Concurrent uterine venous and ovarian arterial prostaglandin F concentrations in heifers treated with oxytocin

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Summary. In preliminary studies 5 of 6 heifers given oxytocin subcutaneously on Days 2–6 of the oestrous cycle had depressed progesterone levels by Day 5, and each had a precocious oestrus and a normal preovulatory LH surge. Oxytocin treatments given once daily on Days 5 and 6, or twice daily on Days 5 and 6 or 4, 5 and 6 resulted in shortened cycles and lower jugular progesterone concentrations in 1 of 5, 2 of 4 and 4 of 5 heifers, respectively.

On the basis of these data, uterine venous and ovarian arterial catheters were inserted into 17 animals on Day 3 of the oestrous cycle and oxytocin treatments were given twice daily on Days 4, 5 and 6. Oxytocin treatment significantly depressed jugular progesterone levels by Day 8 and increased uterine venous prostaglandin (PG) F concentrations between 30 and 240 min after each injection. No changes of PGF concentrations occurred in uterine venous blood in control heifers, or in ovarian arterial blood from oxytocin-treated or control animals. It therefore appears that some factor other than PGF or a mechanism other than local veno-arterial transfer of PGF is involved in mediating the inhibitory effects of oxytocin on bovine corpus luteum function.

Introduction

Armstrong & Hansel (1959) first reported that daily oxytocin administration to cows early in the oestrous cycle resulted in precocious oestrus and shortening of the cycle length to 8–12 days. These inhibitory effects of exogenous oxytocin on the corpus luteum (CL) were absent in hysterectomized heifers. Local, utero-ovarian mechanisms were at least partly involved because oxytocin produced shortened cycles in unilaterally hysterectomized heifers if the remaining uterine horn was adjacent to the CL-containing ovary, but not if the remaining horn was opposite the CL (Ginther, Woody, Mahajan, Janakiraman & Casida, 1967).

Newcomb, Booth & Rowson (1977) reported increased plasma prostaglandin (PG) F concentrations in the posterior vena cava of heifers after oxytocin treatment on Days 2 and 3 of the oestrous cycle, but there was no effect on oestrous cycle length. Mitchell, Flint & Turnbull (1975) found elevated PGF levels in utero-venous effluent in pregnant ewes, and in the posterior vena cava of puerperal ewes, in response to oxytocin. Oxytocin treatment resulted in increased PGF levels in anoestrous ewes primed with oestradiol (Sharma & Fitzpatrick, 1974). Oxytocin addition to ovine endometrial tissue incubated *in vitro* caused release of PGF-2 α in a dose-dependent manner (Roberts & McCracken, 1976). However, comparisons of the effects of

oxytocin in the sheep and cow should be viewed cautiously, since oxytocin does not shorten the ovine oestrous cycle (Milne, 1963).

These interactions between oxytocin and PGF led us to examine whether PGF released from the uterus in response to oxytocin was reflected in changes in ovarian arterial PGF concentrations. To reduce to a minimum the period required for chronic cannulations, several preliminary experiments were performed to determine when and to what degree oxytocin affected plasma progesterone concentrations when given once daily on Days 2–6, and if these changes could be simulated by shorter periods of oxytocin administration.

Materials and Methods

Animals and treatments

Synthetic oxytocin (ICN Pharmaceuticals, Inc., Cleveland, Ohio, U.S.A.) containing 20 i.u./ml water was used for all experiments and each animal was injected s.c. with saline (0.154 M-NaCl) or oxytocin (0.33 i.u./kg body weight) at appropriate times. All animals used were virgin Holstein heifers, except for 2 mature Holstein cows used in Exp. 3. All animals had had 3 normal oestrous cycles (18–23 days) before the experiments.

Experiment 1. Twelve (12) heifers weighing between 389 and 582 kg were used; 6 were injected with oxytocin or saline at 12:00 h on Days 2–6 (Day 0 = day of oestrus, stood to be mounted) of the oestrous cycle. Blood samples were collected via jugular venepuncture once daily on Days 0 and 1, and at 6 h intervals on Days 2–12. Separate aliquots of citrated plasma were made for progesterone and luteinizing hormone (LH) determinations and stored at -20°C until analysed. Heifers were observed for oestrus during 30-min periods four times daily on Days 5–12 and twice daily thereafter until oestrus occurred.

Experiment 2. The objective of this experiment was to produce an inhibitory effect on the CL equal to that found in Exp. 1, but with shorter periods of oxytocin administration. Nineteen (19) heifers weighing between 310 and 515 kg were observed in oestrus and randomly assigned to one of 4 treatment groups: 5 were treated twice daily with saline on Days 5 and 6; 5 were treated once daily with oxytocin on Days 5 and 6; 4 were treated twice daily with oxytocin on Days 5 and 6; and 5 were treated twice daily with oxytocin on Days 4, 5 and 6. Jugular venous blood was collected once daily (12:00 h) on Days 0–3, and twice daily (12:00 and 24:00 h) on Days 4–12. Aliquots of plasma were stored at -20°C until assayed for progesterone concentrations. Heifers were observed for oestrus at least 3 times daily until the occurrence of oestrus.

Experiment 3. Fifteen (15) heifers and 2 cows weighing between 340 and 582 kg were used. Food and water were withheld for 48 and 24 h, respectively, before surgery. The animals were randomly assigned to either a control group or one of two oxytocin treated groups (see Table 1) as they were observed in oestrus, and ovarian arterial, uterine venous and jugular catheters were inserted on Day 3.

Table 1. Placement of catheters and numbers of animals in Exp. 3

Treatment	Ipsilateral uterine vein	Contralateral uterine vein	Ipsilateral ovarian artery	Contralateral ovarian artery
Saline	6			6
Oxytocin	6			6
Oxytocin		5	5	

The sampling ovarian arterial cannula consisted of an inner Silastic (Dow Corning, Midland, Michigan, U.S.A.) catheter (0.76 i.d. $\times$ 1.65 mm o.d.) fed through a protective outer catheter (1.98 $\times$ 3.18 mm). The tip of the inner catheter extended about 2 cm beyond the end of the

outer catheter. The two cannulae were attached with Silastic Medical Grade Adhesive (Dow Corning). A small ring of adhesive was placed 1.5 cm from the tip of the internal catheter to serve as an anchoring site when the catheter was inserted. The inner uterine vein catheter was 1.02×2.16 mm, and the outer protective cannula was 2.64×4.88 mm. Approximately 12 h before use, both the catheters were coated internally with a toluene–petroleum ether solution containing a quaternary salt (tridodecylmethyl ammonium chloride), and 7% heparin (TDMAC–heparin). The TDMAC–heparin solution (Polysciences, Inc., Warrington, Pennsylvania, U.S.A.) released small amounts of heparin when exposed to biological fluids thus reducing the risk of blood clots in the cannula.

Surgery

A jugular venous cannula was inserted into each animal on Day 3. General anaesthesia was then initiated with sodium thiamyl, and continued and maintained with halothane in oxygen. The reproductive tract was exposed by midventral laparotomy and a small uterine vein draining either the uterine horn adjacent or opposite to the CL-bearing ovary was dissected free. A small incision was made in the vessel wall and approximately 1.5 cm of the cannula was inserted downstream to a point where the vessel anastomosed with another uterine vein. Two or three silk ligatures were placed around the vein and catheter and several additional ligatures were placed in the serosa of the uterine body and broad ligament to prevent dislocation of the cannula.

A small branch of the ovarian artery supplying the CL-bearing or the contralateral ovary was dissected free, straightened and cannulated upstream. The branch chosen was always within the hilus of the ovary, and attempts were made to choose a branch that did not lead directly to the CL.

A small incision was made through the anterior vaginal wall, dorsal to the cervix, or in the flank and the catheters were exteriorized. The catheter tips were fitted with 18- or 22-gauge blunt needles and filled with heparinized saline (50 U/ml). Dilute heparinized saline (20 U/ml) was infused into the uterine venous and ovarian arterial catheters via Harvard infusion pumps at a rate of 5–6 ml/h following recovery from surgery and during the experimental period. Flank laparotomies were performed under local anaesthesia to remove the catheters on Day 10.

Sampling

Jugular venous blood was collected twice daily (08:00 and 20:00 h) on Days 0, 1, 2 and 3. On Days 4, 5 and 6, simultaneous samples were collected from the jugular vein (7 ml), ovarian artery and uterine vein (3 ml) at 5 min before and 5, 15, 30, 45, 60, 90, 120, 150, 240 and 550 min after each of the 6 oxytocin or saline treatments. Each 3 ml sample was treated with 0.3 ml 0.1 N-hydrochloric acid to inhibit PG metabolism. All samples were immediately placed in ice, centrifuged within 20 min and plasma aliquots were immediately frozen.

Additional jugular venous samples were collected on Days 7–21. Jugular venous plasma was analysed for progesterone and LH, and ovarian arterial and uterine venous plasma were analysed for PGF.

Assays

PGF. Acidified plasma samples (0.5 ml) were extracted in duplicate with 5 ml diethyl ether. The ether phase and PGF-2 α standards (8–640 pg in ethanol) were evaporated under a current of filtered air while the tubes were in a heated aluminium block (40°C). Antiserum specific for PGs of the F series (Hixon & Hansel, 1974) were diluted 1:4000 in phosphate buffer (pH 7.1) and 0.1 ml PGF antibody solution was added to each tube and shaken, before addition of 0.1 ml [3 H]PGF-2 α (sp. act. ~ 175 Ci/mmol; New England Nuclear Corp., Boston, Massachusetts,

U.S.A.). The tubes were shaken again and incubated overnight at 4°C. The antibody-bound and free PGF were separated by the addition of a dextran-charcoal suspension (1 ml).

In addition to unknown plasma samples, high and low plasma reference samples, solvent blanks, and plasma to which authentic PGF-2 α had been added, were run in each assay. Mean extraction efficiency was 88%, with a range of 85.4–92.6%. The recovery of added authentic PGF-2 α (0, 8, 16, 32, 64, 128, 256, 512, 1028, 2.056 pg) from acidified plasma with an initial level of 78 pg/ml ranged from 94–106% ($n = 70$). The lower limit of sensitivity for the assay was 4 pg/tube. The intra- and inter-assay coefficients of variation were 12.8 and 14.1% respectively.

LH. Concentrations were measured using a previously described radioimmunoassay (Niswender, Reichert, Midgley & Nalbandov, 1969). The sensitivity of the assay was 0.25 ng/ml and the intra- and inter-assay coefficients of variation were 8.9 and 11.1% respectively.

Progesterone. Concentrations were determined as described by Beal, Milvae & Hansel (1980). The limit of sensitivity of the assay was 25 pg/tube and the intra- and inter-assay coefficients of variation were 10.9 and 7.0% respectively.

Statistics

Progesterone and LH concentrations were each compared by one-way analysis of variance (Steel & Torrie, 1960). PGF data were assessed by split-plot analysis of variance for repeated measurements (Gill & Hafs, 1971). Selected contrasts were made with Student's t test.

Results

Experiment 1

Treatment with oxytocin once daily on Days 2–6 resulted in a precocious oestrus on Days 8–10 in 5 of 6 animals. The remaining heifer had a 21-day oestrous cycle. Mean plasma progesterone concentrations (Text-fig. 1) were similar in the treated and control heifers on Days 0–4, but from Day 5 the values were lower ($P < 0.05$) in the treated heifers. There were no differences ($P > 0.05$) in mean LH values between the treated and control heifers, except for the preovulatory peaks that were associated with the precocious oestrus in the 5 heifers having shortened cycles. The LH peaks ranged from 8 to 30 ng/ml for individual heifers.

Text-fig. 1. Mean ($\pm$ s.e.m.) jugular plasma progesterone concentrations in heifers treated once daily (arrows) on Days 2–6 of the oestrous cycle with oxytocin (●; $N = 6$) or saline (□; $N = 6$).

Experiment 2

Only 1 of 5 heifers treated with oxytocin once daily on Days 5 and 6 of the oestrous cycle had a shortened cycle. Treatment with oxytocin twice daily on Days 5 and 6 resulted in 2 of 4 heifers having a shortened cycle, whereas 4 of 5 heifers treated with oxytocin twice daily on Days 4, 5 and 6 had a shortened oestrous cycle. The precocious oestrus was observed on Days 8–10 in all animals in which it occurred while the others had 18–23-day oestrous cycles.

As shown in Text-fig. 2, on Day 6 and thereafter, progesterone levels were lower ($P < 0.05$) in the oxytocin-treated than in the control heifers and indicated reduced luteal function irrespective of the time of the next oestrus. Because treatment with oxytocin twice daily on Days 4–6 had an effect similar to that in Exp. 1, this treatment regimen was chosen for use in Exp. 3.

Text-fig. 2. Mean ($\pm$ s.e.m.) jugular plasma progesterone concentrations in heifers treated with saline (●; $N = 5$), oxytocin once or twice daily on Days 5 and 6 (Δ — Δ ; $N = 9$), or twice daily on Days 4, 5 and 6 ($\square$ — $\square$; $N = 5$) of the oestrous cycle.

Experiment 3

It was not possible to check for oestrus during the experimental period because the animals had to be restrained. Cycle lengths were therefore calculated on the basis of plasma progesterone and LH concentrations. By these criteria, none of the heifers had shortened cycles; treated and control animals each had mean cycle lengths of 20.5 days.

Plasma progesterone concentrations were lower ($P < 0.05$) in treated animals on Days 8–16, indicating an impairment of luteal function. A rather large increase ($P < 0.05$) in jugular plasma progesterone concentrations, from 0.36 ± 0.09 to 1.33 ± 0.16 ng/ml, occurred in all the heifers after surgery on Day 3 (Text-fig. 3). A 3- to 5-fold increase was measured between the pre- and post-surgery sample collected on Day 3 in 13 of 17 animals; this increase did not occur in animals not subjected to surgery (0.44 ± 0.08 to 0.69 ± 0.10 ng/ml; see Text-fig. 1). This increased level of progesterone was then maintained until Day 6 in each of the animals in which it occurred; thereafter progesterone concentration rose more rapidly in the controls.

There were no differences ($P > 0.05$) in the levels of PGF in the samples collected from the uterine veins and ovarian arteries ipsilateral and contralateral to the CL following oxytocin; therefore data for these two groups were combined.

Text-fig. 3. Mean ($\pm$ s.e.m.) jugular plasma progesterone concentrations in heifers treated (arrows) with saline (---; $N = 6$) or oxytocin (—, $N = 11$) twice daily on Days 4, 5 and 6 of the oestrous cycle.

Oxytocin treatment twice daily on Days 4–6 of the oestrous cycle resulted in significant ($P < 0.001$) elevations in uterine venous PGF concentrations, as compared with controls (Text-fig. 4a). Maximum concentrations of uterine venous PGF were found 45–240 min following each of the 6 oxytocin injections. PGF concentrations remained elevated for approximately 2–3 h in

Text-fig. 4. Mean ($\pm$ s.e.m.) plasma PGF concentrations in (a) uterine veins and (b) ovarian arteries in heifers treated (arrows) with saline ($\bullet$; $N = 6$) or oxytocin ($\square$; $N = 11$). When not shown, error bars are within symbols. Stippled bar in (b) is solely for reference, to facilitate comparison of the groups.

each case and mean levels were still slightly higher than means for controls before the 2nd and 4th injections. However, a further rise in uterine venous plasma PGF concentrations still occurred after oxytocin was administered. Mean uterine vein levels in control animals ranged from 288 to 487 pg/ml with no significant ($P > 0.05$) changes occurring during the experimental period.

In contrast, oxytocin treatment did not change ($P > 0.05$) ovarian arterial PGF concentrations at any time (Text-fig. 4b). Mean ovarian arterial PGF concentrations were 196 ± 5 pg/ml and 210 ± 4 pg/ml in treated and control animals respectively. The large increases in uterine venous PGF in response to oxytocin were not reflected in the ovarian arterial concentrations at any time during the experiment.

Discussion

In Exps 1 and 2, oxytocin caused complete inhibition of CL function in 5 of the 6 heifers treated once daily on Days 2–6, as was reported by Armstrong & Hansel (1959), and Harms, Niswender & Malven (1969), and progesterone concentrations were depressed on Days 5–12. The finding that LH concentrations were not affected by oxytocin was similar to that reported by Wilks & Hansel (1971). However, this is the first report indicating that there are preovulatory LH surges associated with the oxytocin-induced precocious oestrus, and that levels of LH attained at this time are similar to those that occur at the onset of oestrus in normal animals (Snook, Saatman & Hansel, 1971).

All animals treated with oxytocin, either once or twice daily on Days 5 and 6, or twice daily on Days 4–6, had depressed plasma progesterone concentrations. Since plasma progesterone levels were lower in all treated heifers, irrespective of cycle length, it appears that the inhibitory action is additive, and not an ‘all-or-none’ phenomenon. Luteal function was impaired by oxytocin even if the dose and duration of treatment were insufficient to cause complete inhibition of the CL.

In Exp. 3, oxytocin treatment increased uterine venous PGF levels in all 11 treated heifers, and the concentrations were similar to those found in the uterine vein of control heifers on Days 15–17 of the cycle, when luteolysis is normally initiated (Shemesh & Hansel, 1975). The finding that the ipsilateral and contralateral uterine horns in treated heifers responded similarly with respect to PGF output was not unexpected; uterine endometrial PGF content has been shown to be equal in the uterine horns in the cow, irrespective of their relationship to the CL (Shemesh & Hansel, 1975). Additionally, no differences were found in PGF concentrations in either ipsilateral or contralateral uterine venous plasma in ewes, implying that there is no local effect of the CL on uterine release of PGF (Pexton, Weems & Inskeep, 1975).

Newcomb *et al.* (1977) reported that intramuscular oxytocin injections given on Days 1–3 resulted in increases of PGF in the posterior vena cava of heifers. They found elevated levels 20 min after treatment, and a larger and more sustained rise 1–2 h following treatment. However, they reported no increase in posterior vena cava PGF concentrations after oxytocin administration on Days 4–6, which is in contrast to our experiments showing great elevations in uterine venous concentrations.

Data from these experiments do not support the hypothesis that transfer of PGF from the uterine vein to the ovarian artery is responsible for the inhibitory actions of oxytocin on the bovine CL. Mean levels of PGF, in both the ipsilateral and contralateral ovarian artery, were unaffected by oxytocin treatment. No increases in ovarian arterial PGF concentrations were found, even when uterine venous levels were greatly elevated. These findings support those of Shemesh & Hansel (1975), who reported that ovarian arterial PGF concentrations did not change at any time during the bovine oestrous cycle, while uterine venous levels increased 20-fold. However, Hixon & Hansel (1974) reported evidence of preferential transfer to the

ovarian artery of the cow following intrauterine administration of large amounts of PGF-2 α on Day 12 or 13 of the cycle. Unless the capacity for veno-arterial transfer of PGF-2 α changes during the oestrous cycle, it would appear that our earlier results may have been due to the relatively large amounts of PGF-2 α placed into the uterine lumen.

Land, Baird & Scaramuzzi (1976) concluded that the concentration of PGF in ovarian arterial blood of ewes was proportional to the log of the concentration in uterine venous blood and estimated that the rate of transfer was only 0.1–0.2%. Our failure to determine PGF concentrations in jugular venous blood or in an additional arterial source precludes any conclusions concerning changes in ovarian arterial levels relative to those in the general circulation. Nevertheless, the failure to find any significant differences between ovarian arterial PGF concentrations of control and treated animals is strong evidence that veno-arterial transfer did not occur in the oxytocin-treated heifers.

The finding that none of the treated animals in Exp. 3 had a shortened cycle, even though CL function, as indicated by depressed progesterone concentrations, was markedly inhibited in all of them, is perplexing, but could have been due to the stress and trauma of surgery, as well as the presence of catheters within the uterine vein and ovarian artery. Black & Duby (1965) have shown that epinephrine injections were effective in negating the ability of oxytocin to shorten the bovine oestrous cycle, and epinephrine stimulates progesterone synthesis when added to bovine luteal tissue (Condon & Black, 1976).

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References

- Armstrong, D.T. & Hansel, W. (1959) Alteration of the bovine estrous cycle with oxytocin. *J. Dairy Sci.* **42**, 533–542.
- Beal, W.E., Milvae, R.A. & Hansel, W. (1980) Oestrous cycle length and plasma progesterone concentrations after administration of prostaglandin F-2 α early in the bovine oestrous cycle. *J. Reprod. Fert.* **59**, 393–396.
- Black, D.L. & Duby, R.T. (1965) Effect of oxytocin, epinephrine and atropine on the oestrous cycle of the cow. *J. Reprod. Fert.* **9**, 3–8.
- Condon, W.A. & Black, D.L. (1976) Catecholamine-induced stimulation of progesterone by the bovine corpus luteum. *Biol. Reprod.* **15**, 573–578.
- Gill, J.L. & Hafs, H.D. (1971) Analysis of repeated measurements of animals. *J. Anim. Sci.* **33**, 331–336.
- Ginther, O.J., Woody, C.O., Mahajan, K., Janakiraman, K. & Casida, L.E. (1967) Effect of oxytocin administration on the oestrous cycle of unilaterally hysterectomized heifers. *J. Reprod. Fert.* **14**, 225–229.
- Harms, P.G., Niswender, G.D. & Malven, P.V. (1969) Progesterone and luteinizing hormone secretion during luteal inhibition by exogenous oxytocin. *Biol. Reprod.* **1**, 228–233.
- Hixon, J.E. & Hansel, W. (1974) Evidence for preferential transfer of prostaglandin F $_2\alpha$ to the ovarian artery following intrauterine administration in cattle. *Biol. Reprod.* **11**, 543–552.
- Land, R.B., Baird, D.T. & Scaramuzzi, R.J. (1976) Dynamic studies of prostaglandin F $_2\alpha$ in the utero-ovarian circulation of sheep. *J. Reprod. Fert.* **47**, 209–214.
- Milne, J.A. (1963) Effects of oxytocin on the oestrous cycle of the ewe. *Aust. vet. J.* **39**, 51–52.
- Mitchell, M.D., Flint, A.P.F. & Turnbull, A.C. (1975) Stimulation by oxytocin of prostaglandin F levels in uterine venous effluent in pregnant and puerperal sheep. *Prostaglandins* **9**, 47–56.
- Newcomb, R., Booth, W.D. & Rowson, L.E.A. (1977) The effect of oxytocin treatment on the levels of prostaglandin F in the blood of heifers. *J. Reprod. Fert.* **49**, 17–24.
- Niswender, G.D., Reichert, L.E., Jr, Midgley, A.R., Jr & Nalbandov, A.V. (1969) Radioimmunoassay of bovine and ovine luteinizing hormone. *Endocrinology* **84**, 116–1173.
- Pexton, J.E., Weems, C.W. & Inskeep, E.K. (1975) Prostaglandin F in uterine venous plasma, ovarian arterial and venous plasma and in ovarian tissue of pregnant and nonpregnant ewes. *J. Anim. Sci.* **41**, 154–159.
- Roberts, J.S. & McCracken, J.A. (1976) Does prostaglandin F $_2\alpha$ released from the uterus by oxytocin mediate the oxytocic action of oxytocin? *Biol. Reprod.* **15**, 457–463.
- Sharma, S.C. & Fitzpatrick, R.J. (1974) Effect of oestradiol-17 β and oxytocin treatment on prostaglandin F $_2$ alpha release in the anestrus ewe. *Prostaglandins* **6**, 97–105.

- Shemesh, M. & Hansel, W.** (1975) Levels of prostaglandin F (PGF) in bovine endometrium, uterine venous, ovarian arterial and jugular plasma during the estrous cycle. *Proc. Soc. exp. Biol. Med.* **148**, 123–125.
- Snook, R.B., Saatman, R.R. & Hansel, W.** (1971) Serum progesterone and luteinizing hormone levels during the bovine estrous cycle. *Endocrinology* **88**, 678–686.
- Steel, R.G.D. & Torrie, J.H.** (1960) *Principles and Procedures of Statistics*. McGraw-Hill, New York.
- Wilks, J.W. & Hansel, W.** (1971) Oxytocin and the secretion of luteinizing hormone in cattle. *J. Anim. Sci.* **33**, 1048–1052.

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