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Effects of a luteolytic dose of oestradiol benzoate on uterine oxytocin receptor concentrations, phosphoinositide turnover and prostaglandin F-2α secretion in sheep*

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Summary. Administration of oestradiol-17β benzoate on Days 9 and 10 of the oestrous cycle resulted in episodic secretion of PGF-2α (as indicated by elevated circulating concentrations of 13,14-dihydro-15-ketoprostaglandin F-2α) and a decline in circulating progesterone. Release of PGF-2α began 35 ± 3 h after first injection of oestrogen and progesterone concentrations declined from 42 ± 3 h. Secretion of oxytocin, which was first observed 26 ± 3 h after oestrogen treatment, preceded secretion of PGF-2α; 69% of pulses of oxytocin coincided with episodes of PGF-2α secretion.

Uterine oxytocin receptor concentrations were raised in ewes treated with oestrogen, increases occurring in caruncular endometrium and myometrium by 12 h after treatment and in intercaruncular endometrium by 24 h. Raised receptor concentrations were followed at 24 h by increases in the incorporation of [^{3}H]inositol into phosphatidylinositol and in the hydrolysis of labelled tissue phosphoinositides in response to oxytocin in slices of caruncular endometrium incubated *in vitro*. The following sequence of events is therefore suggested to occur at oestrogen-induced luteolysis: (a) induction of the oxytocin receptor; (b) increased turnover of phosphoinositides; (c) onset of episodic secretion of PGF-2α; and (d) functional luteolysis.

Introduction

Uterine concentrations of the oxytocin receptor rise at luteolysis in sheep (Roberts *et al.*, 1976), but despite simultaneous measurements of circulating progesterone and the receptor it has not been possible to determine whether the rise in receptor precedes or follows luteal regression (Sheldrick & Flint, 1985). It is not known therefore whether induction of the oxytocin receptor causes the fall in circulating progesterone concentration at luteolysis or results from it. Evidence that it may play a causative role includes the fact that uterine secretion of prostaglandin (PG) F-2α, the luteolysin, is stimulated by oxytocin (Sharma & Fitzpatrick, 1974; Mitchell *et al.*, 1975), presumably through interaction with the oxytocin receptor.

Oestradiol-17β given during the mid-luteal phase of the oestrous cycle causes luteolysis in sheep and cattle (Brunner *et al.*, 1969; Bolt & Hawk, 1975; Hansel & Convey, 1983) and in rats and rabbits the oxytocin receptor is induced in uterine tissues after administration of oestradiol-17β (Soloff, 1975; Nissenson *et al.*, 1978). It is possible therefore that the luteolytic effect of oestradiol-17β may be mediated through induction of the oxytocin receptor. The present experiments were undertaken to determine whether oestradiol-induced luteolysis is associated with raised

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levels of the oxytocin receptor in sheep, and whether any such rise precedes or follows luteal regression and the fall in circulating progesterone. It was anticipated that the data obtained would improve our understanding both of the mechanism of the luteolytic effect of oestrogen and of the role of the oxytocin receptor in spontaneous luteolysis. Uterine phosphatidylinositol cycle activity was determined because its activation by oxytocin has been postulated to represent a source of arachidonic acid for synthesis of PGF-2α (Flint *et al.*, 1986).

Materials and Methods

Animals. Clun Forest ewes kept in a paddock were examined daily for oestrous behaviour; all 31 ewes cycled spontaneously, and each animal had exhibited at least 2 oestrous cycles with a mean (±s.e.m.) length of 16·6±0·2 days before experimentation.

The animals were allocated at random to 2 experiments. Experiment 1 was designed to determine the time course of changes in progesterone, 13,14-dihydro-15-keto-PGF-2α (DHKF-2α) and oxytocin after administration of oestrogen. Ewes were treated with 750 μg oestradiol-17β benzoate (N=5) at 12:00 h on Days 9 and 10 after oestrus (day of oestrus=Day 0) or with arachis oil (N=5), which was the vehicle for oestradiol benzoate. Blood samples were collected for measurement of progesterone, oxytocin and DHKF-2α at 1·5-h intervals beginning 3 h before treatment on Day 9 and continuing until 72 h after the first injection. Concentrations of progesterone were also determined in plasma from blood samples collected 84 and 96 h after first administration of oestrogen or vehicle.

For frequent removal of blood samples, a polyvinylchloride catheter, o.d. 2·1 mm, was inserted into an external jugular vein under local anaesthesia by the method of Seldinger (1953). Catheters were inserted at least 24 h before starting to collect blood samples. Immediately after catheterization, and during sampling, the ewes were kept in metabolism cages under conditions that allowed blood to be obtained without disturbing the animals (Flint & Sheldrick, 1983). The ewes were removed from the cages daily (usually between 12:15 and 13:00 h) for testing for oestrous behaviour with a vasectomized ram, which was housed in the same room as the ewes.

In Exp. 2, cyclic ewes were killed at intervals after administration of 750 μg oestradiol benzoate (given as in Exp. 1) for preparation of uterine oxytocin receptor and measurement of phosphatidylinositol turnover. Twelve animals were killed at 12, 24, 36 or 48 h after the initial oestradiol treatment (3 at each time) and 9 vehicle-treated ewes were killed at 12:00 h on Days 9, 10 and 11 (3 on each day). To ensure that patterns of secretion after treatment were comparable to those observed in Exp. 1, all animals in Exp. 2 were sampled at 1·5 h intervals during the 12 h preceding slaughter, using the catheterization and blood sampling protocol described under Exp. 1.

To obtain uteri, the animals were killed by intravenous administration of a lethal dose of pentobarbitone sodium; tissues were collected onto ice and were dissected immediately.

Determination of uterine oxytocin receptor concentrations. Membrane fractions were prepared from caruncular and intercaruncular endometrium and from myometrium by homogenization in 25 mM-Tris–HCl, pH 7·6 containing 0·25 M-sucrose and 1 mM-EDTA followed by differential centrifugation as described by Sheldrick & Flint (1985). Extracts were stored at −70°C until assay.

Oxytocin receptor concentrations were determined by incubating membrane fractions (10–54 μg protein; mean ± s.e.m., 35·2±1·2 μg) with 5 nM-[3,5-^{3}H-Tyr]oxytocin (sp. act. 36·6 Ci/mmol; New England Nuclear Corp., Nuclear Research Products, Stevenage, Herts) for 15 min at 25°C in the presence of 1 mM-$MnCl_2$. Separation of bound and free [^{3}H]oxytocin was carried out by filtration through Millipore (Durapore, Type GVWP) filters (Sheldrick & Flint, 1985). With reverse-phase high-performance liquid chromatography to confirm the purity of the tracer, >90% of labelled material migrated with authentic oxytocin. As receptor binding was determined at one (saturating) ligand concentration only, no information was obtained on receptor affinity.

Measurement of incorporation of [^{3}H]inositol into phosphatidylinositol. Individual endometrial caruncles dissected from the uterus with scissors were collected into ice-cold 0·9% (w/v) NaCl. Slices (~0·3 mm thick) cut by hand using a razor blade were transferred to ice-cold Krebs–Ringer–bicarbonate-buffered saline (Krebs & Henseleit, 1932) containing 10 mM-glucose (Buffer 1). After blotting and weighing, a number of slices (~100 mg) were transferred to glass scintillation vials and incubated in 1 ml Buffer 1 containing 10 μM-*myo*-[2-^{3}H]inositol (1 μCi/ml; Amersham International, Amersham, Bucks; diluted as required with unlabelled *myo*-inositol; Sigma Chemical Co., Poole, Dorset) with or without 10^{-7} M-oxytocin (Bachem, Torrance, CA, U.S.A.) at 37°C for 2 h. The vials were thoroughly gassed with O_2:CO_2 (95:5 v/v) and closed with a gas-tight stopper. Incubations were ended by removing the medium and homogenizing the slices in 10 ml unacidified chloroform-methanol (2:1, v/v). After separating the methanolic and chloroform phases by addition of 2 M-NaCl, and subsequently washing the chloroform extract 4 times with 2M-NaCl, the radioactivity incorporated into phosphatidylinositol was counted and expressed as d.p.m./g wet wt of slices (Flint *et al.*, 1986). All incubations were in triplicate.

Measurement of hydrolysis of phosphatidylinositol phosphates. Slices of caruncular endometrium were prepared and incubated as above in Buffer 1 containing 10 μM-*myo*-[2-^{3}H]inositol (10 μCi/ml) without oxytocin. After incubation for 2 h the medium was replaced with 2 ml Buffer 1 containing 10 mM-unlabelled inositol, and the slices incubated for a further 30 min. This medium was then removed and replaced with 1 ml Buffer 1 containing 10 mM-LiCl; after

incubation for a further 10 min 10^{-7} M-oxytocin was added, when appropriate, in 10 µl Buffer 1. Incubations were subsequently ended 20 min later by adding 1 ml ice-cold 15% (w/v) trichloroacetic acid.

After keeping on ice for 15 min, slices were removed by centrifugation, supernatants were decanted and trichloroacetic acid was removed by extraction 5 times with 5 ml water-saturated diethyl ether. Residual ether was removed under a stream of nitrogen at 40°C and the extracts were neutralized (to pH 7·0–8·5) by adding 0·5 M-NaOH in 0·5 M-Tris before storage at −20°C.

[^{3}H]Inositol and [^{3}H]inositol mono-, bis- and trisphosphates were separated by using columns of Dowex-1 formate eluted sequentially with solutions of increasing ionic strength (Richards *et al.*, 1979; Downes & Michell, 1981), and results were expressed as d.p.m./g wet wt of slices incubated. All incubations were performed in triplicate. Further details of the procedure are given by Flint *et al.* (1986).

Radioimmunoassays. Progesterone was measured in plasma samples after extraction with diethyl ether as described by Sheldrick *et al.* (1980). Sensitivity of the assays reported here (calculated from 2 × s.d. below the zero mass-added point) was equivalent to 0·12 ng/ml plasma. Intra- and inter-assay coefficients of variation were 7·4 and 9·1% respectively, and mean extraction recovery was 85·6 ± 2·3%.

Oxytocin concentrations were determined after extraction with activated glass powder as validated by Sheldrick & Flint (1981), using antiserum GJ137 (Sheldrick & Flint, 1985). Extraction recoveries of ^{125}I-labelled oxytocin added to plasma samples were 79·6 ± 1·5%, and sensitivity of the assay was 0·625 pg/ml plasma. Intra- and inter-assay coefficients of variation were 9·2 and 14·9% respectively.

DHKF-2α was measured as described by Mitchell *et al.* (1975) after extracting acidified plasma with diethyl ether. Extraction recoveries were 82·9 ± 3·2%; assay sensitivity was equivalent to 60 pg/ml plasma and intra- and inter-assay coefficients of variation were 7·9 and 10·0% respectively.

Values were corrected for extraction losses in all cases. All samples were assayed in duplicate.

Statistics. Differences in progesterone between the groups of animals in Exp. 1 were assessed using a linear model that permitted calculation of statistical probabilities of (a) effects due to oestrogen treatment, (b) effects arising as a result of differences between individual animals in each group, (c) changes with time and (d) the interaction between oestrogen treatment and time. Changes with time and the interaction between treatment and time were not independent variables, because repeated measurements were made on the same ewes, and effects of these parameters were therefore tested using a conservative F-test (Gill, 1978). The time at which concentrations of progesterone began to decline in oestrogen-treated animals was estimated by fitting linear and quadratic regression curves to segments of the data for each animal (Lerman, 1980).

Statistically significant peaks in secretion of DHKF-2α and oxytocin were identified using algorithms which assessed elevations of short duration in a series of observations by performing the following steps: (a) a base line was calculated representing the contribution of circadian rhythms or other long-term trends but not fluctuations of shorter duration; (b) a residual series that contained the secretory episodes, but not long-term trends, was calculated by subtracting base-line values; (c) the residual series was rescaled in terms of standard deviation units by dividing the rescaled values by an estimate of assay noise; (d) the peaks in the rescaled series were then identified by applying criteria of height and duration specified by user-defined cut-off points (G(n)). Peaks were thus identified as an individual subseries if they were elevated by G(n) standard deviation units (where values of G were selected by the user) for peaks of 1–5 datum points (n) in duration. (e) Steps (a)–(d) were repeated until 2 iterations produced the same values for peaks or until the preset limit, 6 iterations, was completed. Assay noise was estimated as a quadratic function of the quantity of hormone measured at each point (Merriam & Wachter, 1982). By using duplicate values for each sample the mean and standard deviation were calculated; the entire series of standard deviations was plotted against the corresponding means and a quadratic model was fitted to relate the 2 parameters. Points in the series where hormone concentrations were below the lower limit of sensitivity of the assay were excluded from this calculation. The numerical values for the quadratic (a), linear (b) and constant (c) terms of the model were: for DHKF-α, $a = 0{\cdot}00$, $b = 0{\cdot}11$ and $c = -0{\cdot}42$; for oxytocin, $a = 0{\cdot}00$, $b = 0{\cdot}08$ and $c = 0{\cdot}22$. The G(n) values were selected by an empirical approach using calibration data sets for each hormone. The following G(n) values were derived for both oxytocin and DHKF-2α: $G(1) = 4{\cdot}2$, $G(2) = 2{\cdot}4$, $G(3) = 1{\cdot}6$, $G(4) = 1{\cdot}0$ and $G(5) = 1{\cdot}0$. These calculations were carried out using a computer program, 'Pulsar' (Merriam *et al.*, 1983).

In Exp. 2, the effects of oestrogen on oxytocin receptor and hormone concentrations in the groups killed at 24 and 48 h (Table 1) were analysed by Student's *t* test. Variables in the oestrogen-treated groups killed at 12 or 36 h were compared to mean values for the control groups killed at 0 and 24 h or 24 and 48 h respectively. Effects of oestrogen and oxytocin on incorporation of labelled inositol into phosphoinositides (Table 2) were analysed by multivariate analysis of variance after logarithmic transformation. Both original and transformed data are presented.

Results

Experiment 1: effects of oestradiol-17β benzoate on luteal function and the secretion of PGF-2α and oxytocin

As expected from previous experience with the treatment used in this study (Hixon *et al.*, 1975), premature luteolysis occurred after administration of oestrogen. Circulating concentrations of

progesterone declined ($P<0{\cdot}01$) in all treated animals (Fig. 1); this decline began $42{\cdot}0\pm3{\cdot}0$ h after oestrogen treatment on Day 9. Progesterone levels were unchanged in control animals (Fig. 2f); concentrations of progesterone in this group at 84 and 96 h after treatment were $2{\cdot}87\pm0{\cdot}42$ and $2{\cdot}71\pm0{\cdot}17$ ng/ml respectively, compared to $0{\cdot}37\pm0{\cdot}14$ and $0{\cdot}45\pm0{\cdot}19$ ng/ml respectively in ewes receiving oestrogen. Oestrus was observed 67 ± 9 h after first administration of oestrogen; in control animals the interval from vehicle treatment to oestrus was 187 ± 9 h ($P<0{\cdot}01$).

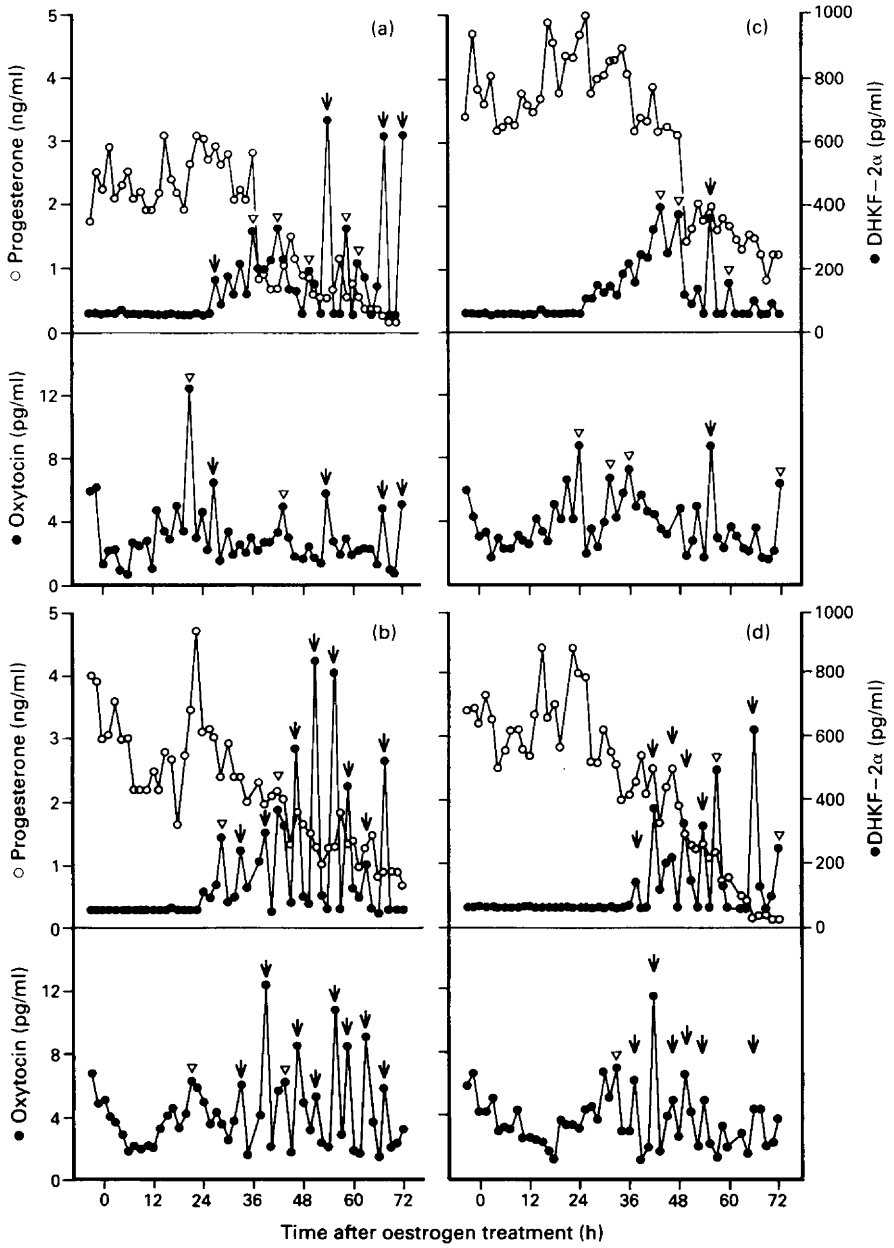

Fig. 1.

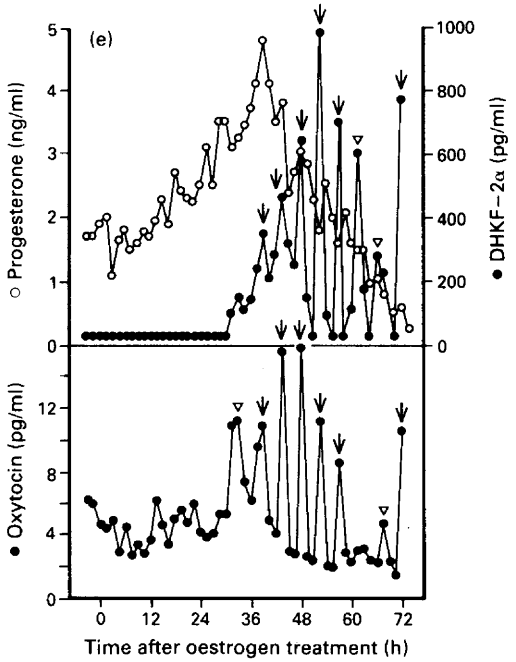

Fig. 1. Concentrations of progesterone, 13,14-dihydro-15-keto-prostaglandin F-2α (DHKF-2α) and oxytocin in jugular venous plasma from individual ewes (a–e) treated with oestradiol-17β benzoate at 12:00 h on Days 9 and 10 of the oestrous cycle. Zero time (0 h) represents 12:00 h on Day 9. Statistically significant episodes of secretion of DHKF-2α and oxytocin are indicated by arrows and inverted triangles; arrows identify synchronous episodes in both compounds, triangles indicate episodes in one compound only.

Episodes of secretion of DHKF-2α were observed in ewes that received oestrogen (Fig. 1), the first peak occurring an average of 35 ± 3 h after injection of oestrogen on Day 9. The mean number of peaks observed/ewe was 8 ± 1 and the mean interval between peaks was $4{\cdot}9 \pm 0{\cdot}2$ h. There were no episodes of release of DHKF-2α in control ewes (Fig. 2g) although concentrations of DHKF-2α occasionally exceeded the lower limit of sensitivity of the assay.

Peaks in the secretion of oxytocin were observed in all ewes receiving oestrogen (Fig. 1) and in 3 of the 5 vehicle-treated ewes (Figs 2a, b & c). The interval of 26 ± 3 h between the start of oestrogen treatment and the first peak in oxytocin was similar ($P > 0{\cdot}10$) to that (29 ± 9 h) for the 3 vehicle-treated ewes in which at least one episode of oxytocin secretion was observed. Because more episodes of oxytocin secretion were observed during the sampling period in ewes treated with oestrogen than in the vehicle-treated animals (7 ± 1 *vs* 3 ± 1; $P < 0{\cdot}01$), the interval between peaks was shorter in the former group (7 ± 1 *vs* 11 ± 2 h; $P < 0{\cdot}05$). The mean amplitude (increment above baseline) for peaks of oxytocin was $5{\cdot}50 \pm 0{\cdot}49$ pg/ml in oestrogen-treated ewes and $4{\cdot}87 \pm 0{\cdot}98$ pg/ml in controls ($P > 0{\cdot}10$). The first episode of oxytocin secretion after start of oestrogen treatment preceded ($P < 0{\cdot}05$) the first peak in DHKF-2α secretion (Fig. 1). However, the average number of peaks/ewe for oxytocin (7 ± 1) and DHKF-2α (8 ± 1) did not differ significantly and 25 of the 36 oxytocin peaks observed were coincident with peaks in the secretion of DHKF-2α.

Experiment 2: effects of oestradiol benzoate on concentrations of progesterone, DHKF-2α and oxytocin receptor and on synthesis and hydrolysis of phosphoinositides in response to oxytocin

Mean concentrations of progesterone during 12-h windows preceding the collection of uterine tissue at 12, 24 and 36 h after oestrogen treatment were similar ($P > 0{\cdot}10$) to those in animals given

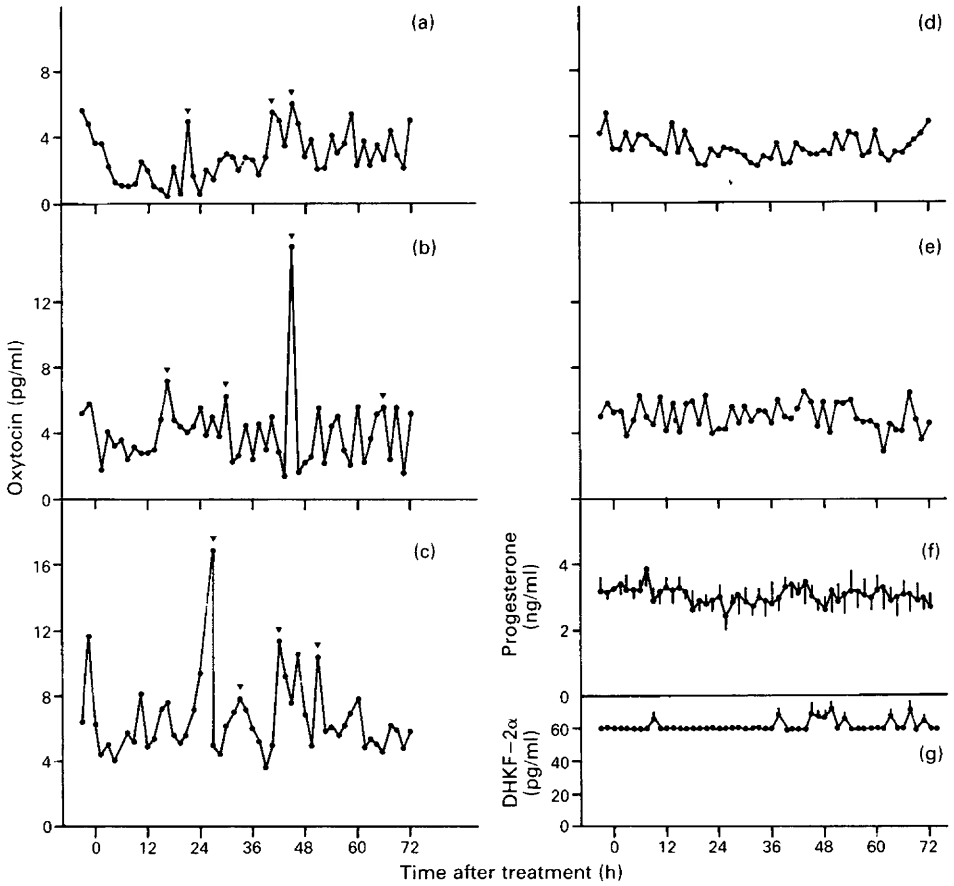

Fig. 2. Circulating concentrations of oxytocin (a–e), progesterone (f) and DHKF-2α (g) in control animals receiving arachis oil at 12:00 h on Days 9 and 10. All hormones were measured in jugular venous plasma. Values for oxytocin are shown separately for each of the control ewes in Exp. 1, as patterns of pulsatile secretion differed between animals. Statistically significant episodes of secretion of oxytocin are indicated by ▼.

arachis oil. However, oestrogen treatment resulted in a lower mean concentration of progesterone preceding slaughter at 48 h than was observed between 36 and 48 h in the vehicle-treated group (Table 1). Two pulses of DHKF-2α (194·6 and 292·7 pg/ml) were observed in 1 of 3 animals treated with oestrogen before slaughter at 24 h. Otherwise the patterns for concentrations of DHKF-2α were similar to those observed in Exp. 1. A pulsatile pattern was evident in all oestrogen-treated ewes before slaughter at 36 and 48 h which resulted in elevated mean concentrations of DHKF-2α (Table 1). Episodes in the secretion of DHKF-2α exceeding the criteria selected for peaks were not observed in ewes that received arachis oil or in oestrogen-treated ewes that were killed at 12 h (Table 1).

When treated with arachis oil, uterine concentrations of oxytocin receptor were similar in animals killed on Days 9, 10 or 11 after oestrus (Fig. 3). The administration of oestrogen increased ($P<0·05$) concentrations of oxytocin receptor in caruncular endometrium and myometrium at 12 h, and receptor concentrations were 4-fold higher in these tissues than in intercaruncular endometrium at this time. At 24 h oxytocin receptor concentrations were raised ($P<0·05$) in intercaruncular tissue from oestrogen-treated ewes relative to those given vehicle, and receptor concentrations

in all 3 uterine tissues from oestrogen-treated animals were higher at 36 and 48 h ($P<0.01$) than in control animals.

Effects of oestrogen treatment with and without oxytocin on incorporation of [^{3}H]inositol into phosphatidylinositol and inositol phosphates are shown in Table 2. Oestrogen treatment resulted in increased labelling of phosphatidylinositol, the maximum increase occurring after 48 h. Oestrogen alone had no effect on labelling of inositol phosphates. Oxytocin had no effect on incorporation of

Table 1. Concentrations of progesterone and 13,14-dihydro-15-keto prostaglandin F-2α (DHKF-2α) in plasma during 12-h periods preceding slaughter of groups of 3 ewes treated with arachis oil (control) or oestradiol benzoate (750 μg) at 12:00 h on Days 9 (0 h) and 10 (24 h) after oestrus (Exp. 2)

Time period (h relative to 12:00 h on Day 9)	Progesterone (ng/ml)	DHKF-2α (pg/ml)
Control		
−12 to 0 h	2·93 ± 0·18	60·2 ± 0·1
12 to 24 h	3·26 ± 0·20	61·4 ± 1·1
36 to 48 h	3·75 ± 0·20	62·5 ± 2·2
Oestradiol		
0 to 12 h	3·72 ± 0·19	61·0 ± 1·0
12 to 24 h	3·70 ± 0·15	87·4 ± 10·3†
24 to 36 h	2·92 ± 0·18	198·2 ± 26·0**
36 to 48 h	2·24 ± 0·34*	246·6 ± 35·8**

Values are means ± s.e.m. ($n = 27$) for samples collected every 1·5 h.
Effects of oestradiol: †$P < 0.10$; *$P < 0.05$; **$P < 0.01$. Values for 0 to 12 h and 24 to 36 h groups were compared to means for the −12 to 0 h plus 12 to 24 h and 12 to 24 h plus 36 to 48 h groups respectively.

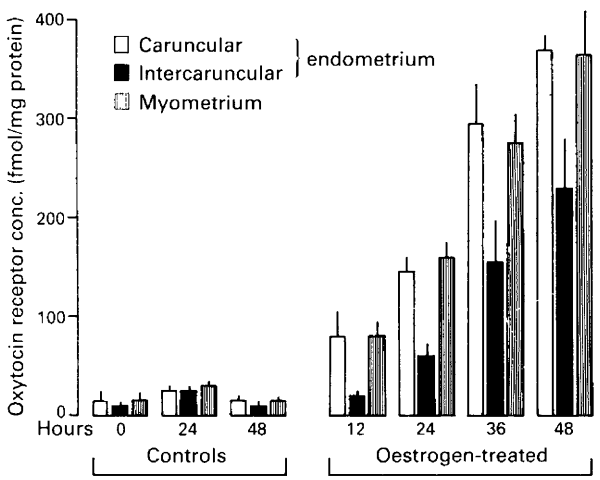

Fig. 3. Concentrations of oxytocin receptor measured in caruncular endometrium, intercaruncular endometrium and myometrium in ewes treated with oestradiol-17β benzoate or arachis oil. Ewes receiving oestrogen were treated at 12:00 h on Days 9 and 10 after oestrus and were killed at 24:00 h on Day 9 (12 h), 12:00 h on Day 10 (24 h), 24:00 h on Day 10 (36 h) or 12:00 h on Day 11 (48 h). Control animals, which received arachis oil at 12:00 h on Days 9 and 10, were killed at 12:00 h on Day 10 (24 h) or 12:00 h on Day 11 (48 h); untreated animals were killed at 12:00 h on Day 9 (0 h). Values are means ± s.e.m. for 3 ewes killed at each time.

Table 2. Phosphoinositide turnover in slices of caruncular endometrium incubated with and without oxytocin (10^{-7} M for 20 min)

Time after in-vivo treatment (h)	Addition of oxytocin *in vitro* (− or +)	10^{-3} × d.p.m./g wet wt incorporated into: PI	IP	IP_2	IP_3
Controls					
0	−	5·7 ± 0·2 (1·75 ± 0·035)	8·9 ± 3·5 (1·99 ± 0·463)	4·5 ± 1·6 (1·29 ± 0·436)	1·9 ± 1·0 (0·32 ± 0·540)
	+	5·2 ± 0·5 (1·65 ± 0·101)	2·9 ± 0·4 (1·04 ± 0·138)	2·3 ± 0·3 (0·83 ± 0·137)	1·0 ± 0·3 (−0·15 ± 0·367)
24	−	8·2 ± 1·4 (2·09 ± 0·179)	6·6 ± 3·7 (1·58 ± 0·530)	4·0 ± 2·1 (1·12 ± 0·499)	3·6 ± 2·6 (0·71 ± 0·740)
	+	9·1 ± 1·5 (2·18 ± 0·151)	5·0 ± 0·9 (1·58 ± 0·214)	1·9 ± 0·7 (0·54 ± 0·346)	2·4 ± 0·8 (0·68 ± 0·500)
48	−	13·1 ± 4·1 (2·48 ± 0·294)	20·9 ± 9·6 (2·84 ± 0·434)	13·6 ± 9·6 (2·07 ± 0·726)	8·5 ± 4·7 (1·83 ± 0·559)
	+	9·0 ± 0·5 (2·19 ± 0·060)	8·2 ± 0·9 (2·09 ± 0·114)	4·7 ± 0·6 (1·53 ± 0·148)	4·7 ± 1·6 (1·44 ± 0·345)
Oestradiol					
12	−	13·8 ± 1·8 (2·61 ± 0·143)	5·1 ± 3·9 (0·99 ± 0·798)	3·3 ± 2·6 (0·41 ± 0·872)	4·7 ± 4·4 (−0·90 ± 2·086)
	+	13·9 ± 4·3 (2·54 ± 0·313)	5·8 ± 2·3 (1·54 ± 0·515)	3·6 ± 1·6 (1·08 ± 0·406)	4·7 ± 3·5 (0·73 ± 1·011)
24	−	9·9 ± 2·1 (2·25 ± 0·204)	6·5 ± 1·3 (1·84 ± 0·197)	2·9 ± 0·6 (1·00 ± 0·257)	1·9 ± 0·8 (0·46 ± 0·400)
	+	14·8 ± 3·5 (2·64 ± 0·223)	20·7 ± 7·7 (2·85 ± 0·459)	6·9 ± 2·3 (1·76 ± 0·464)	5·2 ± 1·9 (1·49 ± 0·405)
36	−	24·3 ± 2·9 (3·18 ± 0·131)	8·2 ± 1·2 (2·08 ± 0·156)	5·3 ± 0·9 (1·65 ± 0·156)	5·0 ± 0·7 (1·60 ± 0·132)
	+	38·3 ± 3·1 (3·63 ± 0·134)	48·5 ± 9·1 (3·84 ± 0·214)	25·3 ± 4·2 (3·20 ± 0·176)	18·1 ± 6·4 (2·78 ± 0·326)
48	−	41·0 ± 6·1 (3·69 ± 0·144)	7·2 ± 0·7 (1·96 ± 0·120)	3·2 ± 0·5 (1·15 ± 0·156)	0·9 ± 0·1 (−0·13 ± 0·075)
	+	45·2 ± 8·7 (3·77 ± 0·213)	42·4 ± 18·9 (3·49 ± 0·542)	23·6 ± 13·0 (2·69 ± 0·771)	12·7 ± 7·5 (1·91 ± 0·938)

Values are means ± s.e.m. for incorporation of [^{3}H]inositol into phosphatidylinositol (PI), inositol monophosphate (IP), inositol bisphosphate (IP_2) and inositol trisphosphate (IP_3). All incubations were in triplicate; N = 3 animals at each time.

Values in parentheses are $\log_e$ transformed; statistical analysis was by multivariable analysis of variance.

[^{3}H]inositol into either phosphatidylinositol or inositol phosphates in the absence of oestrogen ($\chi^2 = 0{\cdot}43$; $P > 0{\cdot}1$), but labelling of inositol phosphates was significantly enhanced by oxytocin in animals killed after treatment with oestrogen ($\chi^2 = 9{\cdot}7$; $P < 0{\cdot}01$).

Discussion

The experiments described here demonstrate that luteolysis induced by oestrogen is preceded by increases in uterine concentrations of the oxytocin receptor and by an increase in the ability of oxytocin to stimulate phosphoinositide turnover *in vitro*. These results, together with measurements of circulating hormone concentrations in a parallel experiment, indicate that the sequence of events after oestrogen administration was: (a) induction of oxytocin receptor (by 12 h); (b) induction of the phosphoinositide response to oxytocin (by 24 h); (c) the onset of episodic PGF-2α secretion (at 35 ± 3 h); (d) functional luteolysis (beginning at 42 ± 3 h), and oestrus (at 67 ± 9 h). Episodes of PGF-2α secretion (as indicated by circulating concentrations of DHKF-2α) were preceded by secretion of oxytocin (first observed 26 ± 3 h after treatment) but this did not appear to be a response to oestrogen treatment since pulses of a comparable amplitude occurred in 3 of the 5 control ewes. A similar episodic release of oxytocin occurs during the mid-luteal phase of the oestrous cycle in cattle (Walters *et al.*, 1984). The coincidence of episodes of secretion of PGF-2α and oxytocin noted in treated ewes confirms observations made at spontaneous luteolysis (Fairclough *et al.*, 1980; Flint & Sheldrick, 1983). Uterine concentrations of the oxytocin receptor observed here 48 h after oestrogen treatment were similar to those reported previously at oestrus (Roberts *et al.*, 1975; Sheldrick & Flint, 1985) except that the increase in the myometrium was more pronounced in the present study. Phosphoinositide turnover parameters were comparable to those reported for ovariectomized ewes treated with progestagen and oestrogen (Flint *et al.*, 1986).

These findings raise the question of whether the induction of oxytocin receptor is responsible for the luteolytic action of administered oestrogen. Evidence that the receptor may play a role in this process includes the fact that oestradiol treatment causes secretion of PGF-2α (Fig. 1; Caldwell *et al.*, 1972; Barcikowski *et al.*, 1974; Ford *et al.*, 1975) which is an established response of the endometrium to oxytocin (Sharma & Fitzpatrick, 1974; Mitchell *et al.*, 1975; Roberts *et al.*, 1976); that immunization against oxytocin delays luteal regression (Sheldrick *et al.*, 1980; Schams *et al.*, 1983) and that uterine concentrations of the oxytocin receptor rise before spontaneous oestrus (Roberts *et al.*, 1975; Sheldrick & Flint, 1985). The present results show that receptor concentrations rise before any fall in the circulating concentration of progesterone in animals treated with oestrogen.

An increase in the responsiveness of the uterus to oxytocin, such as would be anticipated from the induction of the oxytocin receptor, may explain the enhanced sensitivity of the corpus luteum to PGF-2α in animals given oestrogen (Hixon *et al.*, 1975, 1983). Prostaglandin F-2α and its analogues are known to release oxytocin from the corpus luteum in ewes (Flint & Sheldrick, 1982; Heap *et al.*, 1986); administered PGF-2α may therefore stimulate production of additional endogenous PGF-2α, as a result of release of luteal oxytocin and its effect on the uterus, in animals with a raised uterine oxytocin receptor level.

The fact that induction of the oxytocin receptor preceded luteolysis in animals treated with oestrogen raises the possibility that the rise in receptor at the end of the oestrous cycle (Roberts *et al.*, 1976) plays a causative role in spontaneous luteal regression. The interval between receptor induction and reduced progesterone observed here (30 h) is close to the sampling interval (24 h) used in earlier work, which could explain why it was not previously possible to dissociate these events (Sheldrick & Flint, 1985). Gradual induction of the oxytocin receptor towards the end of the cycle may result from an increase in the sensitivity of the uterus to oestrogen (see McCracken *et al.*, 1984). Evidence supporting this suggestion includes the prolongation of luteal function after destruction of ovarian follicles by X-irradiation in sheep and cattle (Karsch *et al.*, 1970; Hixon

et al., 1975; Villa-Godoy *et al.*, 1985) and the failure to identify any significant rise in circulating oestrogen secretion preceding luteal regression (Roberts *et al.*, 1975; Baird *et al.*, 1976).

The mechanism by which oxytocin stimulates uterine prostaglandin synthesis has been suggested to involve increased hydrolysis of phosphatidylinositol phosphates with concomitant production of diacylglycerol, which is subsequently hydrolysed to release arachidonic acid (Flint *et al.*, 1986). In the present work oestrogen treatment induced a marked increase in the ability of oxytocin to stimulate phosphoinositide turnover *in vitro*, which might be expected to result from an increase in oxytocin receptor concentration.

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