Oestradiol-17 β both inhibits and stimulates myometrial activity in ewes *in vivo*

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Summary. Spontaneous intrauterine pressure cycles were abolished for periods of several hours in ovariectomized non-pregnant ewes within 8 h of an injection of 50 μ g oestradiol-17 β . Following the quiescent period, a phase of intense uterine activity ensued for several hours before again being replaced by several hours of quiescence. This active/inactive cycle disappeared after 2–4 days if further daily injections of oestradiol were not given. During the quiescent periods the uterus retained its responsiveness to oxytocin and PGF-2 α . Increasing the dose of oestradiol failed to prolong the periods of quiescence.

Introduction

During daily routine recording of intrauterine pressure for periods of 1–2 h from conscious ovariectomized ewes treated with oestradiol (50 μ g/day s.c.) we noticed that myometrial activity was erratic. For example a ewe might exhibit intrauterine pressure cycles of high amplitude ($\sim$ 45 mmHg) and high frequency (50 cycles/15 min) at 09:00 h and yet have no demonstrable uterine activity at 17:00 h or at 09:00 h the next day. As these observations were made during short (1–2 h) periods of recording we investigated the problems more fully by monitoring intrauterine pressure continuously for longer periods.

Materials and Methods

Ten Clun Cross ewes aged 4–7 years with a history of normal fecundity were ovariectomized bilaterally and equipped with intrauterine recording balloons according to the method described elsewhere (Lye & Porter, 1978). In addition the ewes received an indwelling Silastic catheter (Dow Corning, Midland, Michigan, U.S.A.; i.d. 2.64 mm; o.d. 4.88 mm) in one jugular vein. The animals were injected daily from the time of surgery with 50 μ g oestradiol-17 β (Sigma Chemical, London) in 0.25 ml corn oil. They were allowed at least 4 days to recover from surgery during which time they received antibiotic treatment (7 ml Strypen, May & Baker Ltd, Dagenham, Essex, i.m. daily for 5 days) before intrauterine pressure was monitored by connecting the balloon catheters to Bell and Howell pressure transducers, interfaced with an Ormed MX4 pen recorder (Ormed, Welwyn Garden City, Herts). Intrauterine pressure was monitored daily for several days to determine that spontaneous activity was present before oestrogen treatment was discontinued. The appearance of continuous intrauterine pressure cycles of low amplitude was taken as the time for

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the resumption of oestradiol treatment. Although this interval varied by up to 3 days among ewes, this was inevitable if a consistent level of uterine activity was to be used as a starting point. It is unlikely that this would have led to marked differences in uterine atrophy among the ewes as de Mattos, Kempson, Erdos & Csapo (1967) have shown that the presence of an intrauterine balloon markedly retards the process of uterine atrophy in ovariectomized animals. During the recording sessions, which lasted up to 24 h per day for up to 5 days, the ewes were kept in wooden crates which were large enough to permit them to lie or stand but not to turn round, although some forward and backward movement was possible. The ewes adapted readily to this restraint and showed no behavioural signs of stress. The experiments were conducted between February and April so that the animals received 11–14 h daylight per 24 h. The animals had access to water and hay at all times. Individual ewes were used in several treatment protocols but at least 5 days were always allowed to elapse between the end of one protocol and the beginning of the next. Animals were assigned to one of the following 3 categories.

Treatment I

In 6 ewes which had not received oestradiol treatment for at least 7 days intrauterine pressure was recorded for 2–3 days. Then injections of 50 µg oestradiol-17β in 0.25 ml corn oil s.c. were given daily for 3 days at 08:00 h. In 2 of the ewes at 0, 4, 8, 12 and 15 h after the initial oestrogen injection the chart speed of the pen recorder was increased to 100 mm/sec. This enabled the rate of rise of pressure of each intrauterine pressure cycle to be determined by calculating the ratio of the maximum amplitude of pressure to the time taken for the pressure to peak after rising from the baseline. Whenever possible the mean rate of rise of pressure of 10 cycles was determined.

Treatment II

Four ewes received oestradiol injections as in Treatment I. However, when spontaneous pressure cycles had been abolished each ewe received an injection of 250 µg oxytocin (Syntocinon; Sandoz, Feltham, Middlesex) via the jugular catheter, or, in a separate series of experiments, received an intrauterine infusion of PGF-2α (27 µg Dinoprost/min; Upjohn, Crawley, Sussex).

Treatment III

Four ewes received the following 3 treatment protocols in succession with a 5-day recovery between each: (i) 25 µg oestradiol-17β in 0.25 ml corn oil (s.c.) every 4 h for 4 occasions; (ii) 100 µg in oestradiol-17β in 0.5 ml corn oil (s.c.) at 08:00, 16:00 and 23:00 h; and (iii) 50 µg oestradiol benzoate (Sigma Chemicals, London) daily in 0.25 ml corn oil (s.c.).

Intrauterine pressure records were divided into 15 min intervals and analysed for maximum amplitude and frequency of pressure cycles.

Plasma oestradiol measurements

Method. Blood samples were obtained from 4 additional ewes treated identically to those in Treatment I (except that no intrauterine pressure records were taken) at 0, 1, 2, 3, 4, 5, 6, 8, 12, and 24 h after the first oestradiol injections. The samples were collected in heparinized vacuum tubes and centrifuged at 4°C. Plasma was separated and stored at –20°C until oestradiol-17β was measured by radioimmunoassay.

Plasma samples (1 ml in duplicate) were transferred to test tubes and extracted with 4 ml diethyl ether (Analar, BDH, Poole, Dorset, U.K.) from a freshly opened bottle for 5 min on a Denley multivortex mixer at a speed of 20. Samples were frozen at –15°C for 30 min, before the organic phase was decanted to a second set of tubes and evaporated to dryness. Recovery of oestradiol-17β

during this extraction procedure was estimated as follows: [^3H]oestradiol-17 β (0.1 ml in ethanol) was added to 8 extraction tubes and dried down. Plasma (1.0 ml) was added to each tube and these were mixed and incubated at 30°C for 15 min before ether extraction. A stock solution of 100 μg oestradiol-17 β (Sigma Chemicals, London)/ml ethyl alcohol was diluted to give a solution of 5 ng/ml in phosphate-buffered saline (PBS, 0.1 M, pH 7.1 containing 0.1% of both gelatin and sodium azide). This was serially diluted in buffer to give 9 standards ranging from 500 to 1.9 pg per 0.1 ml plus a tenth containing buffer only; 100 μl of each were pipetted into assay tubes in triplicate. Buffer (100 μl) was added to each of the extracted samples to equalize the reagent volumes before 100 μl antiserum (courtesy Dr J. R. G. Challis; sheep 20–102) were added to all samples and standards at a concentration of 1:13 000. After this 100 μl [2,3,6,7- ^3H]oestradiol-17 β (sp. act. 4 TB $_2$ /mmol: Radiochemical Centre, Amersham, Bucks, U.K.) were added at a concentration of 100 c.p.m./ μl . All tubes were vortexed and left to incubate overnight at 4°C.

To separate bound and free steroid 100 μl 0.5% gelatin in PBS were added to each tube, followed by activated charcoal (Sigma Chemical, London) and 0.1% dextran (clinical grade, Sigma Chemical, London) in 0.5 ml PBS. The tubes were incubated for 10 min at 4°C and centrifuged at 1500 g for a further 10 min at 4°C. The supernatants were decanted into small scintillation tubes, and 2.5 ml scintillation fluid were added to each (0.4% PPO (Fisons, Loughborough, Leics) in toluene). The tubes were incubated at 70°C for 20 min, capped and vortexed, and the labelled oestradiol was allowed to equilibrate between the aqueous and organic phases. The tubes were then counted for 5 min on a liquid scintillation spectrometer (Packard). All samples from the same sheep were analysed in the same assay.

Validation. Steroids tested which had >0.1% cross-reactivity with the antiserum were as follows: oestrone, 9.2%; oestradiol benzoate, 2.7%; oestriol, 0.7%; testosterone, 0.3%; androsterone, 0.3%; dehydroepiandrosterone sulphate, 0.2%. Solvent blanks contained <4 pg/tube, and water blanks <6 pg/tube. Sensitivity, defined as twice the s.d. of the zero value was 4.9 pg (10 assays). The recovery of radioactive oestradiol-17 β from 1 ml aliquants of plasma was $77.7 \pm 3.54\%$ (s.e.m., 8 assays). Accuracy was assessed by adding known amounts of oestradiol-17 β to 1 ml aliquants of plasma from an ovariectomized sheep which contained 2.8 ± 0.17 pg oestradiol-17 β /ml. The mean concentrations for 4 or 5 samples in each determination were: 7.8 pg added, 13.1 ± 3.98 pg measured (124% recovery); 31.2 pg added, 32.3 ± 8.02 pg measured (95% recovery); 125 pg added, 133.8 ± 5.10 pg measured (105% recovery) and 250 pg added, 196.9 ± 8.98 pg measured (78% recovery). The inter- and intra-assay coefficients of variation were 14.1% and 14.5% respectively. Plasma samples taken from one ewe at 5-h intervals during parturition showed a peak in oestradiol-17 β rising from undetectable levels 5 days before parturition to 128 pg/ml at 12 h before and falling again to 3.6 pg/ml by 12 h *post partum*. These values closely resemble those reported by Challis (1971).

Results

All ewes treated with oestradiol exhibited a qualitatively similar myometrial response.

Treatment I

All ewes in this group exhibited a similar pattern of myometrial activity during the period before oestrogen administration. This consisted of an almost continuous series of low-amplitude low-frequency intrauterine pressure cycles. After oestrogen treatment myometrial activity in all ewes declined and the uterus became quiescent. Total quiescence supervened in 306 ± 54 min (mean $\pm$ s.e.m., $n = 4$), and lasted for 431 ± 41 min before uterine mechanical activity reappeared. The activity then increased markedly both in frequency and amplitude and pressure cycles occurred at a higher rate and amplitude than before oestrogen treatment (Text-fig. 1). The periods

of intense activity were limited, having a mean $\pm$ s.e.m. duration of 462 ± 74 min since they alternated with further periods of quiescence of a mean $\pm$ s.e.m. duration of 198 ± 10 min over 48 h. The two uterine horns of each ewe behaved similarly, i.e. both were quiescent or active at the same time. In 2 ewes the rates of rise of intrauterine pressure were determined and were significantly greater during the hyperactive phase following oestradiol treatment (463 ± 16 mmHg/min) than before treatment (245 ± 26 mmHg/min: $P < 0.002$; paired t test).

Text-fig. 1. Frequency of intrauterine pressure cycles in the two uterine horns of an ovariectomized ewe given 50 μ g oestradiol s.c. at (a) 17:00 h and (b) 08:00 h in separate experiments. Note the long periods of quiescence which occur in both horns within 4–6 h of oestradiol administration and the phases of intense activity which interrupt the quiescence.

Treatment II

In all 4 ewes the myometrium responded markedly during the oestrogen-induced quiescent periods to intravenous injections of oxytocin (250 mu) or intrauterine infusions of PGF-2 α (27 μ g/min).

Treatment III

None of the dose regimens adopted for oestradiol-17 β nor the use of oestradiol benzoate appeared to alter the duration of the quiescent periods beyond that induced by a single injection of 50 μ g oestradiol-17 β (Treatment I). Indeed in ewes given 100 μ g oestradiol-17 β every 8 h the duration of the first quiescent period was shorter, i.e. 60–120 min than after 50 μ g (431 min). Moreover, the alternation of active/inactive periods was far less obvious (Text-fig. 2) than at the lower dose. Oestradiol benzoate injection was followed by an initial quiescent period of about 240 min but thereafter the clear distinction between active and inactive periods was again blurred and neither frequency nor amplitude achieved the high levels seen after 50 μ g oestradiol.

Text-fig. 2. Frequency of intrauterine pressure cycles in the two uterine horns of a ewe treated with 3 injections of 100 µg oestradiol-17β s.c. as indicated by arrows. Compare with Text-fig. 1.

Oestradiol measurements

Among the 4 ewes sampled after a single injection (s.c.) of 50 µg oestradiol-17β at 0 h, mean $\pm$ s.e.m. plasma oestradiol titres (pg/ml) were as follows: < 5 at 0 h; 5.2 ± 1.9 at 1 h; 11.4 ± 3.6 at 2 h; 11.7 ± 4.9 at 3 h; 9.8 ± 0.9 at 4 h; 12.9 ± 1.0 at 5 h; 11.6 ± 1.8 at 6 h; 5.9 ± 2.2 at 8 h; 5.7 ± 3.3 at 12 h and < 5 at 24 h.

Discussion

By recording intrauterine pressure continuously from conscious ovariectomized ewes over long periods we have revealed that a distinctive pattern of uterine activity develops in response to injections of oestradiol. When oestrogen is withheld from such ewes the uterus exhibits continuous, low-level activity which usually declines over a period of about 2 weeks. However, if oestradiol is administered the continuous activity gives way to complete inactivity for an initial period of several hours, followed by prolonged periods of intense activity interspersed with further periods of quiescence. The long periods of quiescence or activity explain the apparently erratic behaviour of the uterus which we had observed in ewes in which myometrial activity was recorded only for periods of 1–2 h/day. Although the cycles of active/inactive periods appear to occur at similar times of the day in different ewes after oestradiol injections at 08:00 h, this pattern differed from that which followed an injection time of 17:00 h in pilot experiments. This suggested that it was not an endogenous circadian rhythm but was related to the time of hormone administration.

The first period of total quiescence occurred in the present experiments about 5 h after the oestradiol injections, at a time when plasma titres of oestradiol were elevated, lasted for about 7 h, and then recurred again after a period of intense uterine activity of several hours when oestradiol titres were low. This alternation of behaviour disappeared after 2–4 days if further injections of oestradiol were not given but, from our experience of recording for short sessions from ewes treated with oestradiol daily over a long period, it seems probable that this alternating pattern could be sustained for at least several months.

Although these changes clearly occur in response to oestrogen injections it was not possible to

correlate them with the plasma concentrations of the steroid. However, the measurements made in ewes undergoing Treatment I indicate that the response is related to physiological titres of oestradiol: plasma titres did not exceed 24 pg/ml in any sample taken during the 24 h after oestradiol treatment.

Inhibition of the myometrium by oestradiol has not been reported before in sheep although Coutinho & de Mattos (1968) have described inhibitory effects of the steroid on the uterus in rabbits and Burns (1972), Fuchs (1974, 1976) and Downing, Lye, Bradshaw & Porter (1978) have published similar findings in rats. Although there are some differences in detail in the effects of oestradiol among the three species, in all of them the response to oxytocin appears to be spared when spontaneous activity is abolished or markedly reduced. In the ewe, as in the rat (Downing *et al.*, 1978; Wathes & Porter 1982), during recovery from oestrogen-induced quiescence the rate of rise of intrauterine pressure during spontaneous pressure cycles increased significantly. In the intact pig also, myometrial activity appears to exhibit alternation of activity and quiescence during the period of rising oestrogen levels in early oestrus (M. A. M. Taverne, personal communication).

A surprising feature of the action of oestradiol on the ovine uterus which occurs also in the pig (M. A. M. Taverne, personal communication) is the recurrence of periods of quiescence after the uterus has resumed spontaneous activity after the initial quiescent phase. Although this has not been reported for rabbits and rats it is possible that it may have been missed due to the relatively shorter daily recording periods used for the former (Coutinho & de Mattos, 1968) and in the latter by the very much longer periods of quiescence which are only interrupted from time to time by bursts of activity of short duration (Downing *et al.*, 1978).

The mechanism by which oestradiol exerts its biphasic action is unknown. Although it is possible to postulate mechanisms to account separately for the inhibition, e.g. synthesis of an inhibitory substance in response to oestrogen and for the periods of enhanced activity, e.g. prostaglandin (PG) secretion, or increased population of oxytocin receptors (Alexandrova & Soloff, 1980), it is very difficult to postulate a mechanism which would account for the alternation of these two states. Unpublished experiments (Lye, 1980) in which oestrogen-treated ewes were infused with the PG synthetase inhibitor, meclofenamic acid, suggested that this oestrogen-induced pattern of activity was prostaglandin-dependent, since both the high-activity and quiescent periods were abolished by this drug. The myometrial stimulatory effects of prostaglandins in sheep are well known (see Lye & Porter, 1978) and Lye & Challis (1982) have shown that prostacyclin (PGI-2) can inhibit spontaneous uterine activity in sheep.

Possible mechanisms should take account of catecholamines. Myometrial activity is inhibited by adrenaline in rats (Gaddum, Peart & Vogt, 1949; Levy & Tozzi, 1963; Vesin & Harbon, 1974) and oestrogen increases the uterine content of adrenaline in that species (Spratto & Miller, 1968a, b). However, Downing & Porter (1980) found that neither depletion of uterine catecholamine stores with reserpine nor blockade of α or β adrenoreceptors altered the extent or the time course of oestradiol inhibition in rats. Also, although administration of adrenaline as an intravenous bolus of 0.2 mg (3 ewes) or by intrauterine infusion (1 ewe) of up to 100 μ g/min abolished spontaneous uterine activity, it also blocked the response to oxytocin (S. J. Lye & D. G. Porter, unpublished data).

The suggestion has been made (Porter, 1979) that oestradiol-induced inhibition of myometrial activity may be mediated by relaxin synthesized in the uterus. There is no evidence for or against the existence of such a mechanism in the ewe although relaxin (porcine) inhibits the spontaneous activity of the ovine myometrium *in vivo* (Porter, Lye, Bradshaw & Kendall, 1981) and leaves the response to oxytocin unimpaired. Moreover, uterine synthesis of relaxin has been reported for the guinea-pig (Nagao & Bryant-Greenwood, 1981; Pardo & Larkin, 1982).

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