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Luteal peptides and intercellular communication

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Summary. The variety of peptides synthesized by the corpus luteum (relaxin, vasopressin, oxytocin and oxytocin-related neurophysin) and their possible intracellular effects are reviewed. After luteinization of the granulosa cells and in response to LH and FSH, the output of oxytocin is increased. In addition, insulin-like growth factor is a very potent stimulus of oxytocin secretion. Although luteal cells respond to gonadotrophins by increased production of progesterone, there is no further secretion of oxytocin. Oxytocin is localized in large luteal cells which seem not to be under the direct control of gonadotrophins. Synthesis of luteal oxytocin seems to occur during the early luteal phase according to measurements of oxytocin mRNA. Highest tissue concentrations and secretion under in-vitro conditions were observed during the mid-luteal phase, and so synthesis, storage and secretion are unlikely to occur concomitantly.

Under in-vitro conditions, oxytocin is secreted concomitantly with neurophysin and progesterone, and there appears to be some form of communication between small and large luteal cells for the secretion of progesterone and oxytocin under in-vivo conditions.

Evidence has been obtained that oxytocin may have local effects in the ovary by inhibition of secretion (synthesis ?) of progesterone, especially during the early luteal phase. A mechanism can be suggested whereby, under physiological conditions, oxytocin may delay the increase of progesterone by inhibition of progesterone secretion and therefore delay down regulation of its own receptor. This would prolong the life-span of the CL and the oestrous cycle. Exogenous progesterone given on Days 1–4 shortens the cycle to about 12 days. The best evidence that oxytocin may be involved in controlling luteolysis comes from immunization experiments in ewes and goats, but there is no clear evidence of this type for cattle. Basal concentrations of oxytocin at the end of the luteal phase may interact with oxytocin receptors after the inhibitory effect of progesterone in the uterus is reduced, thus initiating synthesis of PGF-2α.

Introduction

The corpus luteum (CL) occupies a central position in the reproductive process of all mammals. Progesterone is the primary endocrine secretory product, although the CL also secretes prostaglandins and, in some species, oestradiol-17β, and a variety of protein and peptide hormones. The first peptide found in luteal tissue of pigs was relaxin (for review see Bryant-Greenwood, 1982). The primary structure of pig relaxin consists of two peptide chains (A and B) of 22 and 31 amino acids, respectively, covalently linked by two interchain disulphide bonds with an intradisulphide link in the A chain (M_r 6000). Oxytocin and vasopressin have been identified in luteal tissue from sheep (Wathes & Swann, 1982), women (Wathes *et al.*, 1982) and cows (Wathes *et al.*, 1983a). This paper is concerned primarily with luteal peptides in domestic ruminants. It focusses on their synthesis, storage, secretion and possible intercellular function.

Relaxin

Relaxin classically causes uterine quiescence during pregnancy and cervical dilatation before parturition. It may have also some activities during mammary gland development. The common denominator of these activities is its action on the remodelling of connective tissue. There are suggestions that relaxin is a local intrauterine hormone in some species, e.g. woman, and may have some effects during follicular growth and rupture. Our knowledge of relaxin in ruminants is rather limited due to the lack of specific and sensitive assays. Large luteal cells from cows in the middle third of pregnancy stained positively for relaxin with the immunoperoxidase method (Fields *et al.*, 1980). Relaxin has also been isolated from CL of late pregnant cows (Fields *et al.*, 1982).

Relaxin has not been detected immunocytochemically by light or electron microscopy in luteal tissue at the end of pregnancy in sheep. It appears that quantities, if any, are very low (Renegar & Larkin, 1985). Pig relaxin given into the cervical os or by intramuscular injection to beef heifers, beginning 4 days before expected parturition, induced significant dilatation of the cervix 8 and 16 h later and increased growth rate of the pelvic area (Perezgrovas & Anderson, 1982).

Vasopressin

Vasopressin has been extracted from CL of non-pregnant cows (Wathes *et al.*, 1983a, 1984). Concentrations (pg range/g wet weight) were highest during the mid-luteal phase, declined thereafter and were undetectable during pregnancy, following a trend similar to that of oxytocin. Ivell & Richter (1984) found 1000 times less vasopressin mRNA than oxytocin mRNA in luteal tissue.

Oxytocin and neurophysin I

Tissue concentrations

Although evidence for oxytocin in the CL of the goat was published as long ago as 1910 (Ott & Scott, 1910), it is only in the past 4 years that its presence has been confirmed in sheep (Wathes & Swann, 1982; Flint & Sheldrick, 1983a), women (Wathes *et al.*, 1983b) and cows (Wathes *et al.*, 1983a, 1984; Fields *et al.*, 1983; Schams *et al.*, 1984, 1985a, 1987). The material extracted displaced the tracer parallel to oxytocin in a radioimmunoassay, stimulated uterine contractions and eluted at the same position as oxytocin when tested by high-performance liquid chromatography (HPLC). Final confirmation was obtained by purification of oxytocin-like material from sheep CL and identification by sequence analysis (Watkins *et al.*, 1985). Further evidence was given by demonstration that the oxytocin gene is highly transcribed in the cow CL during the mid-luteal phase of the oestrous cycle. The active CL produced up to 250 times more oxytocin mRNA than did a single hypothalamus (Ivell & Richter, 1984). More detailed studies indicated that transcription is maximal at the time of ovulation and decreases rapidly thereafter (Ivell *et al.*, 1985; Schams *et al.*, 1987). Swann *et al.* (1984) measured incorporation of [^{35}S]cysteine into oxytocin and neurophysin I in dispersed cell cultures of CL from cows and sheep.

There is general agreement that oxytocin concentrations in tissue increase up to the mid-luteal phase and decrease thereafter, remaining low after luteal regression and during pregnancy. A positive correlation was found between oxytocin and progesterone until the mid-luteal phase, but not during the late luteal phase. There seem to be breed differences for luteal oxytocin. Maximal content ranged from 0·4 to 1·8 μg/g CL in cows and was about 2 μg/g CL in sheep. Levels of neurophysin I in cows were also maximal during the mid-luteal phase and declined afterwards to low values during pregnancy.

In-vivo studies

Secretion of luteal oxytocin and neurophysin I during the oestrous cycle

Sheep. Measurements of oxytocin during the oestrous cycle of the ewe show that the circulating levels increase and decrease synchronously with changes in progesterone concentration, declining to a minimum at oestrus (Sheldrick & Flint, 1981; Webb *et al.*, 1981; Mitchell *et al.*, 1982; Schams *et al.*, 1982; Flint & Sheldrick, 1983a). Oxytocin concentrations plateau about 2 days earlier than do progesterone values. After ovariectomy of cyclic ewes, circulating oxytocin concentrations fell to the limit of detection of the assay (Schams *et al.*, 1982). Intermittent pulses of the metabolite of prostaglandin (PG) F-2α and neurophysin I/II were measured concomitantly in blood during the period of luteal regression (Fairclough *et al.*, 1980).

Cow. Circulating oxytocin concentration during the oestrous cycle are lower than in the ewe but follow the same trend, with the highest values found in the early and mid-luteal phases and again with a short increase at the time of luteolysis (Schams, 1983; Schams *et al.*, 1985b). More detailed studies after frequent bleeding at different stages of the oestrous cycle and comparison of concentrations of oxytocin and neurophysin I in the posterior vena cava and jugular vein showed that concentrations of oxytocin were similar in both vessels during the non-luteal phase but were higher in the vena cava during the luteal phase, indicating the ovarian origin of oxytocin. Oxytocin was secreted in a pulsatile manner concomitantly with progesterone and neurophysin I, and concentrations of oxytocin also increased concomitantly with these two hormones. Oxytocin concentrations increased parallel with surges of PGF-2α at the time of luteolysis (Walters *et al.*, 1984; Walters & Schallenberger, 1984; Schallenberger *et al.*, 1984; Schams *et al.*, 1985b, c).

The resumption of oxytocin and progesterone secretion during the first luteal phase after parturition in intact as well as hysterectomized heifers shows that oxytocin concentrations increased faster than those of progesterone during the early luteal phase. In hysterectomized animals oxytocin decreased after the first 2 weeks of the luteal phase and remained at low concentrations whereas progesterone values remained high (Schams *et al.*, 1985b).

Goat. A similar pattern of ovarian oxytocin secretion probably occurs in the goat, in which circulating oxytocin concentrations decline from >40 to <10 pg/ml between Days 12 and 16 in non-pregnant and pregnant animals (Homeida & Cooke, 1983) and become undetectable after ovariectomy (Cooke *et al.*, 1984).

Stimulation of luteal oxytocin secretion

Prostaglandins. Injections of cloprostenol, PGF-2α or PGE-2 caused a significant increase of oxytocin secretion in cows (Schallenberger *et al.*, 1984; Schams *et al.*, 1985b) and ewes (Flint & Sheldrick, 1982, 1983b; Watkins *et al.*, 1984). The stimulation of oxytocin release by prostaglandin injection depends on the presence of the ovary and a functional CL. A persistent CL 53–83 days after hysterectomy (performed on Days 6–7 of the cycle) was not able to secrete oxytocin after an injection of cloprostenol (Sheldrick & Flint, 1983). However, a 35-day persistent CL of a hysterectomized heifer released some oxytocin after a prostaglandin challenge, even when unstimulated basal secretion had ceased for weeks. The posterior pituitary seems to release only minute amounts of oxytocin after the injection of prostaglandins.

Gonadotrophins. After infusion of a crude FSH preparation (10 mg) or i.v. injection of 100 i.u. hCG, a simultaneous release of progesterone and oxytocin was observed (Schams *et al.*, 1985b). A high correlation between the number of CL and the increase in oxytocin and immunoreactive neurophysin I was found after stimulation of superovulation with PMSG or pituitary FSH in heifers. Even when PMSG exerted a more pronounced luteotrophic effect on progesterone secretion than did FSH, there was no further stimulation of oxytocin or neurophysin I secretion (Schams *et al.*, 1985c).

In-vitro studies

Follicular oxytocin

There is evidence that the follicle is able to secrete small amounts of oxytocin. Immunoreactive oxytocin has been measured in cow follicular fluid. Concentrations (on average 48–108 pg/ml) increased significantly with the size of the follicle and were significantly higher in histologically verified cysts (190 pg/ml). After culture of follicles the amount of oxytocin released into the medium increased, indicating de-novo synthesis. The granulosa cells were the main source of follicular oxytocin. Secretion increased during luteinization, indicating that luteinization is an important step for production of oxytocin in ovaries (Schams *et al.*, 1985a). Geenen *et al.* (1985) obtained similar results and further demonstrated the production of neurophysin I by bovine granulosa cells. The production of immunoreactive oxytocin was significantly greater in cultures of granulosa cells harvested from large follicles than in those derived from small follicles, but immunoreactive oxytocin was demonstrated immunocytochemically in the granulosa cells of small and large follicles (Kruip *et al.*, 1985). Low levels of oxytocin mRNA were detected in bovine follicles. Contrary to the previous results, Jungclas & Luck (1986) could not measure oxytocin in follicular fluid. They agree that luteinization is the main stimulus for secretion of oxytocin.

To clarify the role of oxytocin for ovarian function, in-vitro studies were undertaken using bovine granulosa and luteal cells. The aim was to find out what stimulates oxytocin, and whether the stimuli effective for progesterone secretion were also operative. Some of the results have been published by Schams *et al.* (1987). Ovaries were collected from the slaughter house.

Stimulation was performed with 10 and 100 ng/ml medium of highly purified sheep LH and FSH (kindly supplied by Dr O. D. Sherwood, U.S.A.).

The effect of growth factors was tested additionally in granulosa cells. IGF-I (somatomedin-C) was kindly supplied by Dr C. H. Li (U.S.A.). Fibroblast growth factor (IGF-II) and nerve cell growth factor were purchased from Sigma, St Louis, MO, U.S.A.

Culture of granulosa cells

Concentrations of progesterone increased in the medium during culture of bovine granulosa cells. LH and FSH exhibited a clear stimulating effect with a 2–8-fold increase over controls. The effect of FSH was more pronounced, especially after 120 h of incubation. Concentrations of oxytocin increased in controls only at the end of incubation (120 h). FSH was clearly more effective especially after 120 h (Fig. 1). FSH stimulated over a 10-fold increase in the output of oxytocin as compared to controls (Table 1). Increase of oestradiol-17β synthesis by addition of aromatase substrate alone (40 ng androstendione) or in combination with FSH was uneffective.

As shown in Fig. 2 insulin-like growth factor-I increased concentrations of progesterone and oxytocin in a dose-dependent manner to values higher than with FSH. IGF + FSH gave no additional effect. The other growth factors used exerted no effect on secretion of progesterone or oxytocin.

Luteal cells

In controls, concentrations of progesterone increased in the medium during incubation of cells obtained from CL of cows at different stages of the oestrous cycle. FSH, and particularly LH, stimulated a further output of progesterone (Table 1). However, although the concentration of oxytocin in the medium increased during incubation no stimulating effect of LH or FSH was observed. In an experiment in which CL from heifers were collected at specific times of the oestrous cycle (Days 3–4, 6–7, 10–12, 15–17 and 19–21), no significant difference was observed. Luteal tissue concentrations of oxytocin mRNA, immunoreactive oxytocin and progesterone were also

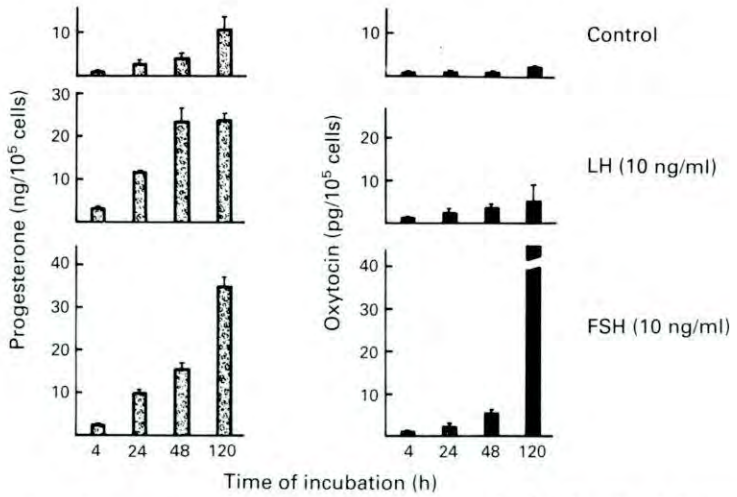

Fig. 1. Secretion of progesterone and oxytocin (mean ± s.d., 4 dishes/dose) during culture of bovine granulosa cells without and with stimulation of gonadotrophins (M 199 + 0·5% BSA). (Modified from Schams *et al.*, 1987.)

Table 1. Effect of stimulation with gonadotrophins (10 ng/ml), expressed as a factor over controls, on hormone secretion by cultured granulosa (120 h) and luteal (4 h) cells of cows

	Progesterone		Oxytocin	
Cell type	LH	FSH	LH	FSH
Granulosa	3·0 ± 1·5 (13)	6·3 ± 3·3 (13)	4·0 ± 3·3 (11)	10·0 ± 10·8 (11)
Luteal	2·2 ± 1·5 (24)	1·4 ± 0·3 (24)	1·0 ± 0·1 (25)	−1·0 ± −0·2 (25)

Values are mean ± s.d. for the no. of experiments indicated in parentheses.

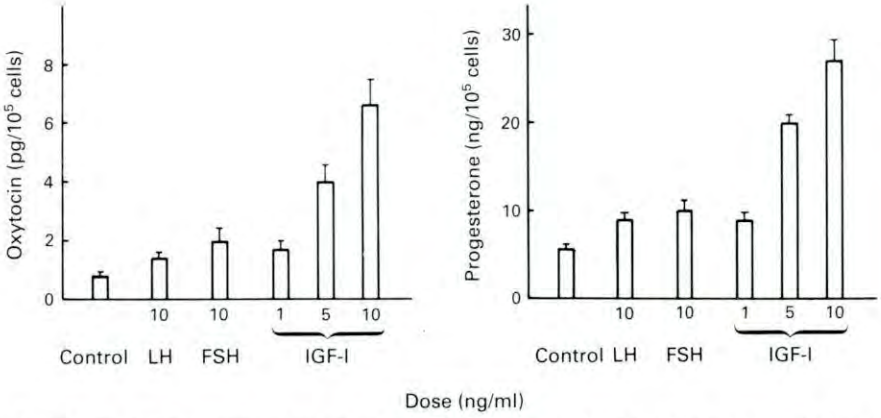

Fig. 2. Culture of bovine granulosa cells in M 199 + 0·5% BSA for 120 h. Effects of LH, FSH and insulin-like growth factor (IGF-I) on secretion of oxytocin and progesterone. Values are mean ± s.d., 4 dishes/dose.

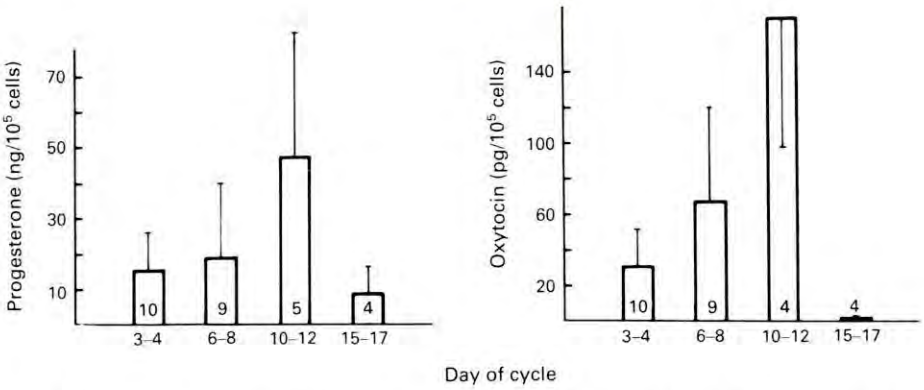

Fig. 3. Secretion of progesterone and oxytocin after incubation (4 h) of bovine luteal cells at different stages of the oestrous cycle. Values are mean ± s.d. for the no. of observations indicated.

measured. Highest oxytocin mRNA levels were found on Day 1 or Days 3–4 with a marked decrease afterwards. In contrast, immunoreactive oxytocin content was highest during Days 10–12 and decreased thereafter. Progesterone content followed the same trend, but was still high on Days 15–17 (Schams *et al.*, 1987). The secretion rate of oxytocin from unstimulated luteal cells showed a similar pattern (Fig. 3).

Conclusions

The in-vitro data clearly demonstrate that luteinization of granulosa cells seems to be the main stimulus for the secretion and, most probably, the synthesis of ovarian oxytocin. This is an agreement with data from Jungclas & Luck (1986), who also found an intercellular communication between theca and granulosa cells. Oxytocin but not progesterone output could be consistently increased by addition of pieces of theca interna tissue, or theca-conditioned medium, to the cultures. In cultured granulosa cells an increase of oxytocin mRNA could not be clearly measured (R. Ivell, unpublished observations). Both gonadotrophins have a stimulating effect, but FSH appears to play a dominating role. Insulin-like growth factor (IGF-I), acting as a potent stimulator of the production of progesterone and oxytocin of granulosa luteal cells is obviously also important. The mechanism involved remains uncertain. Receptors for insulin-like growth factor have been demonstrated in pig granulosa luteal cells (Barranao & Hammond, 1984; Veldhuis & Furlanetto, 1985). The tissue contents of defined luteal material confirmed earlier observations (Ivell *et al.*, 1985; Schams *et al.*, 1987). Oxytocin mRNA content suggests that most of the oxytocin must be synthesized during the early luteal phase and secreted at a high rate. The high oxytocin content during the mid-luteal phase is more likely to reflect storage than synthesis. In the CL of the non-pregnant ewe (Watkins, 1983; Sawyer *et al.*, 1986) and cow (Guldenaar *et al.*, 1984; Kruip *et al.*, 1985), specific staining for both oxytocin and neurophysin I has been demonstrated only in the large luteal cells, which are known to contain secretory granules (Gemmell *et al.*, 1977; Parry *et al.*, 1980). The exocytosis of these granules accelerates during the mid-luteal phase (Gemmell *et al.*, 1977; Gemmell & Stacy, 1979; Quirk *et al.*, 1979). Rice & Thorburn (1985) showed that the oxytocin in ovine CL was associated with a particulate fraction, sedimenting at a density of 1054–1061 g/ml, which contained electron-dense granules with a diameter of 200–250 nm. Theodosis *et al.* (1986) have shown specific staining of similar granules with antisera to oxytocin and neurophysin, using an immunogold labelling technique. Large cells from sheep CL are most active in oxytocin synthesis (Rodgers *et al.*, 1983). The oxytocin tissue content correlates with the number of

large luteal cells in sheep (Schwall *et al.*, 1986). There was a 400% increase in the total number of cells between Days 4 and 8 but no change between Days 8 and 12, and a 70% decrease at Day 16. Alila & Hansel (1984) demonstrated that in the cow the large luteal cells initially develop from the granulosa layer but are soon replaced by the growth of theca-derived cells. Granulosa-derived cells disappear during early pregnancy, while cells of thecal origin persist throughout pregnancy. Large cells from sheep CL contained more receptors for prostaglandins, while the small cells contained more receptors for LH (Fitz *et al.*, 1982). The lack of LH and possibly FSH receptors in large cells may explain why secretion of oxytocin could no longer be stimulated with gonadotrophins. The secretion of oxytocin from large cells seems not to be under the direct control of gonadotrophins. In in-vitro studies using slices of bovine CL, adding prostaglandins or blocking their synthesis by indomethacin did not increase or decrease secretion of oxytocin.

Intra-ovarian effects of oxytocin on steroidogenesis

The first evidence for a direct action of oxytocin on steroidogenesis was obtained by Tan *et al.* (1982a, b; Tan & Biggs, 1984), using short-term incubations of dispersed luteal cells from cows and women. In both cases there was a tendency for lower doses of oxytocin (4–40 mU/ml) to stimulate progesterone production whereas higher doses (>400 mU/ml) inhibited both basal and hCG-stimulated progesterone release. However, Richardson & Masson (1985) were unable to demonstrate such an effect with human luteal cells and Mukhopadhyay *et al.* (1984) also found no effect of a range of oxytocin doses from 10^{-8} to 4×10^{-6} M on basal of hCG-stimulated progesterone production by luteal cells from pseudopregnant rats. Oxytocin added to large and small luteal cell fractions obtained from fully developed sheep CL did not affect progesterone production by either fraction (Rodgers *et al.*, 1985).

We were not able to demonstrate an inhibitory effect on LH-stimulated progesterone production by granulosa cells undergoing luteinization of oxytocin in doses ranging from 0·1 to 100 ng/ml

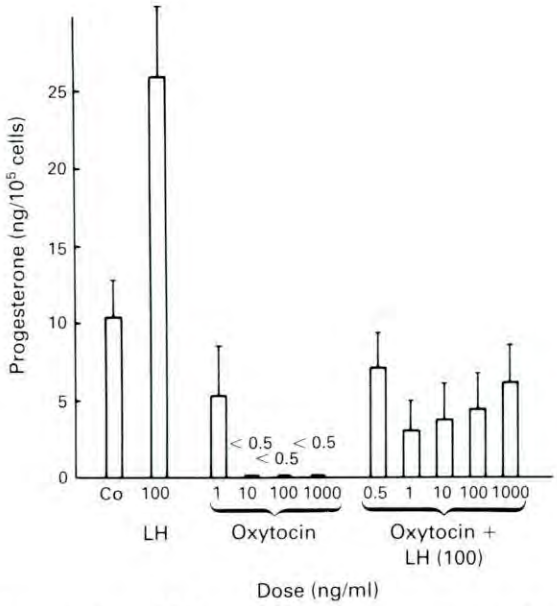

Fig. 4. Effect of LH, oxytocin and LH + oxytocin on the secretion of progesterone by cells obtained from the CL of cows at Days 4–5 of the cycle and incubated for 4 h. Values are mean ± s.d. for 4 dishes.

(Schams *et al.*, 1987). In preliminary experiments FSH-stimulated progesterone production by granulosa cells was depressed with 0·5 ng/ml oxytocin after culture for 120 h. In short-term incubations (4 h) of bovine luteal cells only doses of oxytocin from 1–1000 ng/ml showed a clear inhibitory effect, especially in CL obtained at Day 4–5 of the cycle. LH-stimulated progesterone production was depressed with as little as 0·5 ng oxytocin (see Fig. 4). The data indicate an inhibitory effect of oxytocin on progesterone secretion especially during the early luteal phase. It can be presumed that luteal cells have receptors for oxytocin, but this has not been confirmed by our preliminary experiments.

Luteolysis and ovarian oxytocin

Exogenous oxytocin

The luteolytic action of exogenous oxytocin was first described by Armstrong & Hansel (1959), who found that daily injections of 50–100 units oxytocin in heifers during the first 7 days of the oestrous cycle led to a significant decrease in oestrous cycle length. Hansel & Wagner (1960) reported that oxytocin was only luteolytic between Days 3 and 6 of the cycle. In the goat, oxytocin treatment on Days 3–6 of the cycle also led to a premature return to oestrus (Cooke & Knifton, 1981; Cooke & Homeida, 1982). Exogenous oxytocin, however, does not appear to cause luteal regression in ewes, although it may be followed by a slight depression in progesterone secretion (Milne, 1963; Hatjiminaoglou *et al.*, 1979).

Oxytocin treatment decreased the concentrations of progesterone in jugular venous blood on Day 8 and increased uterine venous PGF-2α concentrations in heifers (Milvae & Hansel, 1980; Oyedipe *et al.*, 1984).

An increase of PGF-2α in response to oxytocin was maximal on Day 3 of the cycle in heifers (Newcomb *et al.*, 1977). Exogenous oxytocin also stimulated the release of PGF-2α from the uterine endometrium in sheep (Roberts & McCracken, 1976) and goats (Cooke & Homeida, 1982). It appears that the luteolytic action of oxytocin is normally mediated by this response, since it can be prevented by hysterectomy in the cow (Armstrong & Hansel, 1959; Anderson *et al.*, 1965; Ginther *et al.*, 1967) and by simultaneous treatment with a PG synthetase inhibitor in the goat (Cooke & Knifton, 1981; Cooke & Homeida, 1983).

Immunization

The best evidence that oxytocin may be involved in controlling luteal regression comes from the active immunization of cyclic ewes against oxytocin (Sheldrick *et al.*, 1980; Schams *et al.*, 1983) and goats (Cooke & Homeida, 1985) in which luteal regression was delayed. However, passive immunization of heifers did not prolong the oestrous cycle (D. Schams, unpublished observations).

Interaction of oxytocin and uterine endometrium

Concentrations of endometrial oxytocin receptor change during the oestrous cycle, reaching a maximum at oestrus and declining to almost undetectable levels during the mid-luteal phase before increasing again on Days 14–15 of the cycle in ewes (Roberts *et al.*, 1976; Sheldrick & Flint, 1985a) or Days 18–19 of the cycle in cows (Schams *et al.*, 1987). Levels of the uterine oxytocin receptor are under the control of steroid hormones. Oestradiol induces and progesterone reduces the formation of receptors for oxytocin (Soloff, 1975; Nissenson *et al.*, 1978). As demonstrated in sheep (for review see McCracken *et al.*, 1984), oestradiol induces the formation of receptors for oxytocin in about 6 h. During the luteal phase, progesterone inhibits the action of oestradiol by blocking the nuclear accumulation of oestrogen receptor and hence the ability of oestradiol to maintain synthesis of oxytocin receptors. However, after about 10 days the action of progesterone in the

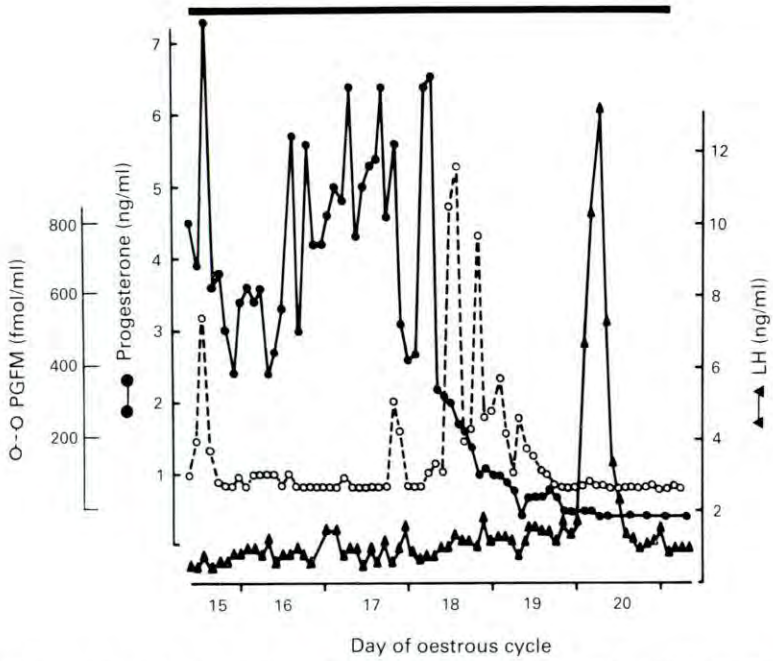

Fig. 5. Concentrations of progesterone, metabolite of PGF-2α (PGFM) and LH in jugular vein plasma during continuous infusion of oxytocin (12 μg/100 kg/day) in a heifer (peripheral oxytocin concentration 25–30 pg/ml plasma). Closed bar indicates time of infusion. Blood was sampled at 2-h intervals.

uterus begins to diminish (possibly due to progesterone-induced loss of its own receptor), and so oestradiol is able to induce synthesis of oxytocin receptors again and also permits oxytocin-induced secretion of PGF-2α. At this time, however, PGF-2α secretion in response to oxytocin is 100-fold greater than before the influence of progesterone. It is suggested that the massive discharge of PGF-2α from the uterus towards the end of the luteal phase is stimulated by oxytocin release, thereby initiating luteolysis. However, it has been shown by Moore *et al.* (1986) that concentrations of PGF-2α in utero-ovarian vein samples in ewes begin to increase before the concentrations of oxytocin and oxytocin-associated neurophysin. This suggests that uterine PGF-2α initiates the release of ovarian oxytocin and oxytocin-associated neurophysin pulses during luteolysis in ewes. This is consistent with the inhibition of pulsatile oxytocin-associated neurophysin release in ewes (Watkins *et al.*, 1984) and oxytocin release in goats (Cooke & Homeida, 1984) after systemic treatment with indomethacin. Pulsatile release of oxytocin was also absent in hysterectomized cows bearing a persistent CL (Schams *et al.*, 1985b). It appears therefore that basal concentrations of oxytocin interact with uterine oxytocin receptors, thus initiating PGF-2α release which induces further release of oxytocin and hence amplifies the release of PGF-2α from the uterus. Earlier suggestions indicate that the pulsatile secretion of PGF-2α may be due to down-regulation of the oxytocin receptor (McCracken *et al.*, 1984; Flint & Sheldrick, 1985). Constant infusion of oxytocin beginning on Day 13 caused prolongation of the oestrous cycle. However, we could not confirm these observations in cattle. Continuous infusion of oxytocin (12 μg/100 kg/day) starting on Day 15 did not prevent induction of oxytocin receptors or prevent luteal regression in cyclic heifers (J. Kotwica & D. Schams, unpublished observations; Fig. 5). Results recently obtained in ewes indicate that the pulsatile secretion of PGF-2α reflects a post-receptor failure of response, possibly due to depletion of prostaglandin precursors (Sheldrick & Flint, 1985b).

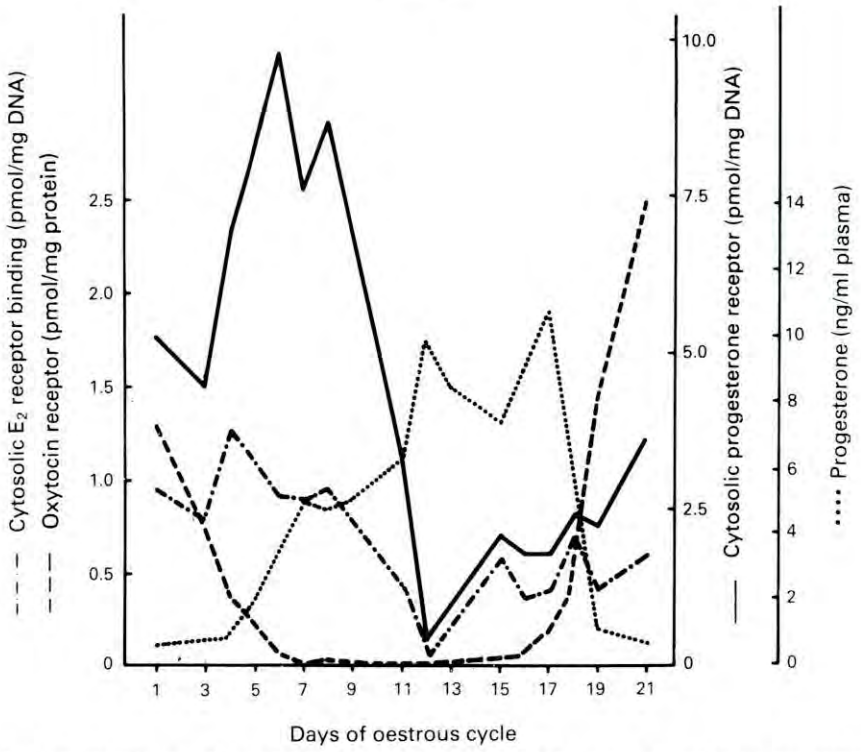

Fig. 6. Concentrations of bovine endometrial receptors for oxytocin, cytosolic progesterone and oestrogen and peripheral blood concentrations for progesterone. Heifers (4/group) were slaughtered at known stages of the oestrous cycle on Days 1–2, 3–4, 6–7, 10–12, 16–17, 18–19.

It may be that the relationships between oxytocin and PGF-2α during luteal regression are different in cattle and sheep. Our studies in heifers (T. Mittermeier, H. H. D. Meyer & D. Schams, unpublished data) give results that are comparable with those in sheep in that initially progesterone may depress the concentrations of receptors for oxytocin, oestrogen and eventually its own receptor. Afterwards, the action of progesterone in the uterus begins to diminish, and so oestradiol may be able to stimulate synthesis of oxytocin receptors and secretion of PGF-2α. The profile of receptors for oxytocin, cytosolic progesterone and oestrogen in the endometrium of heifers slaughtered at defined times (N = 4 on Days 3–4, 6–7, 10–12, 16–17, 18–19 and 21) of the oestrous cycle are given in Fig. 6. The responsiveness of the endometrium in heifers to oxytocin varies with the stage of the oestrous cycle. Experiments with incubated minced endometrial tissue obtained from heifers indicate that basal secretion is lowest during the mid-luteal phase and increases at the time of luteolysis, with the highest sensitivity at oestrus. Exogenous oxytocin stimulated PGF-2α only after luteolysis, especially at oestrus after long-term incubation for 24 h (J. Kotwica, D. Schams & H. H. D. Meyer, unpublished observations).

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