Effect of oestradiol on oxytocin-stimulated prostaglandin $F_{2\alpha}$ release in mares

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The objective of this study was to clarify the role of oestradiol in luteal function by examining its effect on the oxytocin stimulation of 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$ (PGFM) concentrations in cyclic mares. In the first experiment, three groups of mares (4 per group) were given a bolus injection of 17α -oestradiol (1 mg), oestradiol (1 mg) or vehicle on days 7, 9, 11, 13 and 15 of the cycle. Six hours later the mares were challenged with 10 iu oxytocin intravenously and frequent blood samples were taken from 15 min before to 15 min after for measurement of PGFM. Results showed a significant stimulatory effect of oestradiol (five times greater than controls at day 11; $P < 0.05$), but not of 17α -oestradiol, on the oxytocin stimulation of PGFM. As a relatively large dose was given systemically in this experiment, a second experiment was performed to introduce a dose that was more physiological into the uterus. Small Silastic spheres (1 cm diameter) were impregnated with or without oestradiol at a concentration that gave a release rate similar to that of embryos at day 12 (10 ng h^{-1}). These were inserted (one per mare) into the uterus of two groups of mares (five per group) on day 7. The mares were challenged with oxytocin on days 9, 11, 13 and 15 of the cycle and blood samples were taken as before for determination of PGFM. The results showed that oestradiol enhanced (four times greater than controls at day 13; $P < 0.05$) the oxytocin stimulation of PGFM concentrations. These data show that oestradiol enhances the oxytocin stimulation of prostaglandin release at the time of luteolysis. Thus, oestradiol produced by the embryo does not appear to prevent luteolysis or to be the signal involved in maternal recognition of pregnancy.

Introduction

Prostaglandin $F_{2\alpha}$ ($\text{PGF}_{2\alpha}$) is thought to be responsible for luteolysis in mares (Ginther and First, 1971) as in other large domestic animals. A considerable amount of work has been done in sheep (Wathes and Swann, 1982; Flint and Sheldrick, 1983) and cattle (Fields *et al.*, 1983; Lafrance and Goff, 1985) to clarify the role of oxytocin in luteolysis, and it is thought that oxytocin from the corpus luteum is responsible for stimulating the release of uterine $\text{PGF}_{2\alpha}$. In mares there is a pulsatile release of oxytocin at the time of luteolysis (Tetzke *et al.*, 1987) and oxytocin stimulates $\text{PGF}_{2\alpha}$ release at the time of luteolysis and this is inhibited in pregnant mares (Goff *et al.*, 1987).

Maternal recognition of pregnancy and the localized event of attachment or implantation have been suggested to involve the production of chemical signals by the embryo (Dickman *et al.*, 1976; Heap *et al.*, 1979). Evidence for a role of steroid hormones in early pregnancy was initially provided by Huff and Eik-Nes (1966) who showed that rabbit blastocysts can synthesize steroids from an acetate precursor. The pig blastocyst has been used extensively for the study of the role of oestrogens in

maternal recognition of pregnancy. It was originally shown by Perry *et al.* (1973) that porcine blastocyst tissue can aromatize androgen substrates to oestrone and oestradiol. Moreover, this activity appears at about day 12 of gestation (Heap *et al.*, 1981), which is the time of maternal recognition of pregnancy in this species (Dhindsa and Dziuk, 1968). Because the production of oestrogen is much higher in pigs than in many other species (Gadsby *et al.*, 1980) and exogenous oestrogen is luteotrophic (Gardner *et al.*, 1963), oestrogens are thought to be involved in embryonic signalling in this species. The horse embryo synthesizes oestrogen as early as day 6 (Paulo and Tishner, 1985) and produces considerable amounts of oestradiol and oestrone between days 12 and 20 (Zavy *et al.*, 1979; Flood *et al.*, 1979) and it has been suggested that oestrogen is involved in embryonic signalling in this species, too. This synthesis encompasses the time of maternal recognition of pregnancy, which in this species is believed to occur between days 14 and 16 (Hershman and Douglas, 1979) but may be as early as days 11–13 (Goff *et al.*, 1987). These findings resemble those obtained in sows, although it is uncertain whether oestrogens from the conceptus act in similar ways in the two species since there is a controversy as to whether exogenous oestrogens are luteolytic (Allen, 1979) or luteotrophic (Berg and Ginther, 1978) in mares.

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As the oxytocin stimulation of prostaglandin release is significantly reduced in pregnant animals, this system can be used as a model to determine whether particular substances have an antiluteolytic effect and therefore play a role in maintaining luteal function. The object of this study was to test the effect of oestradiol on the oxytocin stimulation of prostaglandin release in cyclic mares.

Materials and Methods

Animals

The experimental animals were 12 light horses, weighing 300–450 kg, that had exhibited normal oestrous cycles before the experiments. The animals were used from May to September. All mares were checked daily for oestrus with a vigorous stallion. During oestrus, ovaries were palpated once a day to detect ovulation.

Blood sampling and oxytocin injection procedures

Daily blood samples for progesterone determinations were taken from the jugular vein into heparinized vacutainers (Beckton-Dickinson, Rutherford, NJ, USA). The frequent serial samples for 15-keto-13,14-dihydro PGF_{2α} (PGFM) determination were taken by syringe through an indwelling catheter (Angiocath; Desert Medical Inc., Sandy, UT) placed in the jugular vein and then transferred to heparinized tubes. The catheter was kept patent by flushing with 2 ml heparinized saline (40 iu ml⁻¹; 10 000 units Hepalean ml⁻¹; Organon Canada Ltd, Toronto) after each sampling. Blood samples were taken every 5 min for 15 min before and then every 5 min for 15 min after the oxytocin injection. All blood samples (7 ml) were immediately kept at 4°C and centrifuged (15 min at 1500 g) within 45 min, and the plasma was stored at -20°C until it was used for hormone analysis. The oxytocin injections were of purified pituitary oxytocin in aqueous solution (Austin Laboratories Ltd, Joliette, Quebec) containing 20 iu ml⁻¹.

Experimental protocols

Experiment 1. The effect of intravenous injection of oestradiol (1 mg in 10 ml saline:ethanol; 9:1; Sigma, St Louis, MO) on the oxytocin stimulation of plasma PGFM was determined on different days of the oestrous cycle. Four mares per group were used in this experiment and were randomly assigned to the treatment groups. On the morning of days 7, 9, 11, 13 and 15, the mares were injected intravenously with 10 ml saline:ethanol (9:1), 1 mg oestradiol in 10 ml saline:ethanol or 1 mg 17α-oestradiol in 10 ml saline:ethanol. Six hours later they were challenged with oxytocin (10 iu).

Experiment 2. This experiment was performed to determine whether the effect of intrauterine administration of oestradiol on the response to oxytocin was different from that of intravenous administration. Silastic (382 medical grade elastomer, Dow Corning, Midland, MI) spheres were fabricated either with or without oestradiol. The spheres containing oestradiol were made by mixing 10 µl oestradiol (1 mg in 10 ml peanut oil) g⁻¹

of Silastic. When 1 cm diameter spheres were made it was determined that they released oestradiol at the rate of approximately 12 ng h⁻¹ when placed in a beaker containing 5 ml saline for 2 h. This rate is similar to the secretion rate of oestradiol by day 12 equine embryos during *in vitro* incubation (A. Goff, unpublished).

One sterilized sphere was introduced into the uterus (five mares per group) using an acrylic catheter (length 60 cm, i.d. 13 mm) on day 7 of the cycle. The catheter was introduced into the cervix under manual intravaginal guidance. With the aid of transrectal manipulation, the catheter was passed from there up to the base of one uterine horn. A sterile acrylic tube (o.d. 10 mm) was used to push the Silastic sphere down the catheter and into the uterus. The presence and position of the spheres in the uterus were monitored each day following insertion by means of real time ultrasonography. The spheres were expelled naturally by the mares during oestrus; if the mares did not return to oestrus during the experiment, oestrus was induced with PGF_{2α} at day 20. Oxytocin (10 iu) was injected on the mornings of days 9, 11, 13 and 15.

Radioimmunoassays

Aliquots (300 µl) of plasma were assayed for concentrations of PGFM by specific radioimmunoassay as described previously (Goff *et al.*, 1987). The antiserum was prepared by immunizing rabbits with 13,14-dihydro-15-keto PGF_{2α} (Upjohn Co., Kalamazoo, MI) conjugated to BSA (Sigma) by the use of the water soluble carbodiimide 1-ethyl-3 (3-dimethyl amino-propyl)/CDI (Sigma) according to the procedure described by Granström and Kindahl (1978). The crossreactivity for this antiserum at 50% displacement is 0.44% for 13,14-dihydro-15-keto PGE₂; 0.01% for 15-keto-PGF_{2α} and less than 0.01% for PGF_{2α}. The sensitivity of the assay was 10 pg ml⁻¹ plasma. The intra- and interassay coefficients of variation were 15 and 18%, respectively.

Aliquots of 50 µl plasma were extracted with petroleum ether and the progesterone content was measured by radioimmunoassay as described previously (Lafrance *et al.*, 1985). Recovery rates were between 80 and 95%. The intra- and interassay coefficients of variation were 12.5 and 14%, respectively.

Statistical analysis

Means were calculated for PGFM concentrations in the samples taken before oxytocin and in those taken after oxytocin. The effects of steroid treatment on the oxytocin stimulation of plasma PGFM concentrations were analysed by analysis of variance with repeated measures for time using the SAS GLM procedure (SAS, 1985).

Results

Experiment 1

The oxytocin stimulation of plasma PGFM during the oestrous cycle, and the effect of intravenous injection of 17α-oestradiol or oestradiol on this response, are shown (Fig. 1). There was a significant effect of day of the cycle ($P < 0.005$),

Fig. 1. Effect of intravenous injection of either 1 mg of oestradiol or 17 α -oestradiol on the oxytocin stimulation of plasma 15-keto-13,14-dihydro-prostaglandin F_{2 α} (PGFM) concentrations. Oestradiol (■), 17 α -oestradiol (▨) or vehicle (controls; □) was injected on the morning of days 7, 9, 11, 13 and 15, and oxytocin (10 iu) was injected 6 h later. Blood samples were taken every 5 min before and after oxytocin injection. Results are expressed as mean ($\pm$ SEM) increase in plasma PGFM during the 15 min after the oxytocin administration, for four mares per group. *Significantly different from control ($P < 0.05$).

oxytocin ($P < 0.001$) and hormone treatment ($P < 0.05$) with a significant interaction between day and oxytocin ($P < 0.0025$). In the control animals the response increased with day of the cycle. There was little effect of oxytocin on plasma PGFM at day 7 (88 ± 14 ng ml⁻¹ before and 99 ± 19 pg ml⁻¹ after oxytocin); however, oxytocin did increase PGFM concentrations (from 104 ± 27 to 523 ± 243 pg ml⁻¹) on day 11. Treatment of the mares with 17 α -oestradiol had no significant effect on the oxytocin stimulation but tended to decrease the basal concentrations of PGFM before the oxytocin injection. However, oestradiol had no effect on the basal concentrations (141 ± 38 pg ml⁻¹ at day 7 and 167 ± 44 pg ml⁻¹ at day 11) but did increase the oxytocin stimulation of PGFM (310 ± 85 pg ml⁻¹ on day 7 and 2520 ± 1320 pg ml⁻¹ on day 11) when compared with controls.

Although oestradiol increased the oxytocin stimulation of plasma PGFM concentrations, it had no significant effect on plasma progesterone profiles (Fig. 2). When the progesterone concentrations in individual mares were examined it can be seen that in half of the mares in each group luteolysis occurred at about the normal time (Fig. 2a), whereas in the other half the luteal phase was extended (Fig. 2b).

Experiment 2

The presence of the control spheres did not appear to affect the response to oxytocin. In the control animals there was an increase in oxytocin stimulated PGFM release at about the time of luteolysis similar to that in normal cyclic animals (Expt 1). The

intrauterine administration of oestradiol, with amounts similar to that released by the equine embryo, significantly increased ($P < 0.05$) the oxytocin stimulation of plasma PGFM concentrations (Fig. 3). The plasma concentrations of PGFM did not change with day for the control or the oestradiol treated group; the overall mean concentration for the control and oestradiol treated groups was 52 ± 6.2 and 60 ± 6.6 , respectively.

As in Expt 1, there was no effect of steroid treatment on the plasma progesterone profiles (Fig. 4). When the profiles of individual mares were examined both the control and treated mares exhibited normal and extended luteal phases and there appeared to be no difference between the two groups. The duration of the oestrous cycle agreed with the plasma progesterone data: for the control group, three mares had cycles of normal duration and two mares had prolonged cycles (> 25 days); for the oestradiol group, three mares had normal cycles, one had a prolonged cycle and one had a short cycle.

The Silastic spheres remained in the uterus in all mares until oestrus (either natural or induced), at which time they were expelled and could not be detected by ultrasound. All spheres moved in the uterus and there was no detectable difference between the two groups, although it is possible that differences might have been observed with more frequent observations.

Discussion

There has been a considerable amount of work done during the last few years on the factors responsible for luteolysis in cyclic

Fig. 2. Effect of intravenous injection of vehicle (○), 1 mg oestradiol (▽) or 17α-oestradiol (●) on plasma progesterone profiles: (a) the profiles of the mares with high progesterone and (b) the profiles of mares in which plasma progesterone dropped below 1 ng ml⁻¹.

Fig. 3. Effect of Silastic spheres containing oestradiol (■) or vehicle (□) on the oxytocin stimulation of plasma 15-keto-13,14-dihydro-prostaglandin F_{2α} (PGFM) concentrations. A sphere was inserted into the uterus on day 7; mares were challenged with oxytocin (10 iu) on days 9, 11, 13 and 15. Results are expressed as mean (± SEM) increase in plasma PGFM during the 15 min after the oxytocin administration, for five mares per group. Owing to the variability in the day when the maximum response was obtained, results were grouped according to the day of the peak response to oxytocin. *Significantly different from control ($P < 0.05$).

animals and how this is prevented when animals are pregnant. Although much is known of these mechanisms in cows, sheep and pigs, there is little information on the mechanisms controlling prostaglandin release and on the nature of the embryonic signal that is responsible for the maintenance of pregnancy in mares. The role of oestrogen, which is produced in large quantities by the equine embryo during days 12–16 (Zavy *et al.*, 1979; Flood *et al.*, 1979), has not been well defined. Oestrogen has been shown to have different effects in different species and the effects appear to be related to embryonic oestrogen synthesis, for example, in pigs, oestrogen is luteotrophic (Gardner *et al.*, 1963) and is produced in large quantities by the embryo (Gadsby *et al.*, 1980), whereas in cows and sheep it is luteolytic (Eley *et al.*, 1979) and little is produced by the embryo (Gadsby *et al.*, 1980). In mares it is not clear whether oestrogen is luteotrophic or luteolytic (Allen, 1979; Berg *et al.*, 1978).

The oxytocin stimulation of prostaglandin release increases at about the time of luteolysis in mares (Goff *et al.*, 1987). This result and the fact that pulses of oxytocin are detected during luteolysis in mares (Tetzke *et al.*, 1987) suggest that oxytocin is involved in luteolysis. As the oxytocin stimulation of PG release is suppressed in pregnant animals, it should be possible to use this system to test the effect of substances produced by the embryo on oxytocin stimulation of prostaglandin release to give some indication of their possible role in maintenance of pregnancy. The equine embryo produces large amounts of

oestrogen and it would be logical to assume that oestrogen has an antiluteolytic effect. However, the results of the experiments reported here show that oestradiol potentiates the stimulatory effect of oxytocin, which means that oestradiol does not have an antiluteolytic effect. This effect is specific for oestradiol since 17α-oestradiol did not affect the response to oxytocin. In addition, the results of Expt 1 would not appear to be artefacts caused by a systemic administration of a relatively large dose of oestradiol, as similar results were obtained when oestradiol was released into the uterine lumen in amounts that should have been in the physiological range. The reason for this apparent paradox of the equine embryo releasing relatively large quantities of oestradiol that potentiates the release of PGF_{2α} and thus is likely to compromise its survival, is unknown.

In both experiments there was a large variance in the stimulation of PGFM concentrations that was due, at least in part, to the different plasma progesterone profiles within groups. It has been shown by Stabenfeldt *et al.* (1981) that during the oestrous cycle the amplitude of the peaks of PGFM increase as progesterone concentrations decline. Thus, the animals that exhibited the normal decline in plasma progesterone concentrations seen at luteolysis had a greater response to oxytocin than those animals with an extended luteal phase.

The progesterone data are difficult to interpret since both control and treated animals exhibited short, normal and extended cycles. The prolonged high progesterone concentrations could be a result of oxytocin injections themselves. It has already been demonstrated in sheep (Flint and Sheldrick, 1985) that constant infusion of oxytocin will prevent luteolysis, and in mares daily injections of oxytocin will also prevent

Fig. 4. Effect of Silastic spheres containing oestradiol or vehicle on the plasma progesterone profiles: (a) individual profiles for each mare in the control group and (b) individual profiles for each mare in the group receiving Silastic spheres impregnated with oestradiol.

luteolysis (Goff *et al.*, 1987). Furthermore, uterine flushes can result in a prolonged luteal phase (Betteridge *et al.*, 1985), which suggests that modifying the release of prostaglandin at critical times during the cycle can also interfere with normal luteolysis. In Expt 2, it is possible that the shortened luteal phase was caused by bacterial endometritis resulting from inadvertent introduction of bacteria at the time of sphere insertion causing premature release of $\text{PGF}_{2\alpha}$ from an inflamed endometrium.

Oestradiol does not appear to be involved in luteal maintenance and the nature of the factor responsible remains to be determined. In cows and sheep, a specific protein (TP-1) is secreted by the embryo and this protein is responsible for maintenance of pregnancy. Although no specific proteins have been isolated from equine embryos, there are data showing that a factor responsible for inhibiting $\text{PGF}_{2\alpha}$ release in mares has a molecular mass of between 3.5 and 6 kDa (Sharp *et al.*, 1988). It is also possible that other steroids, perhaps in conjunction with a protein, play a role in maintenance of pregnancy. Previous data have shown that the equine embryo also synthesizes appreciable amounts of 17α -hydroxy-progesterone, which is metabolized to an as yet unidentified steroid in the endometrium and might affect prostaglandin release (Leduc *et al.*, 1988). Thus the possibility that other steroids affect uterine $\text{PGF}_{2\alpha}$ release remains to be examined.

The potentiation of oxytocin stimulation of PGFM by oestradiol indicates that, as in cows and sheep (Eley *et al.*,

1979), oestradiol could play a role in luteolysis in mares. The progesterone profiles were variable in all treatment groups, as discussed above, and these data do not provide any conclusive evidence that oestradiol is luteolytic. However, it is possible that the uterus becomes responsive to oestradiol only at about the time of luteolysis. If this is the case, then administration of oestradiol might well increase prostaglandin release, as we have shown, but will not significantly affect the time of luteolysis and the duration of the oestrous cycle. Further studies will be required to determine whether this hypothesis is correct.

In conclusion, the results of this study show that oestradiol enhances the oxytocin stimulation of $\text{PGF}_{2\alpha}$ release and therefore does not appear to be the embryonic factor responsible for inhibiting the release of $\text{PGF}_{2\alpha}$ during early pregnancy.

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