Relationship between endometrial oxytocin receptors and oxytocin-induced prostaglandin $F_{2\alpha}$ release during the oestrous cycle and early pregnancy in pony mares

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The effect of transcervical endometrial biopsy on the concentrations of plasma immunoreactive oxytocin and 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$ (PGFM) was studied in 18 pony mares on days 8, 12 and 14 after ovulation, days 12 and 14 of early pregnancy and at oestrus. Five biopsy specimens were taken within 15 min and consecutive specimens from each mare were pooled two (A) and three (B) together for measurement of the number of oxytocin receptors. Blood samples were collected at intervals of 5 min for 15 min beginning just before the initial biopsy. Biopsy procedure elicited prompt oxytocin release in all mares. Pregnancy did not affect the response but day after ovulation had a significant influence on oxytocin release. The greatest increase in plasma oxytocin was observed on day 12 in both nonpregnant and pregnant mares and the lowest on day 8. The concentration of plasma PGFM rose linearly over the 15 min period in nonpregnant mares. This response increased progressively with time after ovulation and was greatest on day 14. There was no increase in circulating PGFM in pregnant mares. Endometrial oxytocin receptor concentration was lowest in mares at oestrus and highest in nonpregnant mares on day 14. Oxytocin receptor density in pregnant mares was similar to that in nonpregnant mares on day 12 but was significantly attenuated on day 14. The affinity of oxytocin receptors was lower in pregnant than in nonpregnant mares. Because of the positive correlation between $PGF_{2\alpha}$ release, endometrial oxytocin receptor density, and plasma oxytocin concentrations in nonpregnant mares, it is assumed that the release of $PGF_{2\alpha}$ was induced by oxytocin and was mediated by oxytocin receptors. Pregnancy-induced inhibition of $PGF_{2\alpha}$ release was not associated with suppression of oxytocin release or oxytocin receptor density. An embryo-derived factor is therefore the most likely cause for the suppression of $PGF_{2\alpha}$ release and interruption of the oxytocin- $PGF_{2\alpha}$ interaction in mares during early pregnancy.

Introduction

The putative interaction of endogenous oxytocin and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in the control of ovarian cyclicity has received much less attention in mares than it has in cattle and sheep (reviewed by Flint *et al.*, 1994). Whereas the luteolytic effectiveness of $PGF_{2\alpha}$ in mares has long been established (Allen and Rowson, 1973; Douglas and Ginther, 1975), the possible role of oxytocin in luteal function during the oestrous cycle is not well understood. However, the participation of the uterus in regulation of luteal regression in mares has been demonstrated (Ginther, 1974). The effect of the uterus is mediated systemically and does not involve a unilateral utero-ovarian relationship; moreover, luteal oxytocin does not appear to be implicated as it is in ruminants (Ginther, 1974; Stevenson

et al., 1991). The effects of exogenous oxytocin on luteal function in mares differ somewhat from those in cows. Injections of 150 iu of oxytocin on days 2–8 after ovulation did not have any effect on luteal function in mares, as determined by plasma progesterone and duration of cycle (Neely *et al.*, 1979a), whereas similar treatment in cows causes shortening of the oestrous cycle (Armstrong and Hansel, 1959). However, 20 iu oxytocin injected on days 10–13 after ovulation resulted in $PGF_{2\alpha}$ release and luteolysis (Goff *et al.*, 1987). The release of $PGF_{2\alpha}$ after oxytocin injections was reduced markedly in early pregnancy (Goff *et al.*, 1987). Similarly, oxytocin added to endometrial strips *in vitro* resulted in $PGF_{2\alpha}$ release when endometrial tissues were obtained from nonpregnant mares at day 14 but not in strips from day 14 pregnant mares (Franklin *et al.*, 1989). These observations suggest that oxytocin receptors in the equine endometrium may be important in the luteolytic cascade in mares. Cervical stimulation and transcervical intrauterine manipulation in mares also cause shortening of the oestrous cycle (Hurtgen and Ganjam, 1979) and

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release of PGF_{2α} (Berglund *et al.*, 1982; Betteridge *et al.*, 1985). The purpose of this study was to determine oxytocin receptor concentrations in the endometrium of cyclic mares and mares in early pregnancy and to monitor changes in concentrations of circulating oxytocin and the metabolite of PGF_{2α} (PGFM) after transcervical uterine manipulation (that is, endometrial biopsy).

Materials and Methods

Animals

Eighteen pony mares belonging to the research herd of the Animal Science Department, University of Florida, Gainesville, were used for this study, which was approved by the Institutional Animal Welfare Committee. The mares were of known fertility, 5–12 years of age and weighed 180–200 kg. They were monitored daily for oestrous behaviour by teasing with a vigorous pony stallion, and for presence of ovarian follicles and occurrence of ovulation by transrectal palpation and ultrasound examination, respectively (Concept; 5–7.5 mH B-mode, real time, linear array diagnostic ultrasound; Products Group International, Boulder, CO). Oestrus was defined as the presence of a follicle > 30 mm in diameter and at least 3 days of oestrous behaviour. The mares were assigned randomly to four groups of nonpregnant (not mated) and two groups of pregnant mares, three mares in each group: day 8 nonpregnant (NP), day 12 NP, day 14 NP, day 12 pregnant (P), day 14 P, and oestrus (day 0 = ovulation).

Cervical/uterine stimulation

Uterine biopsy forceps (Kenney, 1978) were inserted transcervically for removal of five consecutive endometrial biopsies, each of between 50 and 120 mg tissue. Multiple biopsies were taken to assure sufficient tissue for analysis. The procedure was not painful and the mares stood quietly throughout the experiment without need for tranquilisers or analgesics. The first two biopsies were taken within 5 min of first insertion of forceps, and the remaining three biopsies over the subsequent 10 min. The two first biopsy specimens were combined (sample A) and the three subsequent biopsy specimens were combined (sample B). They were immediately frozen in liquid nitrogen and stored at –80°C until shipped in dry ice to New York for receptor assay.

Blood sample collection

Four blood samples of approximately 7 ml each were collected from each mare via indwelling jugular vein catheters. A Becton–Dickinson Angiocath catheter (16 gauge) was installed approximately 2 h before sample collection was begun. The area was washed with 70% ethanol and about 2 ml lidocaine was injected s.c. before venepuncture. The hub of the catheter was kept in place with a stay-suture in the area of the lidocaine block. The catheter was kept patent by flushing after each sample with heparin–saline and maintaining a heparin lock (100 U ml^{–1}) in the capped catheter between the samplings.

The first blood sample was collected just before the biopsy forceps were inserted, and the next three blood samples were taken at 5 min intervals after that. The first two biopsy samples were taken before the second blood sample was collected and the subsequent three biopsies were taken within 5 min after the collection of the second blood samples. All samples were collected in precooled glass tubes containing heparin (100 U ml^{–1}) and placed on ice until plasma was separated by centrifugation for 10–15 min at 4°C at 3000 g. Each sample was frozen in two aliquots for radioimmunoassays (RIA), one for measurement of PGFM and one for oxytocin. The samples were kept at –20°C until assayed (PGFM), or shipped in dry ice to New York for measurement (oxytocin).

Hormone assays

Oxytocin. Oxytocin RIA was performed as described and validated for human, cow, rabbit, rat and mouse blood by Fuchs (1985) and, Fuchs *et al.* (1991), except that a highly specific, well characterized antibody generously provided by T. Higuchi, Fukui Medical Center, Matsuoka University, Fukui, Japan, was used. Two millilitres of acidified, diluted (1:3) plasma were passed through Florisil (Sigma, St Louis, MO) minicolumns, from which oxytocin peptide was eluted with acetone:water (95:5). The dried extracts were dissolved in 250 µl of assay buffer. A 1:100 000 dilution of antibody (10 µl) was added to duplicate aliquots of 100 µl of plasma extract. Iodinated oxytocin, 10 µl, prepared by the lactoperoxidase method and purified on Sephadex-50 columns, was added 24 h later. After incubation for 72 h at 4°C, bound and free ligand were separated by the addition of Pansorbin cells (Calbiochem, Torrance, CA). Oxytocin standards (Fourth International Standard, obtained from the National Bureau of Standards, London) were added to an equal volume of charcoal-treated, acidified horse serum and extracted together with the unknown samples. Standard curves were prepared with the dried extracts, thus eliminating the necessity to correct for extraction losses. The sensitivity of the oxytocin assay was 1.0 µU per tube, and the intra- and interassay variations were < 6% and < 11%, respectively. In preliminary experiments, serial dilutions of horse plasma were assayed in duplicate, and the resulting curves were parallel with standards added to assay buffer. Serial dilutions of standards added to horse plasma also resulted in parallel curves with standards in assay buffer (data not shown).

Prostaglandin F_{2α} metabolite. Concentrations of PGFM were determined using a RIA validated in our laboratory (Cadario *et al.*, 1995). Briefly, plasma samples (500 µl) were assayed in duplicate without extraction or chromatography. Standard curves were prepared by adding 500 µl prostaglandin-free horse plasma to each tube. Prostaglandin-free plasma was obtained by treating a mare in early dioestrus twice with a 12 h interval with an i.v. injection of 500 mg flunixin meglumine (Banamine solution, 50 mg ml^{–1}, Schering–Plough, Kenilworth, NJ); 4 h after the second injection of flunixin meglumine, blood was collected in tubes containing EDTA that were kept in ice until centrifuged at 3000 g at 4°C. The prostaglandin-free plasma was separated and stored in 20 ml aliquots at –20°C. PGFM standard solutions were freshly

made each time from a stock solution of 15-keto-13,14-dihydro-PGF_{2α} (Sigma) in 0.05 mol Tris-HCl buffer l⁻¹, pH 7.5. Tritiated PGFM (5,6,8,11,12,14-[³H]PGFM, 185 Ci mmol⁻¹, Amersham, Arlington Heights, IL) was used as the labelled ligand. The antibody was a gift from K. Kirton, The Upjohn Co., Kalamazoo, MO. The final assay volume was 800 µl. After overnight incubation at 4°C, free and bound ligand were separated by adding 750 µl of cold 40% solution of polyethylene glycol-8000 (Sigma) in water and centrifugation at 3000 g. The pellet was resuspended in assay buffer and the separation procedure was repeated once; the final pellet was resuspended in 1 ml 0.05 mol Tris-HCl buffer l⁻¹ and poured into scintillation vials. Scintiverse-BioHP (Fisher Scientific, Fair Lawn, NJ), 4 ml, was added to each vial and counted in a Packard LS 200 liquid scintillation counter.

Oxytocin receptor assay

Tissue preparation. The frozen tissues were processed for assay as described and validated by Fuchs *et al.* (1990). Briefly, tissue pieces were pulverized while frozen and then homogenized in a 10 mmol Tris buffer l⁻¹ containing 1.5 mmol EDTA l⁻¹, 0.5 mmol dithiothreitol l⁻¹ and 0.002% (w/v l⁻¹) phenylmethylsulfonyl fluoride (PMSF), pH 7.4. Crude microsomal pellets were prepared by centrifugation of 1000 g supernatants at 160 000 g for 30 min. The microsomal pellets were washed and resuspended in Hank's solution at pH 7.0 and stored at -80°C. Protein concentrations were determined according to the method of Lowry *et al.* (1951) with BSA as standard.

Assay procedure. Assays were performed as described previously using tritiated oxytocin as the labelled ligand (spec. act 36.7 Ci mmol⁻¹, New England Nuclear, Boston, MA). Assay buffer consisted of 50 mmol Tris-maleate l⁻¹, 5 mmol MnCl₂ l⁻¹, 0.002% (w/v) PMSF and 0.1% (w/v) BSA, pH 7.4; incubation temperature was 22°C and duration 60 min. Separation of bound and free ligands was achieved by rapid filtration through Whatman GF/F filters using a Brandel cell harvester (Gaithersburg, MD). The protein concentration was 0.5–1.0 mg ml⁻¹, in which range specific binding was correlated linearly with protein concentration. Binding was characterized initially in saturation assays using 8–10 different concentrations of [³H]oxytocin. The data were analysed by a computerized curve fitting program LIGAND (Biosoft, Elsevier Medical Publishers, Cambridge, UK), which gives the parameters K_d , K_d , B_{max} , and their statistical significance. Since the results showed that oxytocin bound to a single population of binding sites, saturation analyses with individual tissues were performed using four different concentrations of [³H]oxytocin. Nonspecific binding was in all instances determined in the presence of 1.1 µmol unlabelled oxytocin l⁻¹ (Bachem, Torrance, CA). For characterization of the ligand specificity of the equine oxytocin receptors, tissues from several samples were pooled for displacement assays with unlabelled agonists and antagonists. All assays were performed in duplicate; results are expressed in fmol microsomal protein mg⁻¹. All binding data were analysed with LIGAND. The oxytocin antagonist, [1-β-mercapto-(β,β-cyclopentamethylene) propionic acid, 2-(O-methyl) tyrosine, 4-threonine, 9-tyrosine-NH₂] ornithine vasotocin (OTA) was a gift from M. Manning, Department of

Biochemistry, Medical College of Ohio at Toledo. Another oxytocin antagonist, Atosiban (AT), [1-desamino-penicillinamine, 2-(O-ethyl)tyrosine, 4-threonine] ornithine vasotocin was received from Ortho Pharmaceuticals, Seaucus, NJ. The other peptides used were purchased from Peninsula Labs, Belmont, CA. They were: isotocin (IT), a highly selective oxytocic agonist; arginine-vasopressin (AVP); [1-(β-mercapto-(β,β-cyclopentamethylene)propionic acid, 2-(O-methyl)tyrosine] arginine-vasopressin ('Manning Compound,' MC), a selective VP1-type antagonist; (2-phenylalanine, 3-isoleucine, 9-ornithine) vasopressin (PAVP), a selective VP1-receptor subtype agonist; and 1-(desamino, 8-D-arginine) vasopressin (DDAVP), a selective VP2-receptor subtype agonist. All other chemicals were purchased from Sigma.

Statistical analysis

Significance of differences among groups was determined by least-squares analysis of variance with the General Linear Models Program of the Statistical Analysis System (SAS Users Guide, 1988). Day of cycle, status and their interaction were considered the independent variables. Differences among individual treatment group means were tested by orthogonal contrasts. Time was also considered as a discrete variable and, in separate analyses, time was considered a continuous independent variable to test for potential differences in time trends among day and status. Differences in time trends were tested for homogeneity of variance using the procedure of Damon and Harvey (1987) and Wilcox *et al.* (1990). Plasma oxytocin and PGFM and oxytocin receptor concentrations are presented as least squares means ± SEM.

Results

Plasma oxytocin

Plasma concentrations of immunoreactive i-oxytocin before cervical manipulation varied from <1.5 µU ml⁻¹ to 15 µU ml⁻¹, mean 3.1 ± 1.25 µU ml⁻¹. Plasma i-oxytocin increased within 5 min after the first biopsy in all mares regardless of day or status (Fig. 1). Pregnancy had no influence on basal or stimulated plasma concentration of i-oxytocin (status effect, $P = 0.61$; day by status interaction, $P = 0.14$). Time of collection after the first biopsy had a highly significant effect ($P = 0.0001$); the concentrations were greatest at 10 min and then declined. Time trend analysis demonstrated heterogeneity of regression among days that was best characterized by fitting separate quadratic regression equations for oxytocin response on day 12 versus day 14. This analysis indicated that peak i-oxytocin concentrations were higher on day 12 than on day 14 ($P = 0.025$).

Plasma PGFM

The concentrations of plasma PGFM were uniformly low before insertion of the biopsy forceps, between 10 and 50 pg ml⁻¹. During the biopsy procedure plasma PGFM increased progressively for the 15 min collection period

Fig. 1. Oxytocin (OT, $\mu\text{U ml}^{-1}$) in peripheral plasma of pony mares before and at 5 min intervals after transcervical biopsy procedure. (▲): oestrus (day 0); (△): day 8 of cycle; (○): day 12 of cycle; (□): day 14 of cycle; (●): day 12 of pregnancy; (■): day 14 of pregnancy. There was no difference between nonpregnant (open symbols) and pregnant mares (closed symbols). Time of sampling had a significant effect ($P < 0.01$, time trend analysis at the quadratic level of data for all groups combined). Oxytocin responses were significantly greater on day 12 than on day 14 ($P < 0.025$).

(Fig. 2). Time trends were best characterized by a linear response ($P < 0.0001$). The PGFM response was greater in mares tested on days 12 and 14 of the cycle than in mares in oestrus or on day 8, and the release of $\text{PGF}_{2\alpha}$ was essentially blocked in pregnant mares on days 12 and 14 (Fig. 3). The effect of status was highly significant ($P < 0.0001$), as were day after ovulation ($P < 0.0001$) and day status interaction ($P < 0.0001$).

Endometrial oxytocin receptors

Characterization. Oxytocin binding to endometrial membrane preparations was to a single population of binding sites as evidenced by a straight Scatchard plot and a Hill coefficient near unity. The affinity of the oxytocin receptors did not vary during the cycle but was significantly lower in pregnant than in nonpregnant mares (Table 1).

Rank order of ligand affinities was, in decreasing order: $\text{OTA} > \text{oxytocin} > \text{AT} > \text{AVP} > \text{PAVP} > \text{MC} > \text{IT} > \text{DDAVP}$ (Table 2). The two oxytocin antagonists, OTA and AT, as well as unlabelled oxytocin displaced [^3H]oxytocin with high affinity, whereas IT, which in pharmacological tests on rat uterus is the purest oxytocic agonist of the naturally occurring oxytocic neuropeptides, displaced with a relatively low affinity. AVP, the VP1 specific agonist PAVP, and the antagonist MC displaced [^3H]oxytocin with relatively high affinity. The VP2 specific agonist, DDAVP, did not displace at all at the concentrations studied (up to $10^{-6} \text{ mol l}^{-1}$). AVP and the VP1 specific agonist appeared to bind to two sites. The higher affinity site for AVP had a significantly lower binding capacity

Fig. 2. Peripheral plasma concentrations of oxytocin (●) and the metabolite of prostaglandin $\text{F}_{2\alpha}$ (PGFM; ◇) in pony mares before, and at 5 min intervals after, transcervical biopsy procedure. Data for all nonpregnant and pregnant mares are combined and grouped according to time of sampling. Time trends for PGFM values were characterized by a steep linear regression ($P < 0.0001$) and for oxytocin by a quadratic regression ($P < 0.01$), that indicated a downward trend by 15 min after the biopsy procedure was initiated.

than the site for oxytocin and OTA binding, whereas the binding capacity of the lower affinity site was similar to that of the oxytocin–OTA site (Table 2). Horse endometrium, therefore, appears to possess a VP1 receptor in addition to the oxytocin receptor.

Concentrations. The oxytocin receptor concentrations shown (Fig. 4), are means for the A and the B samples, which were analysed separately. The coefficient of variation between A and B samples was 22%, and the variations were random with no significant difference between the two means in any of the groups. However, the concentrations of microsomal protein g^{-1} wet mass of tissue were significantly greater in all A samples than in B samples, $7.94 \pm 0.33 \text{ mg g}^{-1}$ versus $5.97 \pm 0.39 \text{ mg g}^{-1}$, $P = 0.005$. There was a significant effect of day on oxytocin receptor concentrations ($P < 0.04$); the lowest receptor densities were observed at oestrus and at day 8 after ovulation and the highest on day 14 of the cycle (NP). In pregnant mares, oxytocin receptor density on day 12 was similar to that of nonpregnant mares but on day 14 oxytocin receptor concentrations in pregnant mares were significantly reduced in comparison with cyclic mares ($P < 0.04$) (Fig. 4).

Discussion

The data presented show that transcervical insertion of biopsy forceps and collection of endometrial tissue is a powerful stimulus for i-oxytocin and $\text{PGF}_{2\alpha}$ release in nonpregnant mares. Pregnancy status did not affect the release of i-oxytocin, but the release of $\text{PGF}_{2\alpha}$ was essentially blocked in all pregnant mares. Whether the release of i-oxytocin was mediated by so

Fig. 3. Prostaglandin $F_{2\alpha}$ metabolite (PGFM) concentration in peripheral plasma before and at 5 min intervals after the biopsy procedure, in the same mares as in Fig. 2, but grouped according to status and day after ovulation. (◆): day of oestrus; (●): day 8 of cycle; (●): day 12 of cycle; (▲): day 14 of cycle; (■): day 12 of pregnancy; (▼): day 14 of pregnancy. Pregnant mares did not respond to the biopsy procedure with release of PGFM. In nonpregnant mares the response increased in magnitude during the luteal phase; PGFM responses on days 12 and 14 of cycle were greater than on day 8 and at oestrus. Overall time trends were characterized by linear regression ($P < 0.0001$), and the effects of status and day after ovulation were highly significant as indicated by differences in the slopes (day: $P < 0.001$; day–status interaction: $P < 0.0001$).

Table 1. Affinity of endometrial oxytocin receptors in non-pregnant and early pregnant pony mares

Group (n)	K_a (l mol ⁻¹)	K_d (mol l ⁻¹)
Nonpregnant (20)	1.06×10^9 $\pm 0.03 \times 10^9$	0.94×10^{-9} $\pm 0.03 \times 10^{-9}$
Early pregnant (12)	0.79×10^9 $\pm 0.04 \times 10^9$	1.26×10^{-9} $\pm 0.06 \times 10^{-9}$
P	< 0.0001	< 0.0001

Values are mean $\pm$ SEM.

called Ferguson reflex (Ferguson, 1941) initiated by cervical stimulation, or was brought about by a reflex arising from uterine innervation, or both, cannot be decided on the basis of these experiments. Endometrial biopsy was found to be much more effective than cervical stimulation in causing shortening of the oestrous cycle in mares (Hurtgen and Ganjam 1979), and uterine manipulation resulted in greater release of PGF $_{2\alpha}$ than both vaginal and cervical dilatation (Betteridge *et al.*, 1985). These findings implicate the uterus as the main source of the stimulus for oxytocin and PGF $_{2\alpha}$ release, and are in agreement with the failure of many authors to observe oxytocin release

Table 2. Rank order of affinities of [3 H]oxytocin binding sites in pooled endometrium from nonpregnant and early pregnant mares to selected oxytocin analogues

Analogue	Rank order	K_a (l mol ⁻¹)	K_d (nmol l ⁻¹)	B_{max} (fmol mg ⁻¹)
OTA	1	2.3	0.43	283
OT	2	0.49	2.0	308
AT	3	0.22	4.5	326
*AVP	4	0.15	6.9	379
*PAVP	5	0.047	24.8	279
MC	6	0.033	30.0	352
IT	7	0.028	35.0	331
DDAVP	8	< 0.0001	> 1000	0

*All values are for one-site model for the binding data; the ligands marked with an asterisk may bind to two binding sites since two-site model gave a better fit for the binding data for these two ligands.

Fig. 4. Oxytocin receptor concentration (fmol mg⁻¹) in endometrial biopsy samples obtained from nonpregnant mares (■) at oestrus (OE) and on days 8, 12, 14 after ovulation, and from pregnant mares (▨) on days 12 and 14 after ovulation, respectively. There was a significant effect of day on oxytocin receptor concentration, with the lowest receptor density at oestrus and the highest on day 14 of cycle. There was also a significant effect of status: receptor concentration on day 14 being lower in pregnant than in nonpregnant mares ($P < 0.04$). *Receptor concentrations are significantly different from values at oestrus. **Receptor concentrations is significantly different from all other groups.

during cervical dilatation or massage in humans (Sala *et al.*, 1965), rabbits (Fuchs *et al.*, 1965), sheep (Peeters and Houvenaghel, 1973), rats (Dreifuss *et al.*, 1976) and cows (Cooper and Foote, 1986). Vaginal distension, however, was found to be a stimulus for oxytocin release in goats and sheep (Roberts, 1971; Peeters and Houvenaghel, 1973; Flint *et al.*, 1975; Blank and DeBias 1977); but not in cows (Cooper and

Foote, 1986) or rabbits (Fuchs *et al.*, 1965), and in rats only under deep anaesthesia (Dreifuss *et al.*, 1976).

The close temporal association between the release of oxytocin and PGF_{2α} seen in the present experiments in pony mares strongly suggests a causal relationship as seen in humans (Fuchs *et al.*, 1981), sheep (Roberts *et al.*, 1976; Sheldrick and Flint, 1985), cows (Mirando *et al.*, 1993; Fuchs *et al.*, 1996), rabbits (Small *et al.*, 1978) and guinea-pigs (Leaver and Seawright, 1982). From the positive correlation between endometrial oxytocin receptor concentrations and the release of PGFM in response to endogenous oxytocin observed in the present study, we infer that prostaglandin release in nonpregnant mares is mediated by endometrial oxytocin receptors. Support for this view is provided by reports on the effect of exogenous oxytocin on the duration of the oestrous cycle in mares (Neely *et al.*, 1979a; Goff *et al.*, 1987). Administration of oxytocin in the mid-luteal phase effectively shortened the oestrous cycle but was ineffective in the early luteal phase corresponding to high and low endometrial oxytocin receptor density, respectively. The data presented here are compatible with the notion that endogenous oxytocin of pituitary origin plays a role in normal luteolysis in cyclic mares. Luteal oxytocin content in mares is low and no cyclic variations in concentrations of plasma ir-oxytocin have been observed in mares (Stevenson *et al.*, 1991); however, the increase in oxytocin receptors in the late luteal phase may sensitize the uterine endometrium to low circulating oxytocin concentrations. Further studies are necessary to clarify this aspect of oxytocin physiology in horses.

Trauma-induced mobilization of arachidonic acid in endometrial tissue could be an alternative explanation for the release of PGF_{2α} after the biopsy procedure. The significantly lower protein concentration in B samples than A samples suggests that an inflammatory response was elicited within minutes of the procedure, resulting in vasodilation and tissue imbibition with fluid. However, there was little or no release of PGF_{2α} at oestrus or on day 8 even though the tissue trauma was similar on all days. Since oxytocin receptor concentrations were low on both days, we interpret the data to indicate that the paucity of endometrial oxytocin receptors was responsible for the poor or absent PGF_{2α} response on those days. In addition, the endometrial capacity to synthesize PGF may require previous exposure to progesterone as demonstrated in sheep by Eggleton *et al.* (1990). This would result in amplification of the PGF response in the mid- and late luteal phases. Indirect evidence for a stimulatory effect of progesterone on prostaglandin synthase activity in nonpregnant mares has been reported (Neely *et al.*, 1979b; Vernon *et al.*, 1981; Douglas and Ginther, 1982). These authors showed that peripheral plasma PGFM, uterine vein PGF_{2α}, endometrial PGF content and production rate *in vitro* increase with time after ovulation. Anecdotally, uterine flushing or oxytocin injections failed to cause PGF_{2α} release in a single ovariectomized mare after treatment with oestrogen, but after subsequent treatment with progesterone for 14 days the release was restored to pre-ovariectomy amounts (Betteridge *et al.*, 1985).

The virtual elimination of PGFM release in response to the biopsy procedure in early pregnant mares in spite of ir oxytocin release and oxytocin receptor concentrations, which equalled those in nonpregnant mares, and the failure of

exogenous oxytocin to induce PGF_{2α} release in early pregnant mares (Goff *et al.*, 1993), suggest that factors produced by the horse embryo cause the inhibition of PGF_{2α} release independent from the density of endometrial oxytocin receptors. By contrast, in ovine and bovine species, suppression of oxytocin receptors (Sheldrick and Flint, 1985; Fuchs *et al.*, 1990) coincides temporally with conceptus-induced inhibition of PGF_{2α} synthesis (Bazer *et al.*, 1986; Fincher *et al.*, 1986; Spencer and Bazer, 1996). The present results suggest that maternal recognition of pregnancy in horses involves suppression of PGF_{2α} synthesis and release without suppression of endometrial oxytocin receptor formation. On the basis of the present results, it is unclear whether this involves an uncoupling of the interaction of oxytocin receptors with PGF_{2α} synthesis, or results from a direct inhibition of prostaglandin synthase gene expression at either the transcriptional or translational level.

The decrease in oxytocin receptor affinity observed in early pregnant mares has not been reported for other species. The horse conceptus may therefore produce substances that alter the interaction of oxytocin with its receptor. The composition of membrane lipids is an important factor for receptor binding (Warr and Branda, 1983), and there is evidence that the binding affinity of oxytocin to myometrial membranes is critically dependent on the concentration of cholesterol in the plasma membrane (Fahrenholz *et al.*, 1995). Mother-embryo interaction in mares may involve a decrease in endometrial cell membrane cholesterol concentration that would result in lower receptor affinity without a change in receptor density.

The characteristics of the oxytocin receptor in equine endometrium are similar to those of the uterine oxytocin receptors in many other species in that high affinity to AVP and VP1-receptor subtype specific agonists and antagonists is exhibited. Binding data suggested the presence in the horse endometrium also of a high affinity VP1-receptor with lower binding capacity than the oxytocin receptor. Minor differences in ligand specificity in comparison to bovine endometrium (Fuchs *et al.*, 1990) were observed: these included lower affinity to isotocin and higher affinity to oxytocin antagonists than in bovine endometrium, and complete lack of binding of the VP2-receptor subtype specific agonist.

Hormonal regulation of oxytocin receptors in equine endometrium appears to differ from that in ruminants and rabbits. In these species endometrial oxytocin binding is maximal at the time of oestrus and is downregulated sharply in the luteal phase (cattle: Fuchs *et al.*, 1990; Jenner *et al.*, 1991; sheep: Roberts *et al.*, 1976; Sheldrick and Flint 1985; rabbits: Small *et al.*, 1978). In women, however, endometrial oxytocin receptor concentrations vary during the menstrual cycle in a manner similar to that in mares, although their density is much lower than in mares (Fuchs and Behrens, 1993). In women, the uterus has no influence on luteal lifespan, owing probably to the low endometrial concentrations of oxytocin receptors. Apparently, there are considerable species variation with regard to hormone control of uterine oxytocin receptor formation *in vivo*. In cattle, the expression of the oxytocin receptor gene and the receptor protein are well correlated, suggesting that regulation occurs mainly at the transcriptional level (Ivell *et al.*, 1995). Further insight into the regulation of oxytocin receptor concentrations in different species awaits advances in

the molecular biology of the receptor genes; such studies are in progress in several laboratories.

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