Effect of continuous infusion of oxytocin on length of the oestrous cycle and luteolysis in cattle*

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Summary. In Exp. I oxytocin (60 µg/100 kg/day) was infused into the jugular vein of 3 heifers on Days 14–22, 15–18 and 16–19 of the oestrous cycle respectively. In Exp. II 5 heifers were infused with 12 µg oxytocin/100 kg/day from Day 15 of the oestrous cycle until clear signs of oestrus. Blood samples were taken from the contralateral jugular vein at 2-h intervals from the start of the infusion. The oestrous cycle before and after treatment served as the controls for each animal. Blood samples were taken less frequently during the control cycles. In Exp. III 3 heifers were infused with 12 µg oxytocin/100 kg/day for 50 h before expected oestrus and slaughtered 30–40 min after the end of infusion for determination of oxytocin receptor amounts in the endometrium. Three other heifers slaughtered at the same days of the cycle served as controls.

Peripheral concentrations of oxytocin during infusion ranged between 155 and 641 pg/ml in Exp. I and 18 and 25 pg/ml in Exp. II. In 4 out of 8 heifers of Exps I and II, one high pulse of 15-keto-13,14-dihydro-prostaglandin F-2α (PGFM) appeared soon after the start of oxytocin infusion followed by some irregular pulses. The first PGFM pulse was accompanied by a transient (10–14 h) decrease of blood progesterone concentration. High regular pulses of PGFM in all heifers examined were measured between Days 17 and 19 during spontaneous luteolysis. No change in length of the oestrous cycle or secretion patterns of progesterone, PGFM and LH was observed. The number of oxytocin receptors in endometrium was not affected by oxytocin infusion around the time of oestrus. These results suggest that luteolytic events were not significantly influenced by a constant infusion of oxytocin.

Keywords: oxytocin; infusion; luteolysis; oestrous cycle length

Introduction

The best evidence that oxytocin may be involved in the events of luteolysis comes from the immunization against oxytocin of cyclic ewes (Sheldrick *et al.*, 1980; Schams *et al.*, 1983) and goats (Cooke & Homeida, 1985) in which luteal regression was delayed. Exogenous oxytocin is able to shorten the oestrous cycle when given between Days 3 and 6 of the oestrous cycle in cattle (Armstrong & Hansel, 1959; Hansel & Wagner, 1960). These inhibitory effects of exogenous oxytocin on the corpus luteum were absent in hysterectomized heifers. Surges of secretion of prostaglandin (PG) F-2α at the time of luteolysis occur simultaneously with those of oxytocin in the ewe (Flint & Sheldrick, 1983) and cow (Schams *et al.*, 1985). A potential source of oxytocin at luteal regression is the corpus luteum itself. Secretion is stimulated by PGF-2α analogues in the ewe (Flint & Sheldrick, 1983) and cow (Schallenberger *et al.*, 1984). Oxytocin is also able to stimulate secretion of PGF-2α

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around the time of luteolysis (Schams *et al.*, 1985). According to a proposed hypothesis for the ewe (McCracken *et al.*, 1984) it is assumed that pulsatile release of PGF-2 α at the time of luteolysis strictly depends on the temporal pulsatile release of oxytocin and down regulation of oxytocin receptor. Continuous intravenous infusion of oxytocin between Days 13 and 21 blocked the rise in uterine oxytocin receptor concentrations and prolonged length of the oestrous cycle in ewes (Flint & Sheldrick, 1985). The present work was undertaken to investigate whether exogenous oxytocin given before the start of luteolysis exerts effects in cows similar to those observed in ewes.

Materials and Methods

Animals and treatment. Heifers of the local Fleckvieh and Braunvieh breeds (550–700 kg body weight) were used. Cyclicity was monitored for at least two consecutive oestrous cycles by observation of symptoms of oestrus, measurement of progesterone at 2-day intervals and of LH in blood samples collected every 8 h at the time of oestrus. The day after the LH surge was designated as Day 1 of the oestrous cycle.

Surgical procedure and blood sampling. On the day before the start of oxytocin infusion the animals were tranquilized with 2.5–3 ml chlorpromazine (23.3 mg/ml Combelene, Bayer, Leverkusen, F.R.G.) given i.m. and medical grade polyvinyl catheters (1.0 mm i.d., 2.0 mm o.d.; Dural Plastics, Dural, NSW, Australia) were inserted into each external jugular vein. One catheter was used for infusion of oxytocin solution and the other for withdrawal of blood samples (5 ml) for hormone analysis. Blood samples were taken at 2-h intervals from the start of oxytocin infusion until 2 days after clear symptoms of oestrus. All blood samples were transferred immediately into centrifuge tubes containing 30 μ M-EDTA (ethylenediaminetetraacetic acid, disodium salt) and 1 mg aspirin, chilled in ice-water for at least 5 min. Then samples were centrifuged at 4°C (3000 g) and plasma was stored at –20°C until analysis.

Oxytocin infusion. Three experiments were performed. In Exp. I heifers (N = 3) were infused with 60 μ g oxytocin (synthetic, 200 i.u./ml: kindly provided by Sandoz, Basle, Switzerland)/100 kg body weight/day diluted in sterile saline (9 g NaCl/l) on Days 14–22, 15–18 or 16–19 of the oestrous cycle. During infusion the concentration of oxytocin in peripheral blood plasma was monitored every 8 h and was for each of the 3 animals 641 ± 99 , 233 ± 43 and 155 ± 6 pg/ml, respectively compared to 4.1–8.8 pg/ml before start of infusion. The concentrations of oxytocin during infusion in peripheral blood were many-fold higher than the highest ones observed during spontaneous episodes of release of oxytocin at luteolysis. Therefore, in Exp. II (N = 5) only 12 μ g oxytocin/100 kg body weight/day were infused from Day 15 until signs of oestrus. Oestrus was checked visually throughout the experiment and confirmed by measuring electrical resistance of vaginal mucus and later by determination of the LH surge. The flow rate of the oxytocin–saline solution was 50 ml/h. The oestrous cycles before and after treatment provided the controls for each animal. Blood samples were taken less frequently during the control cycles. In Exp. III (3 heifers) 12 μ g oxytocin/100 kg body weight/day were infused into the jugular vein for 50 h around the time of oestrus. At about 30 min after the end of the infusion heifers were slaughtered. Endometrium was collected and stored in liquid nitrogen until analysis of oxytocin receptor concentrations.

Oxytocin receptor assay. Concentration of oxytocin receptor was estimated according to a modified method of Nissenson *et al.* (1978). The frozen tissue was homogenized (Micro-Dismembrator[®]; Braun, Melsungen, F.R.G.) at –196°C by shaking the samples for 50 sec at maximal speed and then cooling them for 60 sec in liquid nitrogen. This procedure was repeated 5 times. Then the frozen powder was thawed, suspended in 10 mM-Tris–HCl buffer (pH 7.6) containing 1.5 mM-EDTA, 3 mM-DTT (dithiothreitol) plus 10% glycerine and kept at 0–4°C in the next steps. EDTA was added to remove oxytocin from the receptor (Pearlmutter & Soloff, 1979). DTT inactivates oxytocin by reducing its –S–S– bonds. The suspension of the homogenate was centrifuged at 3000 g for 20 min and the supernatant again at 280 000 g for 60 min. The 280 000 g pellet was resuspended in about 2 ml 10 mM-Tris–HCl buffer to a final protein concentration of 2–4 mg/ml and used for the receptor assay. Specific binding of oxytocin was measured by incubation of this suspension with 5 different (range 1–10 nM) concentrations of [³H]oxytocin (Amersham Buchler, Braunschweig, F.R.G.) in the absence or presence of a 100–2000-fold excess of unlabelled oxytocin. For the assay procedure, 50 μ l of pellet suspension (100 μ g protein) were combined with 50 μ l 45 mM-Tris–HCl, 15 mM-MnCl₂, pH 7.6 containing a variable amount of [³H]oxytocin (3–30 nM) and then 50 μ l of 45 mM-Tris–HCl (pH 7.6 with or without unlabelled oxytocin (3 μ M) were added. Incubation was performed for 60 min at 20°C and terminated by addition of 3 ml ice-cold Tris–HCl buffer followed immediately by rapid filtration (under vacuum) through a 0.2 μ m cellulose acetate filter (Sartorius, Göttingen, F.R.G.). The test tubes and filter were rinsed twice with ice-cold buffer. The filters were placed into scintillation counting tubes, 12 ml scintillation fluid (Rialuma[®] Baker Chemicals, Phillipsburg, NJ, U.S.A.) were added and radioactivity was measured. Concentration of oxytocin receptor was determined by means of Scatchard analysis (1949). Within the calculation, values were corrected for non-specific binding and expressed in terms of fmol/mg protein. Sensitivity of the assay was 130 fmol/mg protein. Inter- and intraassay coefficients of variation for the assay were 13.2 and 11.5%, respectively.

Hormone analyses. PGFM was measured in a direct RIA using highly specific antibodies kindly donated by Dr W. Schlegel (Universitätsfrauenklinik, Münster, F.R.G.). These antibodies showed a slight cross-reaction with PGEM (1.4%), PGF-2 α (0.8%), PGE-2 and PGAM (0.2%) and <0.1% with 6-keto-PGF-1 α , PGA-2 and PGE.

Samples of plasma (50 µl) were assayed in duplicate. PGFM standard was prepared in bovine plasma. The intra- and interassay coefficients of variation (CV) for control samples were 12.3 and 19%, respectively. The limit of sensitivity was 80–200 fmol/ml.

LH was determined according to the method of Schams & Karg (1969) using a specific antibody against bovine LH. Sensitivity of the procedure was 0.4 ng/ml. Interassay CV was in the range 11.5–18.6% and the intra-assay CV was 5.6–8.1%, respectively.

The progesterone assay (Hoffman *et al.*, 1973) was modified to a direct test (unextracted plasma) using 25 µl blood plasma. Standard was prepared in progesterone-free bovine plasma. Sensitivity of the assay was 1 pmol/ml. Interassay CV was in the range between 11 and 16.9%.

Oxytocin was analysed in the plasma samples taken every 8 h by RIA according to the method described by Schams (1983). It was extracted and concentrated from 0.5 ml plasma with SEP-PAK C₁₈ cartridges (Waters Assoc., Milford, MA, U.S.A.), using tetrahydrofuran for elution. Sensitivity of the procedure was 0.3 pg/ml. The interassay CV for control samples was in the range of 7.6–19% and the intra-assay CV was 6.2–6.5%.

Statistical analysis. Differences between means were tested for significance by Student's *t* test.

Table 1. Length of the oestrous cycle in heifers before, during and after treatment with oxytocin in Exps I and II

Exp.	Heifer no.	Length of oestrous cycle (days)		
		Before treatment	During treatment	After treatment
I	111	18	19	19
	112	20	22	20
	113	21	23	20
	Mean ± s.d.	19.7 ± 1.5	21.3 ± 2.0	19.7 ± 0.6
II	11	19	21	20
	12	20	20	20
	13	20	20	20
	24	24	25	24
	25	20	20	20
	Mean ± s.d.	20.8 ± 2.2	21.2 ± 2.2	20.8 ± 1.8

Results

Concentrations of oxytocin increased in Exp. I from 4.1–8.8 pg/ml (obtained from 3 control samples taken from each animal before start of infusion) to 155–641 pg/ml and in Exp. II from 3–5.3 to 16–24 pg/ml during oxytocin infusion. Neither dose of oxytocin had any influence on the length of the oestrous cycle if compared with control cycles (Table 1). Examples for the effect of oxytocin infusion on progesterone, PGFM and LH blood concentrations are given in Fig. 1 (Exp. I) and Fig. 2 (Exp. II). In both experiments luteolysis occurred between Days 17 and 18 of the cycle, as in the control cycles, except in Heifer 111 (Exp. I) which always had a shorter cycle and in which luteolysis was recorded between Days 16 and 17, and Heifer 11 (Exp. II), in which luteolysis was delayed by 1 day when compared with the next cycle. In 4 out of 8 heifers of Exps I and II, the start of oxytocin infusion was followed by a high episode of PGFM followed by some irregular smaller episodes. The first increase of PGFM caused a temporal decrease of progesterone concentrations. After 14–18 h, however, progesterone concentrations in blood recovered (Fig. 2). In the other 4 heifers no or only very small episodes of PGFM were measured with no visible decrease of progesterone concentrations. In 7 heifers spontaneous clear episodes of PGFM occurred at the normal time of luteolysis. The length of the oestrous cycle was not affected by the infusion (Table 1). In Heifer 111 (Exp. I), in which infusion of oxytocin started at the beginning of luteolysis (according to the progesterone values), the first surge of PGFM was followed by others until luteolysis was completed.

Fig. 1. Profiles of progesterone, PGFM and LH concentrations measured in peripheral blood at 2-h intervals during i.v. infusion of oxytocin (Exp. 1, 60 µg/100 kg body wt/day) of 2 heifers. Horizontal bar illustrates time of oxytocin infusion.

Fig. 2. Concentrations of progesterone, PGFM and LH in peripheral blood samples taken at 2-h intervals during i.v. infusion of oxytocin ($12 \mu\text{g}/100 \text{ kg body wt/day}$) from Day 15 until clear signs of oestrus. Horizontal bar represents time of oxytocin infusion.

The effect of a 50-h infusion of oxytocin around oestrus on receptor concentrations of oxytocin in endometrium (Exp. III) is shown in Table 2. Although statistical analysis for concentration of oxytocin receptor was not performed due to the limited number of animals used in this study, there is clear evidence that infusion of oxytocin did not down regulate concentration of its own receptor.

Discussion

Continuous infusion of oxytocin in cattle beginning at least 2 or 3 days before the start of normal luteolysis did not influence length of the oestrous cycle when compared with control cycles. Hansel & Wagner (1960) also show that oxytocin (single injection of a high dose) given on Days 15–22 had no effect on cycle length. Treatment did not affect the pulsatile release of PGF- 2α at the time of spontaneous luteolysis. Milvae & Hansel (1980) found prolonged increases in uterine venous blood after oxytocin treatment during Days 4–6, but no increases in concurrently collected ovarian arterial concentration. Thus, stage of the cycle appears to influence the results obtained. In one animal PGFM seemed to be suppressed for 1 day, but this delay is within the physiological variation.

Table 2. Concentration of oxytocin receptor in bovine endometrium of untreated and oxytocin-infused heifers (12 µg/100 kg body weight/day) for 50 h before slaughter

	Day of oestrous cycle	Oxytocin receptor	
		fmol/mg protein	K _D (nM)
Untreated	21	1576	1.74
	21	2320	1.54
	1	950	1.30
Treated	21	1986	1.35
	21	1408	1.38
	2	660	1.27

These results are in contrast to those obtained for sheep in which infusion of oxytocin beginning about 1 day before luteolysis (Day 13) delayed return to oestrus and blocked the rise of uterine oxytocin receptor concentrations (Flint & Sheldrick, 1985). A temporal down regulation of endometrial oxytocin receptors for the pulsatile secretion of PGF-2α in ewes was assumed by McCracken *et al.* (1984). However, Sheldrick & Flint (1986) have shown that, once formed ovine endometrial oxytocin receptors are not down-regulated by exogenous oxytocin. In this respect, as shown in Exp. III, there appear to be distinct similarities between sheep and cows. From our experiments it is difficult to know whether receptors were present at the beginning of the infusion at Day 15. From another experiment in which heifers were slaughtered at defined days of the oestrous cycle, concentrations of endometrial oxytocin receptor decreased continuously from Day 1 to Days 6–7, remained undetectable during the luteal phase and increased again on Days 17–18 at the time of luteolysis and reached a maximum at oestrus (Schams, 1987). We cannot exclude from that experiment that a few receptors (< 130 fmol/mg protein) formed before Days 17–18. The response of PGFM at the beginning of Exps I and II in 4 animals would allow such an assumption. The non-responding heifers at least would indicate that oxytocin receptors were not formed on Day 15. Episodic release of ovarian oxytocin at 12-h intervals stimulated by a 20-min infusion of PGE-2 on Days 13 and 14 of the oestrous cycle (N = 5 heifers) did not increase sensitivity of the endometrium. Peripheral oxytocin concentrations increased up to 70 pg/ml blood plasma but no response of PGF-2α was measured (D. Schams & J. Kotwica, unpublished data). We assume that down regulation of endometrial oxytocin receptors is not involved during the events of luteolysis in cattle. Another possibility is uterine refractoriness followed after administration of oxytocin as shown in progestagen + oestradiol-primed ovariectomized ewes (Sheldrick & Flint, 1986) in which refractoriness appeared to be due to mechanisms other than the down regulation of the oxytocin receptor.

If such a refractoriness exists in cattle it seems not to be influenced by a constant infusion of oxytocin. The results could indirectly support the hypothesis that endometrial oxytocin receptor during the oestrous cycle is mainly regulated by the ratio of progesterone and oestrogens (Nissenson *et al.*, 1978; Soloff *et al.*, 1983; Fuchs *et al.*, 1983) and the results indicate that, for the release of PGF-2α, ovarian oxytocin is not absolutely necessary in cattle as it seems to be in ewes. Even in sheep the situation is not yet clear. Pulsatile surges of oxytocin at the time of luteolysis appeared a few minutes after PGF-2α pulses (Moore *et al.*, 1986). A pulsatile release of PGFM was observed in ovariectomized ewes treated with oestrogen (Lye *et al.*, 1983). Therefore, if the signal for uterine PGF-2α release comes from the ovary, factors other than oxytocin, perhaps oestrogens, seem to fulfil this function. Extension of luteal function after destruction of ovarian follicles on Days 9, 12 or 15 after oestrus in cattle (Villa-Godoy *et al.*, 1985) would support such a supposition.

However, this assumption implies that the endometrium at that time is oestrogen sensitive. Oestrogen receptor accumulation may be blocked by progesterone as shown in rats (Okulicz, *et al.*, 1981) and so the action of progesterone on the uterus should be at first diminished, probably by inducing loss of its own receptor (McCracken *et al.*, 1984). On the other hand data reviewed by Kindahl *et al.* (1979) indicate that progesterone secretion at the end of the oestrous cycle in cattle may be necessary for pulsatile release of PGF-2 α . This is also supported by the fact that PGF-2 α release continues until progesterone is reduced to basal levels (Figs 1 & 2). Similar results were obtained with untreated animals (Schallenberger *et al.*, 1986). It is also possible that a signal for release of PGF-2 α and initiation of luteolysis originates from the uterus itself. Shortening of the oestrous cycle in sheep (Woody *et al.*, 1967) and cattle (Ginther, 1970) by progesterone injections in the first days of oestrous cycle may support this assumption, indicating that the time of progesterone dominance during the oestrous cycle may be restricted. In the present study, in 4 out of 8 heifers a distinct but single PGFM peak was observed 2 h after the start of oxytocin infusion. Possibly this increase of PGFM can only be observed by giving oxytocin in pulses of short duration, because it was also observed after a single intramuscular injection of high concentrations of oxytocin (Betteridge *et al.*, 1984; Lafrance & Goff, 1985) or intravenous application of a moderate dose (Schams *et al.*, 1985). However, it cannot be excluded that PGF-2 α might be released from the ovary (Milvae & Hansel, 1983) as in in-vitro experiments.

From our results with cows we can conclude that a constant infusion of oxytocin beginning about 2 days before start of spontaneous luteolysis does not influence the length of the oestrous cycle and episodic secretion of PGFM at luteolysis. The participation of ovarian oxytocin in the events of luteolysis remain to be resolved. A permissive action of oxytocin on luteolysis seems to be more likely than a direct one.

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