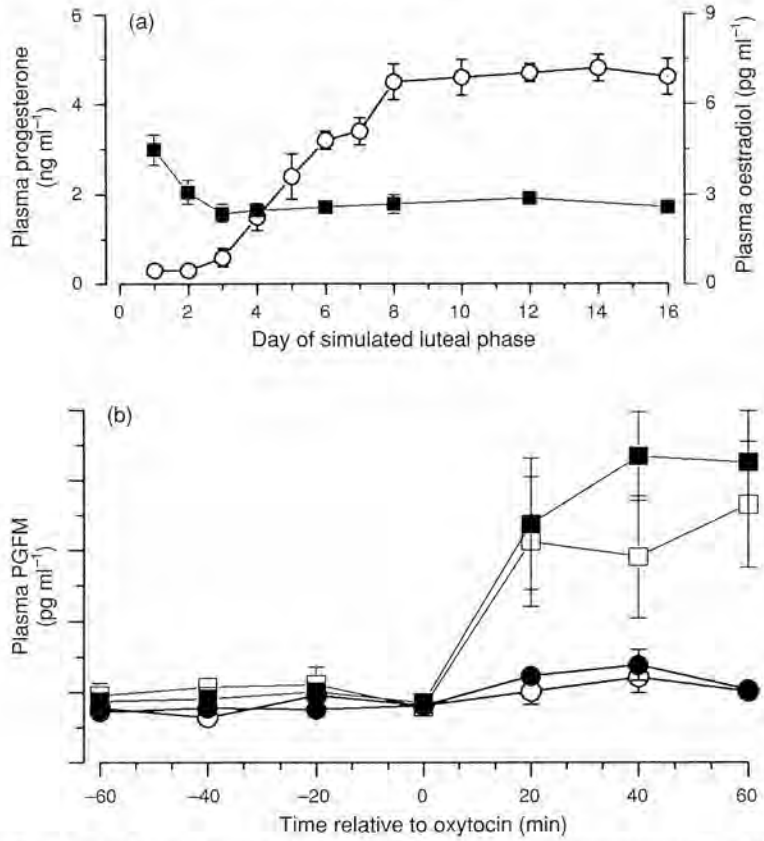

Fig. 2. (a) Mean (± SEM) plasma concentrations of progesterone (○) and oestradiol (■) in steroid hormone pretreated ovariectomized cows treated with progesterone and oestradiol ($n = 5$) to simulate a luteal phase. (b) Mean (± SEM) plasma concentrations of 13,14 dihydro-15-ketoPGF$_{2\alpha}$ (PGFM) before and after oxytocin challenge during a simulated luteal phase in steroid hormone pretreated ovariectomized cows ($n = 5$) treated with progesterone and oestradiol (○ day 8; ● day 12; □ day 16; ■ day 20).

s.c. implant) and either high (12.4 ± 0.8 ng ml^{-1}; $n = 4$) or low (6.0 ± 0.4 ng ml^{-1}; $n = 4$) concentrations of progesterone (via twice daily i.m. injection in corn oil) during a 16 day simulated luteal phase (Fig. 3a). Daily oxytocin challenges were given between days 12 and 16 to monitor the development of the luteolytic signal. Differences in the plasma concentration of PGFM after oxytocin challenge were analysed by analysis of variance.

Results

Experiment 1

Oxytocin receptors were initially present in moderately high numbers (308 ± 12 fmol mg^{-1} protein), but cows did not produce PGF$_{2\alpha}$ in response to oxytocin. In cows treated with oestradiol no response to oxytocin was seen at any time point (data not shown), while in cows treated with progesterone a large ($P < 0.01$) response to oxytocin was seen by day 6 that was maintained to day 18 (Fig. 1b).

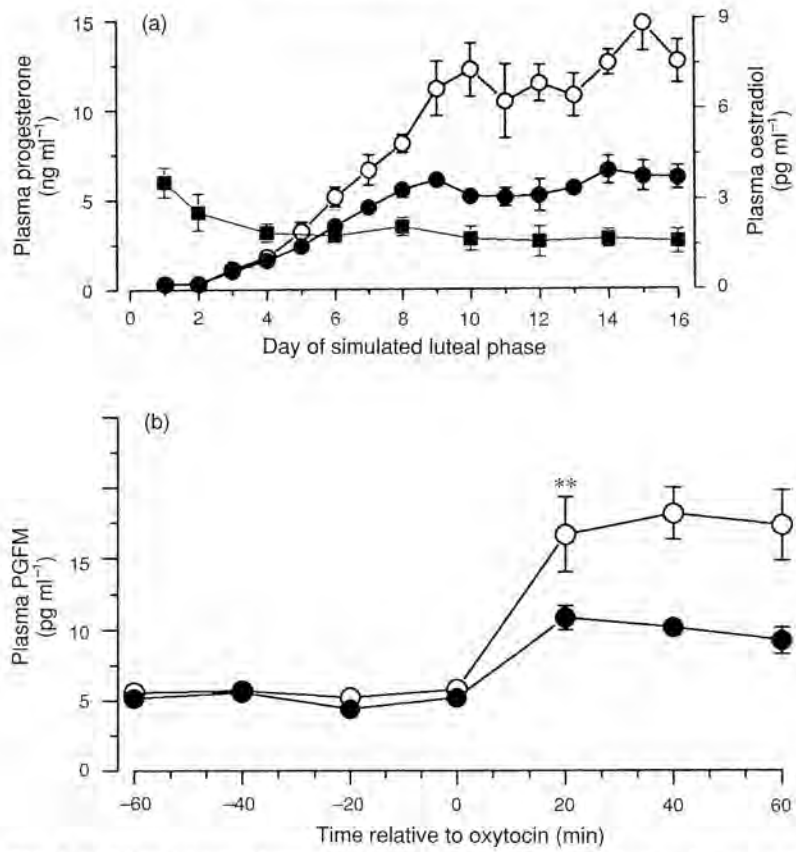

Fig. 3. (a) Mean (± SEM) plasma concentrations of progesterone (○) and oestradiol (■) in steroid hormone pretreated ovariectomized cows treated with oestradiol (■) and either low (●; $n = 4$) or high (○; $n = 4$) levels of progesterone. (b) Mean (± SEM) plasma concentrations of 13,14 dihydro-15-ketoPGF$_{2\alpha}$ (PGFM) before and after oxytocin challenge on day 16 of a simulated luteal phase in steroid hormone pretreated ovariectomized cows treated with either high (●; $n = 4$) or low (○; $n = 4$) concentrations of progesterone. $^{**}P < 0.01$; analysis of variance.

Experiment 2

Oxytocin receptor concentrations had fallen on day 4 after induced oestrus from 325 ± 15 to 72 ± 20 fmol mg^{-1} protein. In the cows given no further hormone treatment, oxytocin receptor concentrations then gradually increased, reaching 132 ± 15 fmol mg^{-1} protein by day 20, but no response to oxytocin was observed at any time point. In the cows given progesterone and oestradiol, oxytocin receptors were suppressed to undetectable values on days 8 and 12 and no responses to oxytocin were observed (Fig. 2b). However, by day 16 oxytocin receptors had reappeared (74 ± 31 fmol mg^{-1} protein) and a large response to oxytocin was observed which was maintained to day 20 when oxytocin receptor concentrations had risen to 169 ± 33 fmol mg^{-1} protein.

Experiment 3

Responsiveness to oxytocin was consistently higher in the low progesterone group than in the high group, and by day 16 a large ($P < 0.01$) difference in the rise in PGFM after oxytocin challenge was observed (Fig. 3b).

Discussion

The results of Expt 1 demonstrate that the action of progesterone is required before oxytocin receptors can express their functional role in the generation of the luteolytic signal. This finding suggests that progesterone (but not oestradiol) is required either for the coupling of post-receptor mechanisms or for the successful functioning of certain aspects of $PGF_{2\alpha}$ production such as substrate availability or enzyme activity. It is likely that progesterone is required for $PGF_{2\alpha}$ production as progesterone is known to induce a variety of important factors such as prostaglandin synthetase activity (Salamonson *et al.*, 1991).

The results of Expt 2 demonstrate that progesterone plays a major role in the control of the development of the luteolytic signal and thus in maintaining regular ovarian cycles. It is thought that progesterone can probably inhibit the development of the luteolytic mechanism until endometrial progesterone receptor activity is lost, by around day 12 in cows (Meyer *et al.*, 1988). The time at which progesterone loses its inhibitory action on the endometrium may determine the time at which development of oxytocin receptors and hence the luteolytic mechanism can begin.

The results of Expt 3 demonstrate that a higher plasma concentration of progesterone exerts a greater inhibition on the development of the luteolytic signal, showing the importance of progesterone in determining the time and strength of this signal. This indicates that in the mated cow the amount of luteal progesterone secreted predetermines the strength of the luteolytic drive and therefore the ability of the embryo to prevent luteolysis, thus providing an explanation as to why early embryo loss in cows is associated with low milk progesterone concentrations.

Conclusions

These results demonstrate that progesterone is important both in the control of oxytocin receptor development and the induction of $PGF_{2\alpha}$ release by these receptors. At the first ovulation after the resumption of cyclicity a failure of appropriate hormonal preconditioning of the uterus often results in the presence of increased numbers of oxytocin receptors early in the luteal phase (Hunter, 1991). In some animals, the first action of progesterone may be to activate these receptors causing premature release of $PGF_{2\alpha}$ and short first cycles. Once cyclicity has been re-established, progesterone can exert its full inhibitory action on the development of the luteolytic mechanism. The concentration of progesterone controls the strength and timing of this development, thus regulating duration of cycle and maintaining regular cyclicity until the animal becomes pregnant.

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