Unilateral control of ovarian oxytocin release and the facilitatory effects of insulin-like growth factor-I in sheep

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Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-induced release of ovarian oxytocin was investigated to determine whether the effect *in vivo* was local. [³H] $PGF_{2\alpha}$ infused downstream into a single ovarian lymphatic was transferred into the adjacent ovarian vasculature (estimated transfer 1.1 and 1.7%, two experiments). When unlabelled $PGF_{2\alpha}$ was infused in a similar manner (76 pmol min^{-1}), there was a prompt eightfold increase in ovarian oxytocin release from the adjacent ovary containing a corpus luteum, but no effect on the opposite corpus luteum, showing that the effect was local. Instillation of 2% lignocaine into the ovarian vascular pedicle did not affect $PGF_{2\alpha}$ -induced oxytocin release, supporting the idea that neural mechanisms are not involved. Repeated doses of $PGF_{2\alpha}$ given close-arterially produced a successive reduction in oxytocin release. This effect was prevented by a prior infusion of insulin-like growth factor-I (IGF-I), which itself gave a small, but significant, increase in oxytocin release. The results show that $PGF_{2\alpha}$ in ovarian lymphatics acts locally and directly to stimulate ovarian oxytocin secretion, that repeated exposure of the corpus luteum to pulses of $PGF_{2\alpha}$ can result in tachyphylaxis, and that this latter effect can be ameliorated by IGF-I infused *in vivo*.

Introduction

The ability of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) to stimulate *in vivo* the release of oxytocin by the corpus luteum in sheep (Flint and Sheldrick, 1982; Sheldrick and Flint, 1983) and cattle (Schallenberger *et al.*, 1984) is well established (Wathes and Denning Kendall, 1992 for review). Other compounds that produce a similar effect include the neurotransmitters acetylcholine and noradrenaline in sheep (Heap *et al.*, 1989) and noradrenaline in cattle (Kotwica *et al.*, 1991). Demonstration of a direct effect of $PGF_{2\alpha}$ *in vitro* has been more difficult (Hirst *et al.*, 1986, 1988; Wathes *et al.*, 1988; McArdle and Holtorf, 1989), although some studies have reported a dose-response effect with luteal slices at day 8 of the cycle. Release has also been inferred from cultured luteal cells (Abdelgadir *et al.*, 1987; Jarry *et al.*, 1992). The present study examines whether other mechanisms are involved *in vivo*, since our previous work showed that $PGF_{2\alpha}$ infused into a uterine lymphatic caused a rapid bilateral release of ovarian oxytocin. This result indicated that $PGF_{2\alpha}$ may act indirectly rather than directly on the corpus luteum (Heap *et al.*, 1989).

Materials and Methods

Animals

Multiparous non-pregnant ewes were obtained from the Institute's flocks of Clun Forest and Friesland sheep. The time of ovulation was regulated by the i.m. injection of $PGF_{2\alpha}$ analogue (cloprostenol: ICI Pharmaceuticals, Alderley Edge, Cheshire) using the technique referred to by Heap *et al.* (1985). Animals were superovulated (number of corpora lutea, 4.5 ± 0.7 , mean $\pm$ SEM) by the i.m. injection of 800 iu serum gonadotrophin (Folligon: Intervet Laboratories Ltd, Cambridge) 8 days after the first injection of cloprostenol (Estrumate: Coopers Animal Health, Crewe, Cheshire). The sheep were kept in paddocks adjacent to the laboratory and housed on the day before an experiment. All experiments were carried out at day 10 after ovulation.

Experimental methods

Unilateral transfer. Previous studies showed that $PGF_{2\alpha}$ infused into a uterine lymphatic caused a rapid bilateral release of ovarian oxytocin (Heap *et al.*, 1989). To test whether this was due to the local stimulation of nerves in the vascular

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pedicle, or to the transfer of $\text{PGF}_{2\alpha}$ across the broad ligament through the utero-ovarian lymphatic network, $\text{PGF}_{2\alpha}$ was infused into a single ovarian lymphatic which restricted its distribution unilaterally. Evidence for local transfer from an ovarian lymphatic into the adjacent ovary was gained using a modification of the technique described by Heap *et al.* (1985). Under pentobarbitone (RMB Animal Health Ltd, Dagenham) anaesthesia (20 ml 60 mg ml^{-1} , i.v.) a single ovarian lymphatic was exposed close to the hilus of the ovary and a polyvinyl catheter (0.5 mm i.d., 0.8 mm o.d.; Dural Plastics, Dural, NSW) inserted downstream. [^3H]Prostaglandin $\text{F}_{2\alpha}$ (specific activity 14.8 Ci mmol^{-1}), D-[^{14}C]mannitol (specific activity 55 mCi mmol^{-1} , marker of paracellular transfer) and ^{36}Cl (sodium chloride solution, [^{36}Cl]Na; > 3 mCi Cl g^{-1} , marker of rapid diffusion) obtained from Amersham International (Amersham, Bucks) were diluted in 0.9% (w/v) NaCl and kept frozen at -10°C until required (approximate concentrations for [^3H]PGF $_{2\alpha}$, [^{14}C]mannitol and [^{36}Cl]Na, 9, 4 and 4 $\mu\text{Ci ml}^{-1}$). Simultaneous infusion (4.1 ml h^{-1}) of all three compounds lasted for 60 min and blood samples from catheters placed in an ovarian vein, utero-ovarian vein and carotid artery were collected simultaneously at 5 min intervals for 120 min after the start of the infusion. Ovarian vein rather than ovarian arterial blood was collected to avoid any disturbance of ovarian haemodynamics.

Unilateral release. The effect of $\text{PGF}_{2\alpha}$ on ovarian oxytocin release was assessed by the unilateral infusion of unlabelled $\text{PGF}_{2\alpha}$ (76 pmol min^{-1} for 10 min, 4.1 ml h^{-1}) into an ovarian lymphatic. Blood samples were collected simultaneously at 2.5, 5 and 10 min after the start of the infusion from right and left ovarian veins (1 ml each) and a carotid artery (5 ml). Other details relating to sampling and measurement of radioactivity were as reported by Heap *et al.* (1989).

Ovarian infusion. Acetylcholine (5 $\mu\text{mol l}^{-1}$), $\text{PGF}_{2\alpha}$ (28 pmol min^{-1}) and recombinant insulin-like growth factor I (7.5 nmol IGF-I min^{-1} , batch no. GTS-2; Monsanto Company, St Louis, MO) were injected (acetylcholine) or infused close-arterially (PGF $_{2\alpha}$, IGF-I) through an indwelling polythene catheter (1.0 mm i.d., 1.5 mm o.d.) introduced into a femoral artery and advanced in a retrograde direction to lie cranial to the origin of the ovarian arteries (about 25 cm from insertion). The end of the catheter was blocked and fine holes were pierced at the end to ensure thorough mixing of injectate into arterial blood. The position of the catheter was confirmed by the infusion of Evans blue (1% in saline) with repeated sampling from the ovarian vein catheters. Analysis of Evans blue in blood of the ovarian vein showed that the mean dilution factor was 382 ± 79 ($n = 15$; Heap *et al.*, 1989). During test periods, blood samples were collected simultaneously from the ovarian vein (1 ml) and carotid artery (5 ml) at 2.5, 5 and 10 min after the start of the infusion, and during control periods at 10, 20 and 30 min after the end of the test period. Blood pressure and heart rate were recorded continuously using a pressure transducer (Transamerican Instruments, Basingstoke, Hants) connected to the carotid arterial catheter.

Assay. Oxytocin was measured using the assay described and validated by Sheldrick and Flint (1983).

Statistical analysis. For unilateral transfer studies, veno-arterial differences were calculated from the plasma concentration of radiolabelled compounds in the ovarian or utero-ovarian vein minus that in the carotid artery. Each value was plotted against time after the start of the infusion, and the area under the curve determined as described by Heap *et al.* (1985). For the release of endogenous oxytocin, a mean value of three samples collected during the 10 min infusion was calculated for each treatment, uncorrected for the carotid arterial value which was < 30 pg ml^{-1} . All endogenous values were transformed to the natural logarithm and the experimental results compared with control values by Student's *t* test.

Results

Ovarian lymphatic infusion of $\text{PGF}_{2\alpha}$

Transfer of $\text{PGF}_{2\alpha}$. [^3H]PGF $_{2\alpha}$, [^{14}C]mannitol and [^{36}Cl] were all transferred from an ovarian lymphatic into the ipsilateral utero-ovarian vein and ovary (as shown by ovarian vein

Fig. 1. Kinetics of transfer of (a) [^3H]PGF $_{2\alpha}$ (b) [^{14}C]mannitol and (c) [^{36}Cl]Na from a single ovarian lymphatic into the adjacent utero-ovarian vein and ovarian vein in a sheep 10 days after oestrus. Veno-arterial differences (utero-ovarian vein minus carotid artery, ovarian vein minus carotid artery) were calculated for blood samples collected during and after a 1 h infusion which was given downstream into an ovarian lymphatic. Ovarian vein values were obtained from blood collected at its origin from the ovary.

Table 1. Ovarian oxytocin release (mean $\pm$ SEM) induced by PGF_{2 α} (76 pmol min⁻¹ for 10 min) infused unilaterally into an ovarian lymphatic

Treatment sequence	Oxytocin concentration (pg ml ⁻¹)		Fold-change (compared with control 1)
	Back-transformed	Log _e	
Ipsilateral corpus luteum (n = 5)			
Control 1	645	6.47 ± 0.37	1.0
PGF _{2α}	5379	8.59 ± 0.55*	8.3
Control 2	1153	7.05 ± 0.38	1.8
Acetylcholine (i.a.) ^a	2298	7.74 ± 0.46	3.6
Contralateral corpus luteum (n = 3)			
Control 1	735	6.60 ± 0.42	1.0
PGF _{2α}	620	6.43 ± 0.23	0.8
Control 2	773	6.65 ± 0.79	1.1
Acetylcholine (i.a.)	3498	8.16 ± 0.77	4.8

Results show mean concentration during infusion period in superovulated non-pregnant sheep.

^aAcetylcholine (5 μ mol) was given as a single injection via a catheter placed cranial to the origin of the ovarian arteries. Statistical analysis of transformed data by paired *t* test comparing each treatment with the previous control value (**P* < 0.05).

Table 2. Ovarian oxytocin release (mean $\pm$ SEM) after repeated close-arterial infusions of prostaglandin F_{2 α} (28 pmol min⁻¹ for 10 min) via a catheter placed cranial to the origin of the ovarian arteries

Treatment sequence	Oxytocin concentration (pg ml ⁻¹)		Fold-change (compared to control 1)
	Back-transformed	Log _e	
Control 1	748	6.62 $\pm$ 0.55	1.0
PGF _{2α} first infusion	11304	9.33 $\pm$ 0.31**	15.1
Control 2	724	6.59 $\pm$ 0.42	0.9
PGF _{2α} second infusion	5054	8.53 $\pm$ 0.15*	6.8
Control 3	621	6.43 $\pm$ 0.42	0.8
PGF _{2α}	3425	8.14 $\pm$ 0.41	4.6

Results show mean concentration during infusion period in superovulated non-pregnant sheep (n = 4).

Statistical analysis of transformed data by paired *t* test comparing each treatment with the previous control value (**P* < 0.05, ***P* < 0.01).

values). Transfer of PGF_{2 α} into the ovary increased linearly over the 60 min infusion and declined steadily when the infusion ceased (*t*_{0.5} = 19 min). In contrast, mannitol and chloride reached a plateau after infusion for about 30 min (Fig. 1). In two sheep, the estimated transfer into ovarian blood was 1.7 and 1.1% for PGF_{2 α} , 0.7 and 0.6% for mannitol and 1.2 and 0.9% for NaCl. Transfer of the three labelled compounds into the utero-ovarian vein was 47.2 and 22.6, 85 and 43, and 75 and 48%, respectively.

Oxytocin release. To determine whether locally transferred PGF_{2 α} stimulated ovarian oxytocin release, unlabelled PGF_{2 α} was infused into a single ovarian lymphatic. This produced an eightfold increase in oxytocin release from the ipsilateral ovary. There was no change in oxytocin release from the contralateral ovary in animals with bilateral corpora lutea demonstrating that the effect was local. A subsequent injection of acetylcholine (intra-arterial) gave a bilateral release of oxytocin which

increased by three- to fourfold indicating that corpora lutea in both ovaries contained oxytocin (Table 1).

Repeated arterial infusion of PGF_{2 α} . To investigate whether repeated administration caused a diminishing oxytocin response, a close-arterial infusion of PGF_{2 α} was given for 10 min through a catheter positioned cranial to the origin of the ovarian arteries. The infusion was repeated at intervals of approximately 30 min and produced a declining response on three successive occasions (15.1, 6.8 and 4.6-fold increase in release). During the third infusion, the 4.6-fold increase was not significantly greater than that of the preceding control period (Table 2). Similar results were obtained when the experiment was repeated in four sheep with bilateral corpora lutea; the third infusion was replaced by a single injection of acetylcholine (5 μ mol; data not shown). Instillation of 2% lignocaine into the ovarian vascular pedicle after the first infusion failed to affect the results obtained.

Fig. 2. Effect of insulin-like growth factor-I (IGF-I, 7.5 nmol min⁻¹ for 10 min) on ovarian oxytocin release induced by PGF_{2α} (28 pmol min⁻¹ for 10 min). Infusions of saline (control), PGF_{2α} and IGF-I were given sequentially and close-arterially via a catheter placed cranial to the origin of the ovarian arteries in a superovulated anaesthetized sheep. Blood was collected from the ovarian vein of an ovary containing two corpora lutea for measurement of oxytocin. Statistical significance of oxytocin concentrations (log_e) after treatment compared with preceding control value (**P* < 0.05; ***P* < 0.01).

Effect of IGF-I on repeated PGF_{2α}-induced release of oxytocin. To test whether IGF-I influenced the release of ovarian oxytocin, the peptide was infused for 10 min after the first and second infusions of PGF_{2α}. Increased release of oxytocin occurred in three of five sheep at the first infusion of IGF-I, and

in all five animals at the second. Thereafter, a third infusion of PGF_{2α} produced a significant increase in oxytocin release (*P* < 0.01, Fig. 2, Table 3).

Discussion

Downstream infusion of PGF_{2α} into a single ovarian lymphatic caused a significant release of oxytocin from the adjacent, but not from the opposite, ovary. The increased release corresponded to the time when the transfer of radiolabelled PGF_{2α} infused into an ovarian lymphatic was first detected in ovarian blood. The estimated amount of PGF_{2α} transferred into the adjacent ovary (1.4%) was greater than that obtained in our previous studies for the transfer from a uterine lymphatic (0.4%). The kinetics of transfer of the paracellular marker, mannitol, and the rapid diffusion marker, sodium chloride, which reached peak concentrations in the ovarian vein within 30 min, corroborated our earlier conclusion that PGF_{2α} is probably transferred by the slower process of facilitated diffusion (Heap *et al.*, 1989). Thus the countercurrent transfer of PGF_{2α} into the ovary from an adjacent ovarian (or uterine) lymphatic provided the local stimulus for oxytocin release.

Failure of an intra-ovarian lymphatic infusion of PGF_{2α} to release oxytocin from the opposite ovary contrasts with our earlier result when an intrauterine lymphatic produced a bilateral response (Heap *et al.*, 1989). Cross-connections between uterine lymphatics on both sides of the broad ligament have been observed at surgery after injection of Evans blue into the wall of one uterine horn (R. B. Heap and I. R. Fleet, unpublished observations). Transfer of PGF_{2α} by this route is the probable explanation for our earlier results, since Evans blue injected into an ovarian lymphatic has never been detected in uterine lymphatics in the broad ligament. Lignocaine instilled into the vascular pedicle had no consistent inhibitory effect on oxytocin release whether induced by intra-lymphatic or close-arterial infusions of PGF_{2α}. Electrical stimulation of the ovary at the base of the hilus failed to

Table 3. Effect of insulin-like growth factor-I (7.5 nmol min⁻¹ for 10 min) on ovarian oxytocin release induced by prostaglandin F_{2α} (PGF_{2α} 28 pmol min⁻¹ for 10 min)

Treatment sequence	Oxytocin concentration (pg ml ⁻¹)		Fold-change (compared with control 1)
	Back-transformed	Log _e	
Control 1	347	5.85 ± 0.66	1.0
PGF _{2α} first infusion	2298	7.74 ± 0.78	6.6
Control 2	473	6.16 ± 0.27	1.4
IGF-I	566	6.34 ± 0.27	1.6
PGF _{2α} second infusion	1808	7.50 ± 0.59**	5.2
Control 3	391	5.97 ± 0.35	1.1
IGF-I	735	6.60 ± 0.13*	2.1
PGF _{2α} third infusion	1274	7.15 ± 0.55**	3.7

Compounds were infused close-arterially via a catheter placed cranial to the origin of the ovarian arteries in superovulated non-pregnant sheep 10 days after ovulation. Results are given as the mean concentrations measured in left and right ovarian veins during the infusion period (mean ± SEM; *n* = 5).

Statistical analysis of transformed data by paired *t* test comparing each treatment with the previous control value (**P* < 0.05, ***P* < 0.01).

stimulate oxytocin release (R. B. Heap, R. G. Dyer and I. R. Fleet, unpublished observations). These findings support the hypothesis that PGF_{2α} stimulates ovarian oxytocin locally and indirectly since there was no evidence from these studies for a neurally regulated mechanism.

Ovarian oxytocin release was quantitatively similar whether it was stimulated by intra-lymphatic or close-arterial infusion of PGF_{2α}. Estimates of the PGF_{2α} dose reaching the ovary indicated that the amount transferred across the vascular pedicle from a lymphatic infusion (0.8 pmol⁻¹ in 10 min) was similar to that which was infused close-arterially (about 0.7 pmol in 10 min). When the close-arterial infusion of PGF_{2α} was repeated three times, a tachyphylaxis occurred. This finding implied the depletion of oxytocin reserves in luteal cells or the downregulation of PGF_{2α} receptors. Luteal content of oxytocin declines gradually after its peak is reached in the mid-luteal phase (Wathes *et al.*, 1986, 1992a). The exponential fall in oxytocin mRNA after days 1–3 of the oestrous cycle (Fehr *et al.*, 1987; Jones and Flint, 1988; Ivell *et al.*, 1990) suggests that luteal oxytocin synthesis would be low by day 10, the time of the present experiments. This conclusion is also supported by the observation that the amount of oxytocin released after a third close-arterial PGF_{2α} infusion was similar to that achieved after the administration of a different agonist, acetylcholine. The effect of acetylcholine was similar when PGF_{2α} had previously been infused into a lymphatic. Since oxytocin is contained within small dense granules located in the large luteal cells (Fields and Fields, 1986; Theodosis *et al.*, 1986), the action of repeated doses of PGF_{2α} may be on exocytotic mechanisms or on post-translational processes acting on a preformed precursor within these granules (Camier *et al.*, 1991). Kotwica and Skarzynski (1993) demonstrated a declining response to repeated doses of noradrenaline in cows and suggested that it represented post-translational processing of the prohormone (Fehr *et al.*, 1987) rather than *de novo* synthesis.

The IGF-I enhancement of ovarian oxytocin release is consistent with studies of dispersed bovine luteal cells after long-term incubation (McArdle and Holtorf, 1989), perfused bovine corpora lutea incubated with high doses (Einspanier *et al.*, 1990), and ovarian IGF-I production in sheep (Wathes *et al.*, 1992b). The dose of IGF-I used in the present study was based on that which produces a physiological vasodilation in the mammary glands of goats *in vivo* (Prosser *et al.*, 1990). The finding that IGF-I sustains the response to repeated stimulation by PGF_{2α} was unexpected. It contrasts with the effect of chronic PGF_{2α} treatment *in vitro* which inhibits IGF-I-stimulated oxytocin release (McArdle and Holtorf, 1989). McArdle and Holtorf (1989), however, gave PGF_{2α} and IGF-I simultaneously, and doses of PGF_{2α} were in the micromolar–millimolar range, rather than the nanomolar range used in our experiments. The mechanism by which IGF-I reduces tachyphylaxis is unclear. IGF-I (and insulin) cause a marked increase in oxytocin mRNA in bovine granulosa cell cultures *in vitro* (Holtorf *et al.*, 1989), but this is an unlikely explanation of our findings since the infusions *in vivo* were short term (10 min). The effect of PGF_{2α} on oxytocin release is mimicked by a protein kinase C activator (McArdle and Holtorf, 1989), so it will be important to ascertain whether IGF-I influences the activity of protein kinase C in the corpus luteum. An intriguing

possibility is that IGF-I and PGF_{2α} mobilize different forms of secretory granules undergoing separately regulated secretion within one cell (Burgess and Kelly, 1987; Kelly, 1991).

In summary, the present findings *in vivo* support those *in vitro* that IGF-I acts on luteal cells to enhance oxytocin release after repeated PGF_{2α} treatment. Under the conditions of the present study, IGF-I influenced the corpus luteum in an endocrine manner and not only in the autocrine–paracrine manner, as proposed by Sauerwein *et al.* (1992).

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