Concentrations of oxytocin in the intercavernous sinus of mares during luteolysis: temporal relationship with concentrations of 13,14-dihydro-15-keto-prostaglandin F_{2α}

D. K. Vanderwall^{1*}, W. J. Silvia^{1†} and B. P. Fitzgerald²

¹Departments of Animal Sciences and ²Veterinary Sciences, University of Kentucky, Lexington, KY 40546-0215, USA

The reproductive tracts of nine thoroughbred mares were examined by ultrasound to determine the day of ovulation (day 0). Mares were fitted with intercavernous sinus cannulae on the day before the start of sample collection of pituitary venous effluent rich in oxytocin. Intercavernous sinus blood samples were collected for at least 36 h at 5 min intervals beginning at noon on day 13 ($n = 2$), day 15 ($n = 5$) or day 16 ($n = 2$) after ovulation. Concentrations of oxytocin and 13,14-dihydro-15-keto prostaglandin F_{2α} (PGFM) in plasma were determined by radioimmunoassay. Three high-magnitude surges of PGFM ($> 1 \text{ ng ml}^{-1}$) were found in these samples. Three high magnitude pulses of oxytocin ($> 200 \text{ pg ml}^{-1}$) were also observed, one associated with each of the PGFM surges. In each of these cases, the oxytocin pulse appeared to follow or coincide with the onset of the PGFM surge. Lower magnitude pulses of both hormones were detected throughout the bleeding period in every mare. The average interval between these pulses was 122.3 min for oxytocin and 121.0 min for PGFM. The interval between pulses for individual mares varied from 90 to 199 min for oxytocin, and from 87 to 213 min for PGFM. However, there was no correlation between PGFM and oxytocin pulse intervals among mares. Within each mare, there was no discernable association between low magnitude pulses of oxytocin and PGFM. From these data, it was concluded that high-magnitude surges of PGF_{2α} are associated with similar surges of oxytocin from the posterior pituitary gland, and that PGF_{2α} may induce their secretion. The posterior pituitary gland also appears to secrete oxytocin in a pulsatile manner at a frequency of approximately 1 pulse every 2 h but these pulses do not appear to be associated with the low magnitude pulses of PGF_{2α} secreted from the uterus.

Introduction

In mares, as in other large domestic species, the uterus is required for luteal regression (Ginther and First, 1971; Stabenfeldt *et al.*, 1974; Squires *et al.*, 1975). It contributes to luteolysis by secreting the luteolytic hormone, prostaglandin (PG) F_{2α} (Sharp *et al.*, 1989). During luteolysis, PGF_{2α} is secreted in a series of discrete pulses (Douglas and Ginther, 1976; Stabenfeldt *et al.*, 1981). The endocrine factors that regulate pulsatile secretion of PGF_{2α} from the equine uterus are not clearly understood.

In ruminants, oxytocin appears to play an important role in this regulation (for reviews see Flint *et al.*, 1990; Silvia *et al.*, 1991). The acquisition of receptors for oxytocin by the endometrium appears to be a critical event in the onset of luteolysis in these species (McCracken, 1980; Stevenson *et al.*,

1994). Oxytocin is released in pulses that coincide synchronously with pulses of PGF_{2α}. Most of this oxytocin appears to be released from the corpus luteum. Oxytocin is a potent stimulus for uterine secretion of PGF_{2α}. Likewise, PGF_{2α} stimulates luteal secretion of oxytocin. Thus, uterine PGF_{2α} and luteal oxytocin comprise a positive feedback loop. Flint *et al.* (1986) proposed that each synchronous episode of oxytocin and PGF_{2α} secretion during luteolysis occurred through the activation of this feedback loop. The precise mechanism by which the loop is activated has not been determined. McCracken *et al.* (1984, 1995) have suggested that the regular pulsatile release of oxytocin from the posterior pituitary gland may be the initiating stimulus for uterine PGF_{2α} release. Pulses of luteal oxytocin during luteolysis are frequently accompanied by synchronous pulses of oxytocin from the posterior pituitary gland (Hooper *et al.*, 1986). However, no attempt has been made to relate uterine secretion of PGF_{2α} to secretion of neurohypophyseal oxytocin. The small amount of secretion and short half life make it difficult to measure neurohypophyseal oxytocin accurately in peripheral blood samples.

*Present address: ARBL, Colorado State University, Fort Collins, CO 80523, USA.

†Correspondence and reprint requests.

Received 7 June 1997.

Oxytocin may play a similar regulatory role in secretion of $\text{PGF}_{2\alpha}$ from the equine uterus. Peripheral concentrations of oxytocin have been measured during the equine oestrous cycle. Burns *et al.* (1981) collected jugular venous samples from mares at four stages of the oestrous cycle. They found that concentrations of oxytocin were high during oestrus and 5 days after ovulation. Concentrations were lower on day 10 and day 15 after ovulation, day 15 being around the expected time of luteolysis. Tetzke *et al.* (1987) sampled very frequently over 8 h sampling periods on days 0, 3, 7 and 15 after ovulation and observed a very irregular pattern of pulsatile secretion at all four stages of the oestrous cycle. In contrast to Burns *et al.* (1981), they found that mean concentrations of oxytocin were greatest on day 15. Thus, the true nature of oxytocin secretion during the equine oestrous cycle remains to be determined.

The equine endometrium has receptors for oxytocin (Stull and Evans, 1986; Sharp *et al.*, 1997). Oxytocin stimulates endometrial secretion of $\text{PGF}_{2\alpha}$, both *in vivo* (Betteridge *et al.*, 1985; Goff *et al.*, 1987) and *in vitro* (King and Evans, 1987; Franklin *et al.*, 1989). Uterine secretion of $\text{PGF}_{2\alpha}$ can also be stimulated by manual dilation of the cervix, presumably through the reflex-induced release of oxytocin from the posterior pituitary gland (Sharp *et al.*, 1997). However, regulation of $\text{PGF}_{2\alpha}$ secretion in mares appears to differ from that in ruminants in at least one critical area. The equine corpus luteum is essentially devoid of oxytocin (Murray *et al.*, 1991; Stevenson *et al.*, 1991). If oxytocin is playing an important role in mares, it must be secreted from an alternative site such as the posterior pituitary gland. The mare is a particularly appropriate animal model in which to study secretion of oxytocin from the posterior pituitary gland because the unique vascular anatomy of the equine head permits the relatively convenient cannulation of the intercavernous sinus (ICS) (Irvine and Alexander, 1987; Silvia *et al.*, 1995). Venous blood draining the pituitary gland flows directly into the ICS. Blood samples collected from the ICS have much higher concentrations of many pituitary hormones (LH, FSH, ACTH and vasopressin) when compared with samples collected simultaneously from the jugular vein (Redekopp *et al.*, 1986; Alexander and Irvine, 1987; Irvine and Alexander, 1987). Concentrations of vasopressin in the ICS can exceed those in the jugular vein by more than 500-fold (Redekopp *et al.*, 1986). The ICS sampling procedure has been used successfully to characterize secretion of oxytocin from the posterior pituitary during parturition (Vivrette *et al.*, 1992).

The objective of the present study was to define the characteristics of neurohypophyseal oxytocin secretion in mares and evaluate its potential role as an initiator of the luteolytic pulses of $\text{PGF}_{2\alpha}$.

Materials and Methods

Animals

Thoroughbred mares, ranging from 3 to 15 years in age and from 350 to 500 kg in weight, were used in these experiments. Experiments were conducted during the breeding season (May–July). The reproductive tract of each mare was examined four times per week by transrectal palpation and ultrasonography (Ginther, 1986). Once an ovarian follicle ≥ 30 mm in

diameter was detected, mares were examined each day until ovulation. Ovulation was defined as the disappearance of an ovarian follicle 30 mm in diameter between two successive daily examinations, and subsequent identification of the corpus luteum by ultrasound. The day of ovulation was designated day 0. The concentration of progesterone was quantified in jugular venous blood samples (10 ml) collected by venepuncture daily on days 0–22 after ovulation to monitor luteal function, and to determine when luteolysis occurred. Plasma was collected and stored at -20°C until assayed for progesterone.

The ICS of each mare was cannulated via the facial vein as described by Silvia *et al.* (1995). Placement of cannulae was verified by radiography at the time of insertion. Procaine penicillin-G (22 000 United States Pharmacopeia (USP) units kg^{-1} bodyweight) was administered i.m. at the time of cannulation and every 12 h thereafter for 48 h. Sterile 0.9% saline (1 ml) containing heparin (100 USP units ml^{-1}), potassium penicillin-G (100 iu ml^{-1}), and streptomycin sulfate (100 mg ml^{-1}) was administered each hour to maintain catheter patency between cannulation and the start of sample collection. Beginning at least 6 h after cannulation, ICS blood samples (5 ml) were collected every 5 min for up to 48 h, depending upon the objectives of the particular experiment. Samples were collected by slow aspiration (approximately 1 min) using a 5 cm^2 syringe so as to minimize trauma induced by severe negative pressure. Samples were transferred to a test tube containing 10 mg EDTA and 250 μg indomethacin in 0.5 ml of water. Plasma was collected and stored at -20°C until assayed for oxytocin. Uterine secretion of $\text{PGF}_{2\alpha}$ was assessed by measuring concentrations of PGFM in the same ICS blood samples. PGFM is a stable, biologically inactive, metabolite of $\text{PGF}_{2\alpha}$ and is the first step in the clearance of $\text{PGF}_{2\alpha}$ from the peripheral circulation (Granström and Kindahl, 1982). Peripheral concentrations of PGFM have been used by other researchers to assess uterine secretion of $\text{PGF}_{2\alpha}$ in mares (Stabenfeldt *et al.*, 1974, 1981; Betteridge *et al.*, 1985; Goff *et al.*, 1987; Vivrette *et al.*, 1992). Immediately after collection of each sample, the cannula was slowly flushed with 8–10 ml of the sterile saline solution described above, except that the heparin concentration was reduced to 20 USP units ml^{-1} . Mares were housed in box stalls throughout the sample collection period, and hay and water were provided *ad libitum*. At the completion of the sample collection period, cannulae placement was verified again by radiography.

Preliminary experiments

Three preliminary experiments using two mares (identification numbers 318 and 3) were conducted to validate various aspects of the ICS cannulation and sample collection procedures for quantification of oxytocin and PGFM. Preliminary Expt 1 was conducted to determine if an acute release of oxytocin could be detected by sampling from the ICS. The ICS of mare 318 was cannulated on the morning of day 12. On day 14, the cervix of the mare was manually dilated to induce release of oxytocin. The cervix was dilated by inserting the index finger to its full length through the cervical os and gently rotating the finger for 2 min. Blood samples were collected from the ICS at 5 min intervals for 2 h beginning 1 h before cervical dilation.

Table 1. The frequency of pulsatile oxytocin and 13,14-dihydro-15-keto-prostaglandin F_{2a} (PGFM) secretion in individual mares during the periluteolytic period

Mare	Days sampled (after ovulation)	Luteolysis complete*	Pulses in 36 h	
			Oxytocin	PGFM
312	15–16	15	21.5	22.6
321	15–16	15	10.8	24.7
322	15–16	15	24.0	19.0
328	15–16	15	17.0	20.0
329	15–16	15	13.9	17.8
342	16–17	15	23.2	19.2
357	16–17	> 22	23.3	10.1
390	13–15	18	22.1	18.5
391	13–15	17	14.6	18.8
Mean			18.9	19.0

*First day after ovulation on which progesterone < 2 ng ml⁻¹.

Preliminary Expt 2 was conducted to verify that the oxytocin released in response to cervical dilation was from the posterior pituitary gland. In this experiment, the ICS of mare 3 was cannulated on the morning of day 12. On day 16, the cervix was manually dilated for 2 min, as in preliminary Expt 1. Blood samples were collected from the ICS at 5 min intervals for 2 h beginning 1 h before cervical dilation. Jugular venous blood samples were drawn simultaneously through an indwelling catheter inserted immediately before the onset of sampling (Silvia *et al.*, 1995). Concentrations of oxytocin in the ICS and jugular vein were determined by radioimmunoassay and compared.

Preliminary Expt 3 was conducted to determine if concentrations of PGFM, the stable systemic metabolite of PGF_{2a}, in ICS samples were an accurate reflection of peripheral PGFM concentrations. On day 15, mare 318 (cannulated on the morning of day 12 and used previously in preliminary Expt 1) was given an injection of oxytocin (10 iu, i.v.). ICS blood samples were collected at 5 min intervals, for 2.5 h beginning 30 min before injection. Jugular venous blood samples were drawn simultaneously through an indwelling catheter inserted immediately before the onset of sampling (Silvia *et al.*, 1995). Concentrations of PGFM in the ICS and jugular vein were determined by radioimmunoassay and compared.

Main experiment

The ICSs of nine mares were cannulated the day before the onset of sample collection. Beginning at 12:00 h on day 13 ($n = 2$), day 15 ($n = 5$) or day 16 ($n = 2$), ICS blood samples were collected at 5 min intervals for 30–48 h. Days on which sampling occurred were selected on the basis of the duration of previous oestrous cycles in an attempt to ensure that some mares were sampled during luteolysis. The days of sample collection and the time of luteolysis for each mare are presented (Table 1). Concentrations of oxytocin and PGFM were determined in the plasma by radioimmunoassay.

Radioimmunoassays

Oxytocin was quantified directly in unextracted plasma using a modification of the procedures described by Robinson (1980). Synthetic oxytocin was used as standard and iodinated using the method of Dawood *et al.* (1978). The principal buffer for this assay was 0.01 mol PBS l⁻¹ with 0.01% (w/v) thimerosal (PBS) and 0.01% gelatin (PBS-gel). Standards (10–10 000 pg ml⁻¹) were prepared in charcoal-extracted gelding plasma. Antiserum (R III₅; a generous gift of I. C. A. F. Robinson) was diluted 1:60 000 in PBS containing 0.05 M EDTA. Standard or sample (200 µl), antiserum (200 µl) and tracer (approximately 30 000 cpm in 100 µl PBS-gel) were added to each tube. The final incubation volume was brought up to 800 µl with PBS-gel and incubated at 4°C for 24 h. Antibody-bound and free tracer were separated by second antibody precipitation at 4°C for 24 h. Crossreactivity of the antiserum was 0.005% for arginine vasopressin, 0.006% for lysine vasopressin and < 0.001% for arginine vasotocin (Robinson, 1980). Serial dilutions of pooled equine plasma ($n = 3$) were parallel to the standard curve. Addition of 25, 100, and 250 pg oxytocin to equine plasma were accurately recovered (mean recovery = 86%). Sensitivity was defined as the concentration of oxytocin required to inhibit binding of radioiodinated oxytocin to 95% of that observed in assay tubes containing no unlabelled oxytocin (B₀). Sensitivity of the assay averaged 11.2 pg ml⁻¹. Values below the assay sensitivity were assigned a value equal to the sensitivity. The intra- and interassay coefficients of variation, determined from aliquots of a plasma pool containing a concentration of oxytocin near the midpoint of the standard curve, were 9.9% and 10.0%, respectively.

PGFM was quantified directly in unextracted plasma using an [³H]PGFM radioimmunoassay developed in our laboratory (Homanics and Silvia, 1988). PGFM standards (50–10 000 pg ml⁻¹) were prepared in charcoal-extracted gelding plasma. Serial dilutions of pooled equine plasma were parallel to the standard curve. Addition of 50, 250, and 500 pg PGFM to equine plasma were accurately recovered (mean recovery = 105%). Sensitivity of the assay was 23.2 pg ml⁻¹. Values below the assay sensitivity were assigned a value equal to the sensitivity. The intra- and interassay coefficients of variation were 10.9% and 17.0%, respectively.

Progesterone was quantified directly in unextracted plasma using a solid-phase ¹²⁵I-labelled progesterone radioimmunoassay kit (Coat-A-Count Progesterone; Diagnostic Products Corp., Los Angeles, CA). Progesterone standards (0.1–40 ng ml⁻¹) were prepared in charcoal-extracted gelding plasma. Serial dilutions of pooled equine plasma were parallel to the standard curve. Addition of 0.1, 0.25, 0.5 and 1.0 ng progesterone to equine plasma were accurately recovered (mean recovery = 107%). Sensitivity of the assay was 0.09 ng ml⁻¹. Values below the assay sensitivity were assigned a value equal to the sensitivity. The intra- and interassay coefficients of variation were 5.8 and 14.8%, respectively.

Statistical analysis

Pulses of oxytocin and PGFM were identified using the Cluster algorithm as described by Veldhuis and Johnson (1986). Minimum peak and trough durations were set at four

Fig. 1. Concentrations of oxytocin in intercaavernous sinus (—) and jugular venous (---) blood samples collected around the time of manual cervical dilation (dilation at time = 0). Note the difference in y axis scales between the two experiments. (a) Preliminary Expt 1 (cervical dilation 14 days after ovulation). (b) Preliminary Expt 2 (cervical dilation 16 days after ovulation).

consecutive samples. This corresponds to durations of 20 min at the 5 min sampling frequency used. The mean number of pulses detected in 36 h was calculated. The presence of regular

Fig. 2. Concentrations of 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (PGFM) in intercaavernous sinus (—) and jugular venous (---) blood samples collected around the time of intravenous injection of oxytocin (injection at time = 0) in preliminary Expt 3. Oxytocin administered 15 days after ovulation.

rhythms in oxytocin or PGFM with periods ranging from 10 min to 16.5 h were determined for each mare using the ARIMA procedures of SAS (1985). Considering the relatively short bleeding time (about 36 h), rhythms with longer periods could not be identified.

Luteolysis was judged to be completed if peripheral concentrations of progesterone had decreased to $< 2 \text{ ng ml}^{-1}$. While this is higher than the 1 ng ml^{-1} used conventionally, it was chosen as a reference point because it was closer to the time of active luteolysis. In our experience, once a fully functional corpus luteum is formed (by day 8 after ovulation)

Fig. 3. Concentrations of oxytocin (—) and 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (PGFM) (shaded area) over the 36 h sampling period in three mares (mare numbers 312, 321 and 328). Sampling began at 12:00 h on day 15 after ovulation for all three mares.

Fig. 4. Concentrations of oxytocin (—) and 13,14-dihydro-15-keto prostaglandin F_{2α} (PGFM) (shaded area) over the 36 h sampling period in three mares (mare numbers 322, 342 and 357). Sampling began at 12:00 h on day 15 after ovulation for mare 322, and at 12:00 h on day 16 for mares 342 and 357.

progesterone concentrations do not fall below 2 ng ml^{-1} until luteolysis is irreversibly underway. The effect of time of sampling relative to luteolysis (that is, samples collected before versus after completion of luteolysis) on numbers of pulses in 36 h was determined by Student's *t* test.

Results

Preliminary experiments

Concentrations of oxytocin increased in ICS samples collected after manual cervical dilation (preliminary Expt 1; Fig. 1a). Concentrations reached a maximum 10 min after dilation and decreased to basal concentrations by 30 min. A similar response was observed after cervical dilation in another mare (preliminary Expt 2; Fig. 1b). A small simultaneous increase in oxytocin was also detected in the jugular vein but was only 1/8 in magnitude of the increase observed in the ICS samples, indicating that the oxytocin released during cervical stimulation was most likely from the posterior pituitary gland. Concentrations of PGFM increased immediately after injection of oxytocin on day 16 after ovulation (preliminary Expt 3; Fig. 2). The increase was similar in timing and in magnitude in both jugular venous and in ICS samples. Therefore, ICS samples can be used to determine peripheral PGFM concentrations accurately.

Main experiment

The patterns of oxytocin and PGFM in ICS samples for up to 36 h from all nine mares are presented (Figs 3–5). Oxytocin

was clearly secreted in a pulsatile manner. The vast majority of these pulses were of relatively small magnitude and were secreted at an average frequency of approximately 1 pulse every 122.3 min (Table 1). These pulses can be seen in more detail in representative, 12 h bleeding windows from six mares (Figs 6 and 7). Concentrations of PGFM also showed a pulsatile pattern of secretion (Figs 3–7), with small magnitude pulses detected at a similar frequency to that observed for oxytocin (1 pulse every 121.0 min; Table 1). The interval between pulses for individual mares varied from 90 to 199 min for oxytocin and from 87 to 213 min for PGFM. There was no correlation between PGFM and oxytocin pulse intervals among mares. There was no discernable temporal association of low magnitude oxytocin and PGFM pulses in any of the mares (Figs 6 and 7).

Three very high magnitude surges ($> 200 \text{ pg ml}^{-1}$) of oxytocin were detected in two mares (mare numbers 322, 329; Fig. 8). Each of these was associated with a high magnitude pulse of PGFM ($> 1000 \text{ pg ml}^{-1}$). In each case, the increase in PGFM appeared to precede or coincide with the increase in oxytocin. The magnitude of the PGFM surges was extremely consistent at almost 2000 pg ml^{-1} . However, the magnitudes of the concomitant oxytocin pulses were extremely variable, ranging from just over 100 pg ml^{-1} (the second pulse in mare 329) to $> 5000 \text{ pg ml}^{-1}$ (the first pulse in mare 329).

Mares could be classified into two groups according to the time of sampling relative to luteolysis (Table 1). Six mares (312, 321, 322, 328, 329, 342) were sampled after luteolysis was complete. The two mares exhibiting greater magnitude

Fig. 5. Concentrations of oxytocin (—) and 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (PGFM) (shaded area) over the 36 h sampling period in three mares (mare numbers 329, 390 and 391). Sampling began at 12:00 h on day 15 after ovulation for mare 329, and at 12:00 h on day 13 for mares 390 and 391.

Fig. 6. Concentrations of oxytocin (—) and 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (PGFM) (shaded area) over a 12 h period in three mares (top panel: mare number 312, corresponding to 720–1440 min in Fig. 3; middle panel: mare number 321, corresponding to 360–1080 min in Fig. 3; bottom panel: mare number 328, corresponding to 600–1320 min in Fig. 3). Horizontal bars across the upper portion of each panel indicate samples that contribute to a pulse series identified by cluster analysis (O, oxytocin; P, PGFM). The highest value within each series is identified by the letter that sits directly above the horizontal line.

Fig. 7. Concentrations of oxytocin (—) and 13,14-dihydro-15-keto prostaglandin F_{2α} (PGFM) (shaded area) over a 12 h period in three mares (top panel: mare number 342, corresponding to 480–1200 min in Fig. 4; middle panel: mare number 390, corresponding to 900–1620 min in Fig. 5; bottom panel: mare number 391, corresponding to 2160–2880 min that do not appear in Fig. 5). Horizontal bars across the upper portion of each panel indicate samples that contribute to a pulse series identified by cluster analysis (O, oxytocin; P, PGFM). The highest value within each series is identified by the letter that sits directly above the horizontal line.

(luteolytic) pulses of PGFM and oxytocin were from this group. Three mares (357, 390, 391) were sampled before the onset of luteolysis. The pulsatile pattern of oxytocin or PGFM did not appear to be affected by the time of sampling relative to luteolysis. No significant rhythms with frequencies ranging from 5 min to 16.5 h were detected for either hormone. On the basis of simple visual examination of the data, there was no evidence of rhythms with longer periods for either hormone.

Discussion

From the preliminary experiments, it is clear that manual cervical dilation induces a prompt release of oxytocin. Sharp *et al.* (1997) have observed a much smaller increase in peripheral concentrations of oxytocin after transcervical uterine biopsy. In preliminary Expt 2, concentrations in samples collected from the ICS were compared with those collected from the jugular vein. The eightfold higher concentrations observed in the ICS versus the jugular vein strongly support the hypothesis that this oxytocin is of neurohypophyseal origin. Taken together, these data confirm the existence of a 'Ferguson reflex' as proposed by Sharp *et al.* (1997). The magnitude of the response was much greater in mare 312 than in mare 3. This may reflect a higher sensitivity of the reflex

system on day 14 versus that on day 16 after ovulation. Sharp *et al.* (1997) showed that the release of oxytocin in response to transcervical uterine biopsy changes over the course of the oestrous cycle.

Oxytocin appears to be secreted in a series of irregularly timed 'pulses' during the periluteolytic period. This pattern is similar to the one described by Tetzke *et al.* (1987) for samples collected from the jugular vein. The origin of these low magnitude pulses cannot be determined because simultaneous ICS and peripheral samples were not collected in this study. The basal pattern of release is somewhat similar to the pattern of vasopressin in mares sampled from the ICS (Redekopp *et al.*, 1986). While these pulses are most likely secreted from the posterior pituitary, other sources of oxytocin such as the uterus cannot be ruled out (Behrendt *et al.*, 1997). Thus, the ICS-cannulated mare appears to be an appropriate animal model in which to study the pattern of oxytocin secretion and acute aspects of its regulation. It should be recognized that slight differences in cannula placement can influence absolute concentrations of oxytocin in samples obtained from the ICS. Data should be interpreted with this concern in mind. Even in the ICS, pulses of both oxytocin and vasopressin are much less clearly defined than gonadotrophin pulses (Alexander and Irvine, 1987; Irvine and Alexander, 1987; Silvia *et al.*, 1995) and may reflect fundamental differences in control of neuropeptide versus gonadotrophin secretion.

Fig. 8. Concentrations of oxytocin (—) and 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (PGFM) (shaded area) over an 8 h period in mares during three high magnitude (luteolytic) pulses. Upper panel of each pair is shown at an expanded scale to give an accurate presentation of pulse magnitude. The lower panel of each pair is shown in a reduced scale so that the small fluctuation preceding each pulse can be visualized.

While it can be difficult to establish causative relationships between secreted hormones because of lag time in responses and differences in clearance rates, there appears to be no relationship between the low magnitude pulses of oxytocin and PGFM detected in these mares. However, this interpretation must be made with some reservation. Even though blood samples were collected from the ICS, many samples had concentrations of oxytocin at or below the sensitivity of the radioimmunoassay. Therefore, the basal concentration of oxytocin from which pulses were identified was very low, with little or no variance. Under these circumstances, very small 'episodes' of oxytocin release are easily identified and may represent an overestimate of true pulsatile secretory activity. It should also be noted that when the true baseline is below the sensitivity of the assay, it is impossible to determine the true onset and completion of individual pulses.

The failure to detect high-magnitude pulses of PGFM in more of the mares in the present study is hard to explain. As in other large domestic species, PGFM pulses typically occur immediately before, and for 24–48 h after, luteolysis in mares (Neely *et al.*, 1979). On the basis of the timing of luteolysis (Table 1), high-magnitude pulses of PGFM would be expected in four other mares (312, 321, 329 and 342) in addition to those in which pulses were observed. It is not surprising that we did not detect high-magnitude pulses of PGFM in mares 390 and 391 since these mares were sampled several days before luteolysis. It is possible that, in the process of sample collection, enough oxytocin was removed from the system to delay the normal luteolytic process in these two mares. However, this

seems extremely unlikely since blood flow through the ICS is approximately 25 ml min^{-1} (Irvine and Alexander, 1987) and we are removing only about 1 ml min^{-1} in the course of collecting samples. Mare 357 appears to be an unusual case of abnormally prolonged luteal function.

Although limited in number, the marked association of high-magnitude surges of oxytocin and PGFM indicates clearly that the release of these two hormones is related. However, there does not appear to be an increase in oxytocin secretion preceding these surges of PGFM. A similar temporal association between PGFM and oxytocin was observed in pregnant mares at parturition (Vivrette *et al.*, 1992). The variability in the magnitude of the oxytocin pulses associated with each surge of PGFM was remarkable. It is possible that this is an accurate reflection of the variability in oxytocin release from the posterior pituitary. As noted above, the responsiveness of the posterior pituitary to physical stimuli changes throughout the oestrous cycle. Similar changes in responsiveness to endocrine stimuli may occur as well. It is also possible that results reflect variation in the placement of the cannula and subsequent dilution of pituitary venous effluent.

Contrary to the model proposed for ewes by McCracken *et al.* (1984), the data presented here suggest that the increase in uterine secretion of $\text{PGF}_{2\alpha}$ (as indicated by an increase in peripheral concentrations of PGFM) may be the trigger for the secretion of oxytocin from the posterior pituitary gland in mares. In an experiment using a single mare, an intramuscular injection of $\text{PGF}_{2\alpha}$ stimulated a prompt release of oxytocin from the posterior pituitary gland (D. K. Vanderwall and

W. J. Silvia, unpublished). In this respect, the posterior pituitary gland appears to serve a similar role to the corpus luteum in ruminants by providing oxytocin during luteolytic surges. Thus, the posterior pituitary gland may contribute to a positive feedback loop that amplifies pulses of PGF_{2α} once they are initiated but it does not appear to be a stimulus for the initiation of these pulses. Such a system may be possible in mares, in which uterine PGF_{2α} reaches the corpus luteum through a systemic route as opposed to the local, counter-current transfer system believed to be present in ruminants (McCracken *et al.*, 1972; Ginther, 1974). Pulmonary metabolism of PGF_{2α} must be much less efficient in mares than in ruminants for a systemic utero-luteal pathway to be effective. This may make it possible for uterine PGF_{2α} to reach the posterior pituitary gland in sufficient quantities to stimulate oxytocin secretion.

This research was supported by the Kentucky Agricultural Experiment Station and published with the approval of the Director (Publication No. 96-07-183). The authors thank S. Vivrette (North Carolina State University) for her invaluable assistance with the ICS catheterization technique, and establishment of the oxytocin radioimmunoassay in our laboratory; I. C. A. F. Robinson (National Institute for Medical Research, London) for generous donation of the oxytocin antiserum; and B. Curd, S. Hayes, C. Holman, J. Light, J. Osten, R. Swope, and A. Willoughby for excellent technical assistance. Finally, the authors would like to thank the two anonymous referees whose constructive criticism made a significant contribution to the quality of this manuscript.

References

- Alexander SL and Irvine CHG (1987) Secretion rates and short-term patterns of gonadotropin-releasing hormone, FSH and LH throughout the periovulatory period in the mare *Journal of Endocrinology* **114** 351–362
- Behrendt CY, Adams MH, Daniel KS and McDowell KJ (1997) Oxytocin expression by equine endometrium *Biology of Reproduction* **56** (Supplement 1) 134
- Betteridge KJ, Renard A and Goff AK (1985) Uterine prostaglandin release relative to embryo collection, transfer procedures and maintenance of the corpus luteum *Equine Veterinary Journal* (Supplement 3) 25–33
- Burns PJ, Kumaresan P and Douglas RH (1981) Plasma oxytocin concentrations in cyclic mares and sexually aroused stallions *Theriogenology* **16** 531–539
- Dawood MY, Raghavan KS, and Pociask C (1978) Radioimmunoassay of oxytocin *Journal of Endocrinology* **76** 261–270
- Douglas RH and Ginther OJ (1976) Concentrations of prostaglandin F in uterine venous plasma of anesthetized mares during the estrous cycle and early pregnancy *Prostaglandins* **11** 251–260
- Flint APF, Sheldrick EL, Theodosis DT and Wooding FBP (1986) Ovarian peptides: role of luteal oxytocin in the control of estrous cyclicity in ruminants *Journal of Animal Science* **62** (Supplement 2) 62–71
- Flint APF, Sheldrick EL, McCann TJ and Jones DSC (1990) Luteal oxytocin: characteristics and control of synchronous episodes of oxytocin and PGF_{2α} secretion at luteolysis in ruminants *Domestic Animal Endocrinology* **7** 111–124
- Franklin KJ, Gross TS, Dubois DH and Sharp DC (1989) *In vitro* prostaglandin secretion from luminal and myometrial sides of endometrium from cyclic and pregnant mares at day 14 postestrus *Biology of Reproduction* **40** (Supplement 1) Abstract 114
- Ginther OJ (1974) Internal regulation of physiological processes through venoarterial pathways: a review *Journal of Animal Science* **39** 550–564
- Ginther OJ (1986) *Ultrasonic Imaging and Reproductive Events in the Mare* pp 133–169 Equiservices, Cross Plains, WI
- Ginther OJ and First NL (1971) Maintenance of the corpus luteum in hysterectomized mares *American Journal of Veterinary Research* **32** 1687–1691
- Goff AK, Pontbriand D and Sirois J (1987) Oxytocin stimulation of plasma 15-keto-13, 14-dihydro-prostaglandin F_{2α} during the oestrous cycle and early pregnancy in the mare *Journal of Reproduction and Fertility Supplement* **35** 253–260
- Granström E and Kindahl H (1982) Species differences in circulating prostaglandin metabolites: relevance for the assay of prostaglandin release *Biochimica et Biophysica Acta* **713** 555–569
- Homanics GE and Silvia WJ (1988) Effects of progesterone and estradiol-17β on uterine secretion of prostaglandin F_{2α} in response to oxytocin in ovariectomized ewes *Biology of Reproduction* **38** 804–811
- Hooper SB, Watkins WB and Thorburn GD (1986) Oxytocin, oxytocin-associated neurophysin, and prostaglandin F_{2α} concentrations in the utero-ovarian vein of pregnant and nonpregnant sheep *Endocrinology* **119** 2590–2597
- Irvine CHG and Alexander SL (1987) A novel technique for measuring hypothalamic and pituitary hormone secretion rates from collection of pituitary venous effluent in the normal horse *Journal of Endocrinology* **113** 183–192
- King SS and Evans JW (1987) Effects of arachidonic acid and oxytocin on equine endometrial PGF_{2α} during normal estrous cycles and pseudo-pregnancy *Journal of Equine Veterinary Science* **7** 303–308
- McCracken JA (1980) Hormone receptor control of prostaglandin F_{2α} secretion by the ovine uterus *Advances in Prostaglandin and Thromboxane Research* **8** 1329–1344
- McCracken JA, Carlson JC, Glew ME, Goding JR, Baird DT, Green K and Samuelsson B (1972) Prostaglandin F₂ identified as a luteolytic hormone in sheep *Nature (New Biology)* **238** 129–134
- McCracken JA, Schramm W and Okulicz WC (1984) Hormone receptor control of pulsatile secretion of PGF_{2α} from the ovine uterus during luteolysis and its abrogation in early pregnancy *Animal Reproduction Science* **7** 31–55
- McCracken JA, Custer EE, Lamsa JC and Robinson AG (1995) The central oxytocin pulse generator: a pacemaker for luteolysis. In *Oxytocin* pp 133–154 Eds R Ivell and J Russell. Plenum Press, New York
- Murray MA, Evans JW, Dees WL and Forrest DW (1991) Immunocytochemical localization of oxytocin in the equine corpus luteum *Proceedings of the Equine Nutrition and Physiology Symposium* **12** 185–186
- Neely DP, Kindahl H, Stabenfeldt GH, Edqvist L and Hughes JP (1979) Prostaglandin release patterns in the mare: physiological, pathophysiological and therapeutic responses *Journal of Reproduction and Fertility Supplement* **27** 181–189
- Redekopp C, Irvine CHG, Donald RA, Livesey JH, Sadler W, Nicholls MG, Alexander SL and Evans MJ (1986) Spontaneous and stimulated adrenocorticotropin and vasopressin pulsatile secretion in the pituitary venous effluent of the horse *Endocrinology* **118** 1410–1416
- Robinson ICAF (1980) The development and evaluation of a sensitive and specific radioimmunoassay for oxytocin in unextracted plasma *Journal of Immunoassay* **1** 323–347
- SAS (1985) *SAS User's Guide* Statistical Analysis System Institute Inc., Cary, NC
- Sharp DC, McDowell KJ, Weithenauer J and Thatcher WW (1989) The continuum of events leading to maternal recognition of pregnancy in mares *Journal of Reproduction and Fertility Supplement* **37** 101–107
- Sharp DC, Thatcher MJ, Salute ME and Fuchs AR (1997) Relationship between oxytocin receptors and oxytocin-induced prostaglandin F_{2α} release during the oestrous cycle and early pregnancy in pony mares *Journal of Reproduction and Fertility* **109** 137–144
- Silvia WJ, Lewis GS, McCracken JA, Thatcher WW and Wilson L, Jr (1991) Hormonal regulation of uterine secretion of prostaglandin F_{2α} during luteolysis in ruminants *Biology of Reproduction* **45** 655–663
- Silvia PJ, Meyer SL and Fitzgerald BP (1995) Pulsatile gonadotropin secretion determined by frequent sampling from the intercavernous sinus of the mare: possible modulatory role of progesterone during luteolysis *Biology of Reproduction* **53** 438–446
- Squires EL, Wentworth BC and Ginther OJ (1975) Progesterone concentration in blood of mares during the estrous cycle, pregnancy and after hysterectomy *Journal of Animal Science* **39** 759–767
- Stabenfeldt GH, Hughes JP, Wheat JD, Evans JW, Kennedy PC and Cupps PT (1974) The role of the uterus in ovarian control in the mare *Journal of Reproduction and Fertility* **37** 343–351
- Stabenfeldt GH, Kindahl H, Hughes JP, Neely DP, Lin I and Pascoe (1981) Control of luteolysis in the mare *Acta Veterinaria Scandinavica* (Supplement 77) 159–170

- Stevenson KR, Parkinson TJ and Wathes DC (1991) Measurement of oxytocin concentrations in plasma and ovarian extracts during the oestrous cycle of mares *Journal of Reproduction and Fertility* **93** 437–441
- Stevenson KR, Riley PR, Stewart HJ, Flint APF and Wathes DC (1994) Localization of oxytocin receptor mRNA in the ovine uterus during the oestrous cycle and early pregnancy *Journal of Molecular Endocrinology* **12** 93–105
- Stull CL and Evans JW (1986) Oxytocin binding in the uterus of the cycling mare *Journal of Equine Veterinary Science* **6** 114–119
- Tetzke TA, Ismail S, Mikuckis G and Evans JW (1987) Patterns of oxytocin secretion during the oestrous cycle of the mare *Journal of Reproduction and Fertility Supplement* **35** 245–252
- Veldhuis JD and Johnson ML (1986) Cluster analysis: a simple, versatile, and robust algorithm for endocrine pulse detection *American Journal of Physiology* **250** E486–E493
- Vivrette SH, Kindahl H, Munroe C and Stabenfeldt G (1992) The initiation of parturition in the mare: prostaglandin $F_{2\alpha}$ or oxytocin *Biology of Reproduction* **46** (Supplement 1) Abstract 178