Control of endometrial oxytocin receptors and prostaglandin $F_{2\alpha}$ production in cows by progesterone and oestradiol

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We have investigated changes in endometrial oxytocin receptor concentrations and prostaglandin $F_{2\alpha}$ release in response to exogenous oxytocin treatment in ovariectomized cows treated with progesterone and oestradiol, and made comparisons with similar treatment in cyclic cows. In long-term ovariectomized cows, endometrial oxytocin receptors were present (300 fmol mg^{-1} protein), but no prostaglandin $F_{2\alpha}$ was released in response to oxytocin treatment until after the administration of progesterone. Subsequent administration of a concentration of oestradiol sufficient to induce oestrus resulted in the downregulation of these receptors and the loss of oxytocin responsiveness, which did not reappear within 20 days in the absence of further hormone treatment. When induced oestrus was followed by further treatment with luteal phase concentrations of progesterone and oestradiol, both oxytocin receptors and oxytocin-stimulated release of prostaglandin $F_{2\alpha}$ reappeared by day 16 after oestrus, in a pattern similar to that seen during the luteal phase of cyclic cows. These results demonstrate how progesterone and oestradiol control the development and responsiveness of endometrial oxytocin receptors in cows, and provide a valuable model in which to investigate further the precise control of the oxytocin receptor in this species.

Introduction

In ruminants, the precise control of the timing of luteal regression is the key event in determining the duration of the oestrous cycle, while the prevention of luteal regression is the key feature of the maternal recognition of pregnancy. To understand why pregnancy recognition often fails, we need to understand the precise mechanisms controlling luteolysis. In cattle and sheep, this occurs as a result of the release of luteolytic episodes of uterine prostaglandin $F_{2\alpha}$ ($\text{PGF}_{2\alpha}$) in response to the binding of ovarian oxytocin to newly developed endometrial oxytocin receptors (McCracken, 1984). After numerous studies in both cyclic and steroid-treated ovariectomized ewes, the importance of progesterone and oestradiol in the control of the luteolytic mechanism in sheep is now well established (for a review see Silvia *et al.*, 1991). However, equivalent studies are required in cattle because of differences in the timing of luteolysis and cycle duration in these species.

We carried out a series of experiments in ovariectomized cows in which cyclic patterns of oestradiol and progesterone were replaced to determine how they control the development of the luteolytic mechanism, and to provide a model in which to investigate the precise hormonal control of luteolysis and the maternal recognition of pregnancy.

Materials and Methods

Experimental design

Three consecutive experiments were carried out to investigate the control of oxytocin receptor development and responsiveness by progesterone and oestradiol.

Experiment 1. Five mature blue-grey cows that had been ovariectomized several months previously received a steroid hormone pretreatment regimen designed to mimic a previous luteal and follicular phase and to induce oestrus. The day of induced oestrus was designated day 0 and all treatments are described relative to this. Progesterone was administered via a single intravaginal progesterone releasing device (CIDR-B: SmithKline Beecham Animal Health, Tadworth) inserted for 14 days (days -15 to -1). Oestradiol treatment was initiated at the time of CIDR withdrawal at 08:00 h on the morning of day -1 , and consisted of six i.m. injections of a solution of oestradiol (Sigma Chemical Co., Poole) in corn oil (25, 50, 75, 100, 100 and $100 \mu\text{g}$ oestradiol, respectively) at 8 h intervals. Behavioural oestrus was observed on the second day of oestradiol treatment. During the subsequent 20 day period, animals received no further steroid treatment. Oxytocin challenges were given and, where possible, endometrial biopsies collected prior to (day -16) and on the last day of (day -2) progesterone treatment, and on days 1, 4, 12 and 20 following

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induced oestrus. Plasma samples were collected from all animals at various time points to monitor progesterone and oestradiol concentrations.

Experiment 2. Five long-term ovariectomized blue-grey cows were pretreated with progesterone and oestradiol as described in Expt 1, and then treated for 20 days with further oestradiol and progesterone in a pattern designed to simulate a normal luteal phase. Oestradiol was administered via a single implant inserted s.c. on day 1. Progesterone was administered via a total of six s.c. implants. The first implant was inserted on day 3 with additional implants inserted on days 4, 5, 6, 7 and 8 to produce a gradual rise in plasma progesterone concentration to simulate the normal rise after ovulation. Oxytocin was given and, where possible, endometrial biopsies collected on days 8, 12, 16 and 20 of the simulated cycle. Plasma samples were collected at 1–2 day intervals to monitor plasma progesterone and oestradiol concentrations.

Experiment 3. An experiment was carried out in four naturally cyclic Friesian cows to provide a comparison to the time of appearance and magnitude of responses to oxytocin treatment during our simulated luteal phase in ovariectomized cows. Oxytocin challenges were administered on days 14, 15, 16 and 17 of the cycle (the period of development of the luteolytic mechanism).

Blood sampling and oxytocin challenges

Before the insertion of jugular cannulae for the oxytocin challenges, blood samples were collected from the ovariectomized cows for steroid determination by jugular venepuncture. On the day preceding the first oxytocin challenge, in both intact and ovariectomized cows, a jugular vein of each animal was cannulated under local anaesthesia (2 ml lignocaine s.c. (Lignovet: C-Vet Ltd, Bury St Edmunds)) with a 30 cm indwelling jugular catheter (i.d. 1.2 mm, o.d. 1.8 mm; Secalon Universal Tubing: BOC Health Care, Swindon) using a 12 gauge needle and an 80 cm guide wire. The cannulae were maintained for the duration of the experiment. During oxytocin challenges, blood samples (10 ml) were collected at 20 min intervals for 1 h before and at 10 min intervals for 1 h after injection of a single i.v. bolus of 100 iu oxytocin (Sigma Chemical Co.) in 10 ml saline, flushed in with a further 5 ml saline. Blood samples were collected into heparinized tubes, centrifuged at 1500 g for 10 min and the plasma was stored at -20°C until assayed for 13,14 dihydro-15-keto prostaglandin $\text{F}_{2\alpha}$ (PGFM).

Endometrial biopsy collection

In animals in which the cervix was sufficiently patent, samples of endometrium were collected using mare punch biopsy forceps by a transcervical technique, previously shown not to affect the development of the luteolytic mechanism in cyclic cows (Mann and Lamming, 1994). The cervical canal of ovariectomized cows is small and, on welfare grounds, the use of excessive force in attempting to penetrate the cervix was avoided. As a result, biopsies were obtained from only 2–3 of

the 5 animals in which attempts were made at the various time points. For biopsy collection, animals were sedated by an i.m. injection of 20 mg xylazine (Rompun: Bayer UK Ltd, Cambridge) and the biopsy forceps were guided through the cervix by transrectal manipulation. Once in the uterus a single sample of 300–600 mg endometrial tissue was collected. Animals were then given a prophylactic i.m. injection of penicillin (Duplocillin LA: Mycofarm UK, Cambridge). Endometrial samples were placed on ice and transported to the laboratory for the preparation of subcellular fractions of oxytocin receptors by the method of Sheldrick and Flint (1985). Samples were then stored at -196°C before receptor determination.

Hormone implants

Progesterone implants were made using a Silastic MDX-4-4210 Medical Grade elastomer kit (Dow Corning Corp., Midland, MI). The implants produced contained 20% progesterone (Sigma Chemical Co.) and were 8 cm long with a diameter of 12 mm. Oestradiol implants were made by filling 6 cm lengths of Silastic tubing (i.d. 7.5 mm, o.d. 9.0 mm: Dow Corning Medical Grade Elastomer, Bibby Sterelin Ltd, Stone) with oestradiol (Sigma Chemical Co.) and filling the ends with Silastic rubber adhesive. Before insertion, implants were incubated at room temperature for 48 h in several changes of distilled water to remove surface accumulation of hormone, and then sterilized by soaking for 20 min in 0.5% (w/v) chlorohexadine (DePuy Healthcare, Leeds). Implants were inserted subcutaneously over the shoulder, under local lignocaine anaesthesia (Lignovet), in cavities produced by blunt dissection, and the opening sutured after application of prophylactic iodine (Betadine: Napp Laboratories, Cambridge).

Assays

Plasma samples were assayed for PGFM after extraction with acidified diethyl ether by radioimmunoassay (Kaker *et al.*, 1984). Intra- and interassay coefficients of variation were $< 15\%$ and 14.6% , respectively. Progesterone concentrations were measured in plasma samples after extraction with petroleum ether by radioimmunoassay (Haresign *et al.*, 1975). Intra- and interassay coefficients of variation were $< 10\%$ and 12.3% , respectively. Oestradiol concentrations were measured in plasma using a modified radioimmunoassay kit (Serono Diagnostics Ltd, Woking) (Mann *et al.*, in press). The sensitivity of the assay was 0.5 pg ml^{-1} and the intra- and interassay coefficients of variation were $< 10\%$ and 8.4% , respectively. Oxytocin receptor concentrations were measured using a binding assay described by Sheldrick and Flint (1985), modified for use in bovine samples (Jenner *et al.*, 1989). Results were expressed as fmol oxytocin bound mg^{-1} total protein (Lowry *et al.*, 1951). The sensitivity of the assay was 20 fmol oxytocin bound mg^{-1} total protein, and the interassay coefficient of variation ($n = 3$) of a quality control sample collected at oestrus was 7.9% .

Statistical analysis

Increases in the plasma concentration of PGFM following oxytocin challenges were analysed by repeated sample analysis

Fig. 1. Mean ($\pm$ SEM) plasma concentration of progesterone ($\circ$) and oestradiol ($\bullet$) generated in ovariectomized cows ($n = 5$) during (a) progesterone treatment for 14 days; (b) oestradiol treatment for 2 days, and; (c) 20 days during which no further hormone treatment was administered.

of variance on untransformed data. Differences in oxytocin receptor concentrations were analysed using Student's t tests on data that had been log transformed to reduce heterogeneity of variance.

Results

Experiment 1

Before treatment, plasma progesterone concentration was below the sensitivity of the assay ($< 0.3 \text{ ng ml}^{-1}$) (Fig. 1). After insertion of the CIDR-B progesterone releasing device, concentrations rose to 9 ng ml^{-1} and were maintained above 5 ng ml^{-1} for the duration of the treatment period. After CIDR-B removal, progesterone concentrations fell and were undetectable by day 0. Mean ($\pm$ SEM) oestradiol concentration during the progesterone pretreatment period was $0.6 \pm 0.1 \text{ pg ml}^{-1}$. After the initiation of injections, the oestradiol concentration rose markedly to a mean, midway between injections, of $12.4 \pm 0.3 \text{ pg ml}^{-1}$ over the second day of treatment (day 0). For the subsequent 20 days plasma progesterone remained undetectable ($< 0.3 \text{ ng ml}^{-1}$). Despite falling after the last oestradiol injection, the plasma concentration of oestradiol was still high on days 1 ($2.7 \pm 0.4 \text{ pg ml}^{-1}$) and 2 ($1.5 \pm 0.2 \text{ pg ml}^{-1}$) but was subsequently maintained at a concentration similar to that seen before oestradiol treatment ($0.7 \pm 0.1 \text{ pg ml}^{-1}$).

Before progesterone treatment (day -16), there was no response to oxytocin, but after progesterone pretreatment (day -2), a large ($P < 0.01$) response was observed (Table 1). This response was significantly ($P < 0.05$) reduced on day 1, after oestradiol injection, and by day 4 no significant response was present. Furthermore, no response to oxytocin was seen on either day 12 or 20.

Measurements of oxytocin receptor concentration were obtained for two cows before progesterone pretreatment and from the same two cows and an additional cow at the other time points (Table 1). Before progesterone treatment, concentrations of oxytocin receptor in the two cows were moderately high ($280\text{--}320 \text{ fmol mg}^{-1} \text{ protein}$) and after progesterone

Table 1. Mean ($\pm$ SEM) increase in plasma concentration of 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ following oxytocin challenge and mean ($\pm$ SEM) concentration of endometrial oxytocin receptors measured in biopsy samples before (day -16) and after (day -2) progesterone treatment for 14 days and following the induction of oestrus (on day 0) by oestradiol treatment for 2 days (days 1, 4, 12 and 20) in ovariectomized cows (Expt 1)

Time (day)	Response to oxytocin challenge (% pretreatment baseline) ($n = 5$)	Oxytocin receptor ($\text{fmol mg}^{-1} \text{ protein}$)
-16	114.1 ± 8.5^a	280 and 310 ($n = 2$)
-2	243.6 ± 33.1^c	325 ± 15 ($n = 3$)
1	145.8 ± 8.6^b	72 ± 20 ($n = 3$)**
4	117.0 ± 6.0^a	104 ± 15 ($n = 3$)
12	111.5 ± 5.4^a	131 ± 20 ($n = 3$)*
20	108.3 ± 9.8^a	132 ± 15 ($n = 3$)*

Values with different superscript letters differ significantly: ^{ab, bc} $P < 0.05$; ^a $P < 0.01$.

** $P < 0.01$ compared with day -2 ; * $P < 0.05$ compared with day 1.

pretreatment, the oxytocin receptor concentration was similar to that seen before treatment ($325 \pm 15 \text{ fmol mg}^{-1} \text{ protein}$). Receptor concentrations fell significantly ($P < 0.01$) on day 1 after oestradiol injection and were still low on day 4. However, by day 12 the receptor concentration had risen significantly ($P < 0.05$) compared with that seen on day 1, and was still high on day 20, although the concentration was still lower than that seen before the start of hormone treatment.

Experiment 2

The mean plasma concentration of oestradiol was still high from oestrus oestradiol treatment on day 1 ($4.5 \pm 0.5 \text{ pg ml}^{-1}$) but between day 3 and day 20 it was maintained at a concentration of $2.4 \pm 0.3 \text{ pg ml}^{-1}$ (Fig. 2). Progesterone was undetectable on days 1 and 2, began to rise on day 3, and reached a plateau of $4.8 \pm 0.2 \text{ ng ml}^{-1}$ between day 8 and day 20.

Fig. 2. Mean ($\pm$ SEM) plasma concentration of progesterone ($\circ$) and oestradiol ($\bullet$) during 20 days of oestradiol and progesterone treatment in ovariectomized cows initially pretreated with progesterone and oestradiol ($n = 5$).

Table 2. Mean ($\pm$ SEM) increase in plasma concentration of 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ following oxytocin challenge and mean ($\pm$ SEM) concentration of endometrial oxytocin receptors measured in biopsy samples during a simulated luteal phase in ovariectomized cows (Expt 2)

Time (day)	Response to oxytocin challenge (% pretreatment baseline) ($n = 5$)	Oxytocin receptor concentration (fmol mg^{-1} protein) ($n = 3$)
8	135.7 $\pm$ 20.7	< 20
12	130.7 $\pm$ 16.3	< 20
16	292.1 $\pm$ 39.9 ^a	74 $\pm$ 31
20	338.3 $\pm$ 46.5 ^a	169 $\pm$ 33*

^aSignificant response $P < 0.01$; * $P < 0.05$ compared with day 16 value.

There was no significant increase in PGFM concentration after oxytocin challenge on day 8 or day 12 (Table 2). However, by day 16 a large response to oxytocin was seen ($P < 0.01$) and maintained to day 20.

Oxytocin receptor measurements were obtained from three of the five cows (Table 2). Oxytocin receptor concentrations were below the sensitivity of the assay ($< 20 \text{ fmol mg}^{-1}$ protein) on days 8 and 12 of the cycle. However, by day 16 receptor concentrations had started to rise, and on day 20 they were significantly ($P < 0.05$) higher than on day 16.

Experiment 3

No significant response to oxytocin was seen on days 14 or 15 of the cycle. On day 16, a small response was observed ($180.4 \pm 20.3\%$ increase above the pretreatment baseline; $P < 0.05$), and on day 17 a larger response was present ($247.4 \pm 31.7\%$; $P < 0.01$).

Discussion

These studies demonstrate for the first time that replacement of oestradiol and progesterone in ovariectomized cows can produce changes in oxytocin receptor concentrations and their responsiveness to oxytocin that closely approximate those changes seen in intact cyclic animals, allowing investigation of the hormonal control of luteal regression and cycle duration in this species.

Before long-term ovariectomized cows were treated with hormones, endometrial oxytocin receptor concentrations were high (300 fmol mg^{-1} protein), although no $\text{PGF}_{2\alpha}$ secretion was stimulated in response to oxytocin until after progesterone treatment. This suggests that progesterone is required for either the coupling of post-receptor mechanisms or the successful functioning of certain aspects of $\text{PGF}_{2\alpha}$ production, such as substrate availability or enzyme activity. For example, in ewes, prostaglandin synthetase activity is induced by progesterone (Salamonsen *et al.*, 1991).

In Expt 1, after the removal of the progesterone and the administration of high concentrations of oestradiol, oxytocin receptor concentrations fell and responsiveness to oxytocin was lost, as is the case following natural oestrus in cyclic cows. This loss of oxytocin receptors cannot be attributed to the inhibitory action of progesterone, as concentrations of this hormone were minimal at this time, nor can it be the result of the withdrawal of progesterone, as a similar decline in receptor concentrations is seen after simulated oestrus in ovariectomized ewes given no previous progesterone treatment (Vallet *et al.*, 1990). It must therefore have resulted from the high concentrations of oestradiol seen during the simulated follicular phase as this would provide an explanation for the fall in oxytocin receptors seen after oestrus in cyclic animals. While the high concentration of oestradiol at oestrus caused the initial down-regulation of oxytocin receptors, subsequent treatment with progesterone in Expt 2 caused further suppression of these receptors, demonstrating the role of progesterone in

inhibiting oxytocin receptors during the middle stages of the cycle.

In Expt 2, replacement of physiological concentrations of oestradiol and progesterone in the absence of all other ovarian factors resulted in a pattern of inhibition, and the subsequent development of oxytocin receptors, equivalent to that previously reported in intact cyclic cows (Meyer *et al.*, 1988; Jenner *et al.*, 1989; Fuchs *et al.*, 1990). In cyclic cows, luteolysis occurs when endometrial oxytocin receptors have risen to about 100 fmol mg⁻¹ protein (Mann and Lamming, 1993). In ovariectomized cows in Expt 2 a luteolytic type response to oxytocin had developed by day 16, when oxytocin receptors had risen to 74 ± 31 fmol mg⁻¹ protein. This response was greater than that seen on day 16 in the intact cows in Expt 3 but comparable to that seen on day 17, suggesting that, while oestradiol and progesterone may be key hormones involved in controlling the development of the luteolytic mechanism, other factors such as ovarian oxytocin may have some influence on the precise timing of this development. Alternatively, the sensitivity of the uterus to oestradiol and progesterone, or the pattern of development of steroid hormone receptors, may differ slightly in ovariectomized cows, altering the temporal pattern of steroid hormone influences on the development and responsiveness of oxytocin receptors.

In ewes, exposure to progesterone for 10 days is required before luteolysis occurs (Bray and Hecker, 1976) and in ovariectomized ewes, 10 days of progesterone treatment is required before full responsiveness to oxytocin challenge is achieved (Vallet and Lamming, 1991; Beard and Lamming, 1994). The cows in the present study displayed a luteolytic-type response to oxytocin on day 16, after the plasma progesterone concentration had been maintained above 1 ng ml⁻¹ for 12 days. The oestrous cycle of cows is 4 days longer than that of sheep, and it appears that at least part of this difference can be accounted for in terms of a longer period of progesterone downregulation of the luteolytic mechanism, with the remaining difference the result of a later postovulatory rise in progesterone.

The results of this study demonstrate that steroid-treated ovariectomized cows provide a valuable model in which to study the hormonal control of the oestrous cycle. While low concentrations of oestradiol are thought to stimulate oxytocin receptor development, the high concentrations seen at oestrus result in the loss of oxytocin receptors and a reduction in receptor activity. After oestrus has been induced in ovariectomized cows, treatment with progesterone and oestradiol results in a pattern of further oxytocin receptor inhibition and subsequent receptor development and PGF_{2α} release comparable to that reported during luteolysis in intact cyclic cows.

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References

- Beard AP and Lamming GE (1994) Oestradiol concentration and the development of the oxytocin receptor and oxytocin induced prostaglandin F_{2α} release in ewes *Journal of Reproduction and Fertility* **100** 469–475
- Bray AR and Hecker JF (1976) Role of progesterone in regulating the length of the oestrous cycle in the sheep *Journal of Reproduction and Fertility* **46** 522–523
- Fuchs AR, Behrens O, Helmer H, Liu C, Barros CM and Field MJ (1990) Oxytocin and vasopressin receptors in bovine endometrium and myometrium during the oestrous cycle and early pregnancy *Journal of Endocrinology* **83** 613–615
- Haresign W, Foster JP, Haynes NB, Crighton DB and Lamming GE (1975) Progesterone levels following treatment of seasonally anoestrous ewes with synthetic LH-releasing hormone *Journal of Reproduction and Fertility* **43** 269–279
- Jenner LJ, Parkinson TJ and Lamming GE (1989) Uterine oxytocin receptors in cyclic and pregnant cows *Journal of Reproduction and Fertility* **91** 49–58
- Kaker ML, Murray RD and Dobson H (1984) Plasma hormone changes in cows during induced or spontaneous calvings and the early post partum period *Veterinary Record* **115** 378–382
- Lowry OH, Rosebrough NJ, Farr AL and Randall RJ (1951) Protein measurement with the folin phenol reagent *Journal of Biological Chemistry* **193** 265–275
- McCracken JA, Schramm W and Okulicz WC (1984) Hormone receptor control of pulsatile secretion of PGF_{2α} during luteolysis and its abrogation in early pregnancy *Animal Reproduction Science* **7** 31–55
- Mann GE and Lamming GE (1993) Monitoring endometrial oxytocin receptor development in the cow using a biopsy technique *Journal of Reproduction and Fertility Abstract Series* **11** Abstract 172
- Mann GE and Lamming GE (1994) Use of repeated biopsies to monitor endometrial oxytocin receptors in the cow *Veterinary Record* **135** 403–405
- Mann GE, Lamming GE and Fray MD Plasma oestradiol and progesterone during early pregnancy in the cow and the effects of treatment with Buserelin *Animal Reproduction Science* (in press).
- Meyer HHD, Mittermeier Th and Schams D (1988) Dynamics of oxytocin, oestrogen and progesterone receptors in the bovine endometrium during the oestrous cycle *Acta Endocrinologica* **118** 96–104
- Salamonsen LA, Hampton AL, Clements JA and Findlay JK (1991) Regulation of gene expression and cellular localization of prostaglandin synthase by oestrogen and progesterone in the ovine uterus *Journal of Reproduction and Fertility* **92** 393–406
- Silvia WJ, Lewis GS, McCracken JA, Thatcher WW and Wilson I (1991) Hormonal regulation of uterine secretion of PGF_{2α} during luteolysis in ruminants *Biology of Reproduction* **45** 655–663
- Sheldrick EL and Flint APF (1985) Endocrine control of uterine oxytocin receptors in the ewe *Journal of Endocrinology* **106** 249–258
- Vallet JL and Lamming GE (1991) Ovine conceptus secretory proteins and bovine recombinant interferon α₁-1 decrease endometrial oxytocin receptor concentrations in cyclic and progesterone-treated ovariectomized ewes *Journal of Endocrinology* **131** 475–482
- Vallet JL, Lamming GE and Batten M (1990) Control of endometrial oxytocin receptor and uterine response to oxytocin by progesterone and oestradiol *Journal of Reproduction and Fertility* **90** 625–634