Effects of progesterone and oestradiol-17 β on oxytocin-induced release of prostaglandin F-2 α in heifers

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Summary. Seven bilaterally ovariectomized heifers were used in 4 experiments and received: (1) saline injections, as control; (2) one injection of oestradiol (3 mg; i.v.); (3) two i.v. injections of oxytocin (100 i.u.) 6 h apart; or (4) one oestradiol injection 30 min after the first oxytocin injection and a second oxytocin injection 6 h later. All experiments were performed without progesterone and then after 7, 14 and 21 days of progesterone treatment. Frequent blood samples were taken for 1 h before and 7 h after the first injection of oxytocin or oestradiol for the measurement of 13,14-dihydro-15-keto-PGF-2 α (PGFM) by radioimmunoassay. After 7, 14 and 21 days of progesterone priming, oestradiol caused a significant increase ($P < 0.001$) in plasma PGFM after 6 h but not before. After 7, 14 and 21 days of progesterone, there was a significant increase ($P < 0.005$) in PGFM after the first oxytocin injection and a similar increase following the second. The oxytocin-induced increase in PGFM after 14 and 21 days of progesterone was significantly higher ($P < 0.001$) 6 h after oestradiol injection than before the oestradiol injection. There was no significant effect of oestradiol on the response to oxytocin in animals that received no progesterone or in those animals that received progesterone for only 7 days. These results show that, under the influence of progesterone, oestradiol enhances the oxytocin-induced release of PGF-2 α , and suggest a possible synergistic action of these hormones for the induction of luteolysis in heifers.

Keywords: luteolysis; oxytocin; PGF-2 α ; progesterone; oestradiol; heifer

Introduction

The regression of the corpus luteum in the cyclic cow is now thought to result from an interaction between oxytocin, released by the corpus luteum (and perhaps the posterior pituitary), and the uterine endometrium resulting in the pulsatile release of the luteolysin prostaglandin (PG) F-2 α (Ginther *et al.*, 1967; Milvae & Hansel, 1980; Schams *et al.*, 1983; Wathes, 1984; Oyedipe *et al.*, 1984; Lafrance & Goff, 1985). Furthermore, there is evidence that follicular oestrogens are also involved in the luteolytic process. In sheep and cattle there is a release of oestradiol-17 β from the ovary coincident with PGF-2 α output from the uterus (Nancarrow *et al.*, 1973; Barcikowski *et al.*, 1974; Cox *et al.*, 1974) and exogenous oestrogens shorten the lifespan of the corpus luteum in cattle if administered during the luteal phase of the oestrous cycle (Eley *et al.*, 1979). The normal luteal regression in sheep is delayed by the destruction of growing follicles by X-irradiation (Karsch *et al.*, 1970) or by mechanical destruction (Ginther, 1970). The destruction of bovine follicles on Days 9, 12 or 15 after oestrus resulted in a heavier corpus luteum at Day 24 after oestrus compared to control animals (Villa-Godoy *et al.*, 1981).

Oestrogens are thought to act via the uterus since they are not effective in hysterectomized heifers (Brunner *et al.*, 1969). Several studies have shown that oestrogens can modulate uterine PGF-2 α production (Caldwell *et al.*, 1972; Barcikowski *et al.*, 1974; Baird, 1978). Bartol *et al.*

(1981) showed that oestradiol administered in the mid-luteal phase of the cow stimulated uterine synthesis and/or release of PGF into the uterine flushings and increased the peripheral plasma concentration of 13,14-dihydro-15-keto-PGF-2 α (PGFM). The luteolytic effect of oestradiol in the ewe is augmented by prior administration of progesterone (Caldwell *et al.*, 1972; Warren *et al.*, 1973; Louis *et al.*, 1977). However, there is a paucity of information on the hormonal requirements for the oxytocin-induced release of PGF-2 α in the cow. The objective of the present study was to determine the effects of progesterone and oestradiol on the oxytocin stimulation of PGF-2 α in ovariectomized heifers.

Materials and Methods

Animals. The experimental animals were Holstein heifers, about 37 months old and weighing 503–635 kg, that had been bilaterally ovariectomized 4–6 weeks before the start of the experiments.

Hormone treatments and blood sampling. Daily samples were taken for progesterone determinations from the coccygeal vein into heparinized vacutainers (Becton-Dickinson, Rutherford, NJ, U.S.A.) the day before, and for the 7 days following, the insertion of a progesterone-releasing intravaginal device (PRID; Ceva Co., Paris, France). The PRID, which maintained constant levels of progesterone, was replaced after a period of 7 days. The frequent serial samples for PGFM determinations were taken by syringe through an indwelling cannula in the jugular vein and then transferred into heparinized tubes (Becton-Dickinson). The cannula, tygon microbore tubing (i.d. 0.10 cm; Northern, Akron, OH, U.S.A.) was inserted into the jugular vein through a 13-gauge needle and was kept patent by flushing with 10 ml heparin (40 i.u./ml; Hepalean, Organon Canada Ltd, Toronto, Canada) in saline (0.85% sodium chloride) after each sampling. For all experiments the cannula was inserted 90 min before the injection of oestradiol or oxytocin, via the cannula, and frequent blood samples were collected beginning 30 min after cannulation as described in the experimental protocol. All blood samples (7 ml) were immediately placed in iced water and then centrifuged. The plasma was stored at -20°C until used for hormone analysis. The oestradiol injections were of 3 mg oestradiol (Sigma Chemical Co., St Louis, MO, U.S.A.) in 5 ml saline-ethanol (1:1 v/v). The oxytocin injections were of purified pituitary oxytocin in aqueous solution (Austin Laboratories Ltd, Joliette, Québec, Canada) containing 20 i.u./ml (USP).

Experimental protocol. All experiments were performed without progesterone priming and then after 7, 14 and 21 days of progesterone treatment. The same 7 animals were used in all experiments and were randomly assigned to experiments. Between experiments, animals were left for a period of 21 days without treatment before progesterone repriming.

Experiment 1: control. Animals received two saline injections 6 h apart and one injection of saline-ethanol (1:1 v/v) 30 min after the first saline injection. Blood samples were collected at 15-min intervals for 1 h before each injection of saline and at 5, 15, 30, 45 and 60 min after each injection. Three blood samples were also taken at 15-min intervals during the 3rd and 4th hour after the first saline injection.

Experiment 2: effect of oestradiol. The heifers received one i.v. injection of oestradiol (3 mg). Blood samples were taken at 15-min intervals for 1 h before injection of oestradiol via the cannula and then at 5, 15, 30, 45 and 60 min after injection. Three samples were also taken at 15-min intervals during the 3rd, 4th, 5th and 6th hour after oestradiol. The increase in PGFM is expressed as the mean of the concentration in the samples taken within each hour divided by the mean basal concentration before oestradiol for each animal.

Experiment 3: effect of oxytocin. The animals received two i.v. doses of oxytocin (100 i.u.) 6 h apart and an injection of saline-ethanol (1:1 v/v) 30 min after the first oxytocin injection. Blood samples were taken at 15-min intervals for 1 h before each injection of oxytocin and at 5, 15, 30, 45 and 60 min after each injection. Three blood samples were also taken at 15-min intervals during the 3rd and 4th hour after the first oxytocin injection. In this experiment and in Exp. 4, the increase in PGFM after oxytocin for each animal is expressed as the mean of the concentrations for the hour following oxytocin divided by the mean basal concentration before each oxytocin injection.

Experiment 4: effect of oestradiol and oxytocin. The heifers were treated with oestradiol (3 mg i.v.) 30 min after the first oxytocin injection (100 i.u.) and then received a second oxytocin injection (100 i.u.) 6 h later. Blood samples were taken at 15-min intervals for 1 h before each injection of oxytocin and at 5, 15, 30, 45 and 60 min after each injection. Three blood samples were also taken at 15-min intervals during the 3rd and 4th hour following the first oxytocin injection.

Radioimmunoassays. Concentrations of PGFM were determined directly in 300 μl samples of plasma using the method described by Granström & Kindahl (1982). The sensitivity of the assay was 20.8 pg/ml plasma. The intra- and inter-assay coefficients of variation were 16% and 18% respectively. Exogenous standards of 250 and 100 pg, when added to 1 ml PGFM-free plasma, gave values of 255 ± 11 and 91 ± 4 pg/ml (mean $\pm$ s.e.m., $n = 16$). Concentration

of progesterone was measured in 50 μ l plasma after extraction using petroleum ether. Recovery rates were between 85 and 94%. The antibody used was raised in rabbits against 11 α -hydroxyprogesterone 11-hemisuccinate-bovine serum albumin conjugate (Sigma Chemical Co.). The cross-reactivity of this antiserum at 50% displacement is: 45% for 5 α -pregnan-3,20-dione; 2.2% for 5 α -pregnan-3 α -ol-20-one; 0.9% for pregnenolone; 0.4% for 17 α -hydroxyprogesterone, oestradiol and oestrone; 0.2% for 20 α -dihydroprogesterone; and <0.05% for testosterone, androstenedione and hydrocortisone. The sensitivity of the assay was 62.5 pg/ml plasma. The intra- and inter-assay coefficients of variation were 12% and 13% respectively.

Statistical analysis. The results were analysed by analysis of variance, with repeated measures on all factors. Differences between individual means were tested using Duncan's New Multiple Range Test.

Results

In all experiments, plasma progesterone was <1 ng/ml before insertion of the PRID, and after insertion of the PRID the concentration remained constant, in samples taken every other day, at 3–4 ng/ml for the full 21 days of treatment.

There was no change in PGFM concentration at any time in the control animals (mean basal concentration = 115 ± 17 pg/ml). In Exp. 2 (Fig. 1), heifers not receiving progesterone were not responsive to oestradiol. However, after priming of the heifers with progesterone for 7, 14 and 21 days, plasma PGFM was significantly ($P < 0.001$) increased by 1.4-, 2.5- and 2.7-fold respectively 6 h after oestradiol administration, reaching concentrations of 280 ± 75 , 340 ± 92 and 332 ± 98 pg/ml.

Fig. 1. Increases in plasma PGFM concentration (mean $\pm$ s.e.m.) as % of mean concentration before oestradiol at 1, 3, 4, 5 and 6 h after an intravenous injection of 3 mg oestradiol in 7 heifers after 0, 7, 14 and 21 days of progesterone priming. * $P < 0.001$, compared to concentrations before oestradiol administration.

In Exp. 3 (Fig. 2), there was no significant increase in PGFM after the first or the second injection of 100 i.u. oxytocin in the animals not primed with progesterone. After 7, 14 and 21 days of progesterone treatment, there was a significant increase ($P < 0.005$) in plasma PGFM after

Fig. 2. Increases in plasma PGFM concentration (mean $\pm$ s.e.m.) after a first intravenous injection of 100 i.u. oxytocin and after a second similar injection 6 h later in 7 heifers receiving no progesterone priming (a) and after 7 (b), 14 (c) and 21 (d) days of progesterone treatment. The arrow indicates the time of oxytocin injection.

the first oxytocin injection (1.8 ± 0.2 -, 2.3 ± 0.4 - and 2.4 ± 0.4 -fold respectively) and a similar increase after the second dose (1.9 ± 0.3 -, 2.5 ± 0.3 - and 2.5 ± 0.4 -fold). There was, however, no significant difference between the response to oxytocin after 7, 14 or 21 days of progesterone priming. The concentration of PGFM increased within 5 min, reached peak levels between 15 and 45 min and was still significantly higher ($P < 0.05$) than time zero levels at 60 min after oxytocin. Plasma PGFM concentration returned to basal levels within 3 h after the first oxytocin injection. The concentrations of PGFM reached 30 min after the first and the second oxytocin injections were, respectively, 202 ± 33 and 185 ± 26 pg/ml for 7 days of progesterone, 232 ± 76 and 261 ± 55 pg/ml for 14 days of progesterone, and 284 ± 80 and 290 ± 52 pg/ml after 21 days of progesterone priming. The mean basal concentration of plasma PGFM was slightly higher (101 ± 4 pg/ml) before the first oxytocin injection than before the second (87 ± 2 pg/ml).

In Exp. 4 (Fig. 3), there was no significant effect of oestradiol on the response to oxytocin in animals that received no progesterone or progesterone for 7 days. However, after 14 and 21 days of progesterone there was a significant ($P < 0.001$) interaction between oestradiol and oxytocin on the stimulation of PGFM concentration with the oxytocin stimulation of PGFM greater 6 h after oestradiol injection (3.9 ± 0.5 - and 4.6 ± 0.5 -fold respectively) than before oestradiol (2.4 ± 0.3 - and 2.3 ± 0.3 -fold). The concentrations of PGFM reached 30 min after the first and the second oxytocin injections were, respectively, 311 ± 65 and 543 ± 80 pg/ml for 14 days of progesterone, and 255 ± 66 and 426 ± 66 pg/ml for 21 days of progesterone treatment.

As in Exp. 3, the mean basal concentration of plasma PGFM in Exp. 4 was higher (1.17-fold) before the first oxytocin injection than before the second. Comparison of the concentrations of PGFM 5 h after oestradiol in Exps 2 and 4 (i.e. before the second oxytocin injection in Exp. 4) showed that the first injection of oxytocin prevented the oestradiol-induced increase in plasma PGFM in animals primed with progesterone for 14 and 21 days.

The progesterone priming of the heifers had no significant effect on the basal levels of PGFM during the first hour of blood sampling in any of the experiments.

Discussion

In the sheep, both progesterone and oestradiol are necessary for the oxytocin stimulation of PGF- 2α . After a period of progesterone priming, oestradiol induces the synthesis of oxytocin receptors in the endometrial cell membranes. Subsequent binding of oxytocin to these receptors activates the

Fig. 3. Increases in plasma PGFM concentration (mean $\pm$ s.e.m.) after a first intravenous injection of 100 i.u. oxytocin and after a second similar injection 6 h later. An injection of oestradiol (3 mg) was administered 30 min after the first oxytocin dose in 7 heifers receiving no progesterone priming (a) and after 7 (b), 14 (c) and 21 (d) days of progesterone treatment. The arrow indicates the time of oxytocin injection.

arachidonic acid cascade resulting in PGF-2a release (McCracken *et al.*, 1984). In the cow, as in the sheep, there is a pulsatile release of oxytocin, presumably from the corpus luteum (Walters *et al.*, 1984), which appears to be responsible for the pulsatile release of PGF-2a at the time of luteolysis (Flint & Sheldrick, 1983; Vighio & Liptrap, 1986). The uterus is responsive to oxytocin stimulation at the end, but not during the middle, of the luteal phase of non-pregnant heifers (Lafrance & Goff, 1985). This increasing sensitivity to oxytocin may therefore be important for the timing of luteolysis. However, the hormonal control of prostaglandin release, especially in response to oxytocin, has not been resolved.

Many studies (Caldwell *et al.*, 1972; Warren *et al.*, 1973; Ford *et al.*, 1975; Louis *et al.*, 1977) have demonstrated that progesterone priming is necessary for PGF release by the uterus. However, progesterone alone does not seem to be responsible for provoking the surges of PGF-2a seen during luteolysis (Fairclough *et al.*, 1983). This is supported in the present study which has demonstrated that there is no significant difference in the basal concentrations of PGFM after different periods of progesterone priming. Plasma concentrations of PGFM have been used to monitor uterine PGF-2a release since Kindahl *et al.* (1976) have shown that changes in the concentration of PGFM in peripheral plasma reflect changes in PGF-2a secretion. The precise role of progesterone is not known but it has been proposed that the priming effect could be related to increasing the prostaglandin synthetase enzyme system and/or making available fatty acid precursors in the uterus (Brinsfield & Hawk, 1973; Barcikowski *et al.*, 1974), thus making the uterus sensitive to acute stimulation by other factors. Results of the present study show that progesterone priming for 7 days induces the responsiveness of the uterus to oxytocin, although 14 days are required to obtain the maximum response. These results vary slightly from observations on intact animals (Lafrance & Goff, 1985), in which no response to oxytocin was seen at Day 13 (i.e. after ~ 10 days of elevated progesterone concentrations). This suggests that in the cyclic animal the interactions between these hormones are more complex, the changes in oestradiol and progesterone that occur at the

beginning of the cycle possibly affecting events later on. Differences between intact and ovariectomized sheep have also been reported; for example, oestradiol does not stimulate prostaglandin release in intact cyclic animals without progesterone priming of the uterus but some studies have shown that it is able to do so in ovariectomized (Ford *et al.*, 1975) or anoestrous (Sharma & Fitzpatrick, 1974) animals.

Oestradiol also presumably plays an important role in prostaglandin release in the cow. An intravenous injection of oestradiol increased plasma PGFM concentration in the experimental heifers after progesterone priming of the uterus for 7 days. This agrees with the previously reported effects of oestradiol on PGF-2 α release in intact animals. Bartol *et al.* (1981) and Thatcher *et al.* (1984) have reported that the same dose of oestradiol, when given intravenously during the mid-luteal phase of the oestrous cycle, caused a rise in PGFM concentration that began at 3 h and reached a peak by 6 h before returning to basal levels by 9 h after oestradiol administration. The time course of the oestradiol stimulation differs from that reported for the sheep when an almost immediate increase in PGF-2 α release was seen (Roberts *et al.*, 1975) suggesting that oestradiol may be acting via different mechanisms in these species. Oestradiol has been shown to increase the activity of the PGF synthetase enzyme system (Ham *et al.*, 1975; Huslig *et al.*, 1979) and is therefore probably responsible for stimulating de-novo synthesis and release of prostaglandins. The possibility that oestradiol causes a release of oxytocin, from the posterior pituitary, which subsequently causes the increase in PGF-2 α release, cannot be ruled out. It is known that oestradiol treatment increases the reflex release of oxytocin in response to vaginal stimulation (Roberts & Share, 1969). However, Roberts *et al.* (1975) have shown that oestradiol does not increase oxytocin concentration in the absence of vaginal stimulation. Furthermore, the observed synergistic action of oestradiol and oxytocin on PGF-2 α release could not be explained if oestradiol was simply causing a release of oxytocin. It is therefore unlikely that oestradiol is indirectly stimulating prostaglandins via oxytocin.

In sheep, oestradiol is essential for inducing the response to oxytocin (McCracken *et al.*, 1984). However, progesterone priming for at least 10 days is required before oestradiol is able to exert its effect. The results of the present study show that exogenous oestradiol is not essential for the oxytocin stimulation of PGF-2 α release in the cow since progesterone priming alone for 7 days is able to induce the responsiveness of the uterus to oxytocin. Although oxytocin is able to stimulate PGFM concentration after 7 days of progesterone priming, acute treatment with oestradiol did not significantly increase this effect. There is a synergistic effect of oestradiol with progesterone on the oxytocin-induced release of uterine PGF-2 α only after the uterus has been primed for more than 7 days. The increase in prostaglandin release in response to oestradiol is abolished if oxytocin is administered before the oestradiol. This does not appear to be due to a lack of precursors or a decrease in enzyme activity since a second injection of oxytocin is able to elicit a large release of prostaglandin. This does, however, support the opinion that, at least after more than 7 days of progesterone priming, oestradiol stimulates prostaglandin release through a mechanism different from that of oxytocin, possibly by increasing the number of oxytocin receptors and thus increasing the sensitivity of the uterus to oxytocin stimulation, as occurs in the sheep endometrium (McCracken *et al.*, 1984).

It therefore seems reasonable to conclude that this synergism between progesterone and oestradiol is important for the oxytocin-induced release of PGF-2 α at the time of luteolysis. The present study demonstrates that a mechanism similar to that reported in the sheep may also exist in the cow. However, the exact mechanism whereby luteolysis is initiated remains unknown and requires further study.

We thank Mr Jacques Breault for technical assistance with the animals, Dr Patrick Guay and Dr Daniel Bousquet for ovariectomy of the heifers; Dr Hans Kindahl of the Swedish University of Agricultural Sciences, Uppsala, Sweden, for the gift of PGFM antiserum; and the NSERC of Canada for financial support. M.L. received a fellowship from the FCAR of Québec.

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Received 16 February 1987