Local and systemic control of myometrial contractile activity during labour in the sheep

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Summary. The relative contribution of systemic versus local (intrauterine) factors in the activation and stimulation of the sheep myometrium during labour was examined using an in-vivo myometrial explant preparation. Myometrial tissue alone (MYO) or with attached endometrium (ENDO/MYO) was removed from the pregnant uterine horn, sutured to a stainless-steel frame and placed into the omental fat. After 7–10 days the explants developed a pattern of electromyographic activity qualitatively similar to that of the uterine myometrium. Induction of preterm labour by infusion of ACTH (66·6 ng/min for 15 min every 2 h) to the fetus resulted in a reduction in plasma progesterone concentrations and increases in values of oestradiol-17β and 13,14-dihydro 15-keto PGF-2α in maternal plasma. The onset of labour, which followed these endocrine changes, was characterized by an increase in EMG burst frequency and reduction in burst duration occurring simultaneously in both the uterine myometrium and in the explants. The response of the uterine and explant myometrium to oxytocin also exhibited a parallel significant increase over the 24-h period leading to delivery. No differences were apparent between the explants containing myometrial tissue alone or those comprising endometrial and myometrial tissue. There was no significant change in uterine or explant EMG activity, or oxytocin responsiveness, after saline administration to the fetus. The pattern of EMG activity changes during spontaneous labour were not distinguishable from those during ACTH-induced labour. As with oxytocin, the responsiveness of the explants to electrical stimulation increased significantly at labour compared to pre-labour. These data suggest that factors within the systemic circulation play a major role in both the onset of labour contractions and the increased response to electrical or hormonal (oxytocin) stimulation during parturition in sheep.

Keywords: myometrium; contraction; labour; parturition; sheep

Introduction

The sheep, like many other species, exhibits two distinct patterns of uterine contractile activity during pregnancy. From around Day 28–60 of gestation until the onset of labour, the uterus is virtually quiescent except for the presence of regular low-amplitude (~2–5 mmHg), low-frequency (0·5–3/h) contractions which have a duration of 5–8 min (Nathanielsz et al., 1976; Van der Weyden et al., 1981; Harding et al., 1982; Sigger et al., 1984) and which have been termed contractures by Nathanielsz et al. (1976). We have investigated this pattern of contractile activity in vivo during pregnancy using our explant model (Lye & Freitag, 1988), in which myometrial tissue is removed from the uterus, attached to a stainless-steel frame and sewn into the omental fat. Under these conditions the explant develops regular bursts of spontaneous activity within 7–14 days which are
very similar in appearance to the uterine contractures. Data obtained using this preparation led us to suggest that this underlying pattern of activity is not due to neural or direct systemic endocrine stimulation but to an intrinsic activity of the myometrium itself (Lye & Freitag, 1988), an observation consistent with the report of Sigger et al. (1984).

In contrast, labour is characterized by a change to high-frequency, high-amplitude contractions with a relatively short duration (~1 min; Harding et al., 1982; Lye et al., 1983a). We have suggested that this pattern of activity during labour is due to two separate but related events (Challis & Lye, 1986). Firstly, an activation of the myometrium, resulting in an increase in responsiveness, communication and ability to develop contractile activity of this muscle. Secondly, an increase in the production of substances (e.g. prostaglandins) that act to stimulate this already activated myometrium. We have suggested (Challis & Lye, 1986) that the process of activation is probably mediated by steroids (particularly an increase in oestrogen) and involves increased synthesis of gap junctions, receptors, and contractile proteins. Stimulation occurs as a result of increased production/release of agonists (oxytocin, stimulatory prostaglandins). It is generally accepted that a change in placental steroid metabolism leading to a fall in progesterone and an increase in oestrogen production is a prerequisite for the events that lead to normal labour (Thorburn & Challis, 1979; Challis & Lye, 1986). This change in maternal and intrauterine steroid metabolism is also associated with an increase in fetal and maternal plasma concentrations of the stimulatory prostaglandins (PGF, PGE) that precedes, and is believed to be responsible for, the onset of myometrial contractile activity indicative of labour (Olson et al., 1984). The elevated concentrations of prostaglandins are due to increased production within each of the intrauterine compartments (amnion, chorion, allantois, fetal/maternal cotyledons and myometrium: Olson et al., 1984). A critical question, however, is how do the steroids and prostaglandins reach the myometrium. Two routes are possible: (a) direct diffusion across the fetal membranes/endometrium/placenta (paracrine stimulation), or (b) transport via the uterine venous drainage and systemic circulation (systemic or endocrine stimulation). Evidence in man suggests that PGs can directly cross the fetal membranes providing a local or paracrine stimulatory route (Nakla et al., 1986). However, in the sheep uterine contractions occur simultaneously in both uterine horns of twin pregnancies, despite different local hormone environments (Brooks et al., 1988), arguing against a paracrine stimulation. In this study we have used our in-vivo explant model to determine whether the myometrial activation and myometrial stimulation during labour involve paracrine or endocrine pathways.

Materials and Methods

Surgical preparation

Twelve crossbred sheep of known single insemination dates were used. Surgery was performed at Days 110–113 of gestation under general anaesthesia (N₂/O₂/halothane). In 8 of the animals strips (2.5 × 3.5 cm) of myometrium alone (MYO explant) and endometrium with attached myometrium (ENDO/MYO explant) were removed from the pregnant uterine horn attached to stainless-steel frames and transplanted to sites within the omental vasculature as previously described (Lye & Freitag, 1988). In the other 4 sheep only myometrial explants were prepared. We have previously demonstrated (Lye & Freitag, 1988) that MYO explants so prepared are devoid of endometrial tissue, as determined by histological examination. Each of the explants was instrumented with two pairs of multi-filament stainless-steel (Cooner Wire, Chatsworth, CA, USA) electrodes attached at either end of the muscle strip to record electromyographic (EMG) activity (or provide electrical stimulation). Reference electrodes were sutured into the omental fat.

The fetal head and neck were then delivered through the uterine incision and polyvinyl catheters (V4 Bolab, Lake Havasu City, AZ, USA) inserted into the jugular vein and carotid artery to allow for infusion and blood sampling. A catheter was also inserted into the amniotic cavity to enable intrauterine pressure (IUP) to be monitored. The incisions in the fetal membranes and uterus were closed and a pair of EMG electrodes were sutured into the antimesometrial surface of the myometrium. Catheters (V11, Bolab) were inserted 20 cm into the maternal femoral artery and vein such that the catheter tips lay in the abdominal aorta and vena cava respectively, about 5 cm proximal to the bifurcation of these vessels.

The animals received post-surgical analgesia and prophylactic antibiotics to the ewe and fetus (Lye et al., 1983a). Catheters were flushed daily with heparinized saline (4 U/ml), under aseptic conditions.
Experimental protocol

Two protocols were used. In one the pattern of spontaneous myometrial activity and responsiveness to oxytocin of the MYO and ENDO/MYO explants were examined during labour induced by fetal administration of adrenocorticotrophin (ACTH). In the second the spontaneous activity of the MYO explants and their sensitivity to direct electrical stimulation was assessed during normal term labour.

Adrenocorticotrophin-induced labour. The 8 sheep prepared with MYO and ENDO/MYO explants were used in this experiment. Beginning on Day 125 of pregnancy, blood samples were collected daily from the fetal artery (2 ml), and the maternal artery and vein (5 ml each) and placed immediately on ice. The plasma was separated by centrifugation (1500 g for 10 min) and stored at −20°C until assayed. Part of the fetal sample was used to measure blood Po2, PCO2, pH and haemoglobin as an indication of fetal well being. The responsiveness of the explants and uterine myometrium to oxytocin was also examined daily, commencing on Day 124 as described below. On Day 127 the sheep were divided into two groups of 4 animals each. One group received a pulsatile infusion (via the fetal jugular vein) of ACTH (66.6 ng/min for 15 min every 2 h). We have previously shown that, using this regimen, labour contractions first appear in 91.7 ± 8.7 h after the start of the ACTH infusion and labour is fully developed by 99.0 ± 4.1 h when administered to singleton fetuses (Lye et al., 1983a), whereas in twin pregnancies labour occurs after 204.0 ± 29.5 h (Brooks et al., 1988). In this study mixed singleton and twin pregnancies were used and the mean duration of ACTH administration to labour was 174.0 ± 22.7 h. The control group received 0.5 ml saline over a 15-min period every 2 h for 192.0 ± 9.8 h.

To assess changes in the responsiveness of the explant and uterine myometrium to hormone stimulation during the induction of preterm labour, oxytocin was administered to the sheep, daily, in a dose response fashion beginning on Day 124. Oxytocin (Syntocinon: Sandoz, Dorval, Quebec: 50, 100 and 200 mU in 0.2, 0.4 and 0.8 ml saline (0.9% w/v NaCl) respectively. In preliminary studies the response of the myometrium to oxytocin was partly dependent on the time from the previous spontaneous contracture that oxytocin was administered (Lye & Freitag, 1988). Consequently, EMG activity was monitored until bursts were observed in both explants and the uterus at approximately the same time. Oxytocin, at the appropriate dose was then administered as a bolus via the maternal femoral vein catheter (usually between 5 and 10 min after the spontaneous EMG bursts). Since the periodicity of spontaneous contractures at this time of gestation was ~23 min for both ENDO/MYO and MYO explants and ~40 min for the uterine myometrium, administration of oxytocin was conducted at a time when spontaneous activity would not have been expected.

Spontaneous term labour. Out of the 12 animals, 4 were allowed to continue to full term (approx. 145 days). In this part of the study we were interested only in the pattern of myometrial EMG activity and the sensitivity to electrical stimulation in the explant during spontaneous labour. Consequently, blood samples were not collected for hormone analysis. Recording of spontaneous EMG activity of explants and uterine myometrium were begun on Day 125.

Beginning on Day 130 measurements of the sensitivity of the explants to direct electrical stimulation were made at 2-3-day intervals until the onset of labour at which time all animals were tested. More frequent testing was not conducted to prevent any possibility of damage to the tissue. Electrical stimulation of the explant was achieved by passing a DC pulse of ~20 msec duration down one of the two pairs of electrodes sewn into each end of the explant myometrium. The sensitivity of the explant was tested at +5 + 10 min after a spontaneous EMG burst and the response was monitored with the remaining pair of electrodes. If no response was elicited the voltage was increased and the myometrium stimulated after the next spontaneous EMG burst. The polarity of the stimulus was alternated between tests. The threshold voltage was defined as the lowest voltage eliciting a response. Attempts to stimulate the uterine myometrium using this technique were not successful, probably due to the very high resistance within this tissue.

Recording of myometrial activity in vivo

Electromyographic activity from explants reaches a stable pattern within 10 days of surgery (Lye & Freitag, 1988). Consequently, EMG activity from the myometrial electrodes (which probably reflect contractile events) and intrauterine pressure (IUP: from the amniotic fluid catheter) were monitored continuously from Day 124 onwards throughout the experimental period. The EMG signal from the uterus and explants was processed through a Grass wide-band AC preamplifier. Model 7P511J using a half-amplitude low frequency filter of 0.3–1 Hz and a half-amplitude high frequency filter of 10 kHz. IUP was measured by connecting the amniotic fluid catheter to a Statham P23 pressure transducer and processing the signal through a Grass low-level DC preamplifier (7P1). Both signals were recorded using a Grass 78 polygraph.

Analysis of chart records

Spontaneous activity. Traces were divided into 2-h segments. The frequency and duration of EMG bursts and the frequency, duration and maximum amplitude of uterine pregnancy contractions (contractures) and labour contractions per 2-h period were recorded. An EMG burst was defined as a continuous firing of EMG electrodes of amplitude >50 µV (for the explants) or 200 µV (for the uterine myometrium) and a duration of >30 sec separated by at least 15 sec from a previous burst. Uterine pregnancy contractions were defined as increases in IUP of at least 2 mmHg.
lasting for >3 min and associated with an EMG burst from the uterine electrodes, while uterine labour contractions were characterized by a duration of elevated IUP of <1 min.

**Oxytocin responsiveness.** The response of the myometrium to oxytocin was analysed over the 30-min period after administration of oxytocin. The response of the MYO explant and the uterine myometrium was assessed by integrating the EMG signal using a Grass polygraph integrator (7P10). The EMG and integrator calibrations within each tissue were maintained constant over the course of the experiment. However, since explant and uterine calibrations were different the absolute values cannot be directly compared between these tissues. The response of the ENDO/MYO explant was measured using arbitrary units (frequency x duration x amplitude of EMG bursts).

**Electrical stimulation.** The threshold voltage necessary to induce a burst of EMG activity was recorded for each test. An induced response was defined as a firing of the non-stimulated electrode occurring within 1 sec of the stimulus artefact.

**Hormone assays**

Progesterone, oestradiol-17β and 13,14-dihydro 15-keto prostaglandin F-2α (PGFM) were measured by specific radioimmunoassays that have been previously validated for the sheep (Challis & Patrick, 1981; Challis et al., 1981; Olson et al., 1985). Within this study the interassay and intra-assay coefficients of variation ranged between 10 and 12%. The sensitivities of the three assays were: progesterone, <0.2 ng/ml; oestradiol-17β, <5 pg/ml; PGFM, <20 pg/ml.

**Statistical analysis**

The results are expressed as mean (± s.e.m.). Since the duration of ACTH administration varied between the animals the data from each animal were tabulated for the 3 days before ACTH/saline administration and the 7 days leading to the onset of labour. Differences between groups were examined for statistical significance by analysis of variance, having first confirmed homogeneity of variance. Means were subsequently separated using Duncan's multiple-range test.

**Results**

**ACTH-induced preterm labour**

**Spontaneous contractile activity.** As we have previously described (Lye & Freitag, 1988), a regular pattern of myometrial EMG activity developed in both the myometrial (MYO) and endometrial/myometrial (ENDO/MYO) explants over a period of 7–10 days. During the pre-infusion period (Days 124–127) the frequency of EMG bursts (per 2 h) in the ACTH and Saline groups was 4.91 ± 0.30 and 4.65 ± 0.25 (MYO), 3.3 ± 0.61 and 3.79 ± 0.28 (ENDO/MYO), and 2.05 ± 0.18 and 2.43 ± 0.13 in the uterine myometrium, respectively (Fig. 1a, b, c). Within each tissue there was no significant difference between the two groups. However, with the exception of the ENDO/MYO explant in the ACTH group, the frequency of EMG bursts was significantly higher in the explants than in the uterine myometrium. Similarly, the duration of EMG bursts (min) in the ACTH and Saline groups for each tissue was not significantly different from each other (4.7 ± 0.2 and 4.6 ± 0.4 [MYO]; 5.2 ± 0.3 and 4.6 ± 0.4 [ENDO/MYO]; 7.3 ± 0.2 and 7.5 ± 0.3 [uterine], respectively: Fig. 1a, b, c), although the duration was significantly less in explants than in the uterine myometrium during this preinfusion period.

Induction of premature labour in animals receiving ACTH resulted in a marked change in the characteristics of EMG bursts from the explants as well as from the uterus (Fig. 1). Uterine contractions (EMG bursts) exhibited the characteristic increase (P < 0.05) in frequency (to 26.6 ± 9.5 per 2 h) and reduction in duration (to 2.3 ± 0.5 min) (Fig. 1a). Associated with this change in uterine contractile activity there was also a significant increase (P < 0.05) in the frequency (to 16.7 ± 4.7 bursts per 2 h) and decrease in the duration (to 2.4 ± 0.5 min) of EMG bursts in the MYO explants (Fig. 1b) and in the ENDO/MYO explants (to 12.8 ± 3.7 bursts per 2 h; and to
Systemic control of labour in sheep

Fig. 1. Mean (± s.e.m.) frequency (Δ ▲) and duration (○ ●) of EMG bursts from (a) the uterine myometrium, (b) the MIO explant and (c) the ENDO/MIO explant. △ ○, saline infusions; ▲ ●, infusion of ACTH to the fetus.
3·0 ± 0·6 min) (Fig. 1c) with the onset of labour. At this time there was no significant difference in either the frequency or duration of EMG bursts between the explants and the uterine myometrium.

The increase in contraction parameters induced by administration of ACTH to the fetus only became significant during the 36 h before labour, and the timing of these changes showed some difference between the tissues. Changes in EMG burst activity (both frequency and duration) in the MYO explant reached significance on the day before labour was established. In contrast, in ENDO/MYO explants these changes reached significance only on the day of labour. In the case of the uterine myometrium, while burst frequency was only significantly elevated on the day of labour, burst duration declined significantly on the day before labour. Although the changes in frequency and duration of EMG bursts of the explants paralleled those in the uterine myometrium during labour, the occurrence of individual bursts in these tissues remained asynchronous.

The spontaneous EMG activity of explant and uterine myometrial tissue in control animals receiving saline alone showed no significant changes throughout the infusion period (Fig. 1a, b, c). Vaginal delivery of the fetus was associated with a period of extensive EMG activity recorded in the explants and the uterine myometrium that was coincident with abdominal straining movements of the ewe (Fig. 2).

**Oxytocin responses.** The uterine myometrium and both explants displayed a dose-dependent increase in EMG activity following administration of oxytocin. The magnitude of the daily oxytocin response (measured as integrated EMG activity over the 30 min following oxytocin) did not change throughout the infusion period, except in animals receiving ACTH, in which case the response was significantly elevated only on the day of labour (Fig. 3).

**Plasma hormone concentrations.** In the sheep randomized to the ACTH group plasma hormone concentrations were 13·4 ± 5·7 ng progesterone/ml, 24·6 ± 7·5 pg oestradiol/ml and 529 ± 84·6 pg PGFM/ml, respectively (Fig. 4). After the start of ACTH infusion to the fetus there was a significant fall in progesterone, and a significant increase in both oestradiol and PGFM in the maternal plasma of these animals. On the day of labour the plasma concentrations of progesterone, oestradiol and PGFM were 4·3 ± 1·4 ng/ml, 109·1 ± 9·4 pg/ml and 2068 ± 241 pg/ml, respectively. No significant changes occurred in plasma hormone values in the saline-infused animals during the course of the study.

**Spontaneous term labour**

**Spontaneous contractile activity.** The pattern of myometrial activity recorded from both the explants and the uterus was similar to that seen in ACTH-induced labour. There was a significant increase in the frequency of EMG bursts in both the explants and the myometrium and a significant decrease in the duration of bursts with the onset of labour.

**Electrical stimulation.** The sensitivity of the myometrial explants to electrical stimulation remained relatively constant until the day of labour at which time there was a significant reduction in the stimulation threshold. The voltage needed to elicit a response from the explant myometrium was 14·8 ± 4·0 V 3 days before labour and 4·3 ± 1·3 V on the day of labour (P < 0·05).

**Discussion**

In this study we sought to determine whether the increased contractile activity of the ovine myometrium, in terms of activation and stimulation, at the time of labour, is due to a local paracrine stimulation, as has been suggested for the human (Nakla et al., 1986; Casey & MacDonald, 1988; Lye & Challis, 1990), or to stimulatory factors in the systemic circulation. Our findings support a major role for the systemic circulation in the passage of stimulants to the myometrium during labour in sheep. In addition, our data provide evidence to suggest that factors within the
**Fig. 2.** Trace of electromyographic activity from the MYO explant, the ENDO/MYO explant and EMG activity and intrauterine pressure from the uterus during labour and delivery. Major fluctuations in intrauterine pressure represent occasions when the animal stood or lay down. Intense rapid pen excursions occurred during abdominal straining at delivery.

**Fig. 3.** Mean (± s.e.m.) integrated EMG activity of the MYO explant (●) and uterine myometrium (△) during the 30-min period after administration of oxytocin to ewes. Myometrial responsiveness to oxytocin increased significantly in both tissues, but only on the day of labour.
systemic circulation are also responsible for the process of myometrial activation (as indicated by an increase in in-vivo myometrial responsiveness to oxytocin and sensitivity to electrical stimulation).

Our conclusion with respect to spontaneous contractions supports the findings of Sigger et al. (1984), who examined the electrical activity of isolated uterine tissue in nonpregnant and pregnant sheep. Nevertheless, the data presented in this report differ from and extend these findings in several important respects. Our explant model maintains the in-situ (uterine) degree of stretch on the myometrium. This is especially important since tension is widely recognized as a critical factor in determining the ability of the myometrium to develop contractile activity (Finn & Porter, 1975; Wathes & Porter, 1982). Our model also permits the investigation of the paracrine role of the endometrium in the stimulation of the myometrium during labour. The report of Sigger et al. (1984) provided a descriptive account of an increase in the EMG activity of isolated uterine tissue in 3 ewes in spontaneous labour. In the present study we have conducted a quantitative assessment of the changes in spontaneous activity and responsiveness of isolated myometrial tissue during labour induced prematurely by administration of ACTH to the fetus. In addition to the considerable data on the temporal changes in uterine myometrial activity in ACTH-induced labour, this protocol also allowed the pattern of explant activity in control animals, matched for gestational age, to be examined. Finally, we have compared the temporal relationship between the activity of the isolated myometrial tissue and endocrine changes in the systemic circulation.

The changes in spontaneous EMG activity in the explants and uterine myometrium, with the approach of labour (decrease in duration of EMG bursts and an increase in burst frequency), were similar qualitatively and temporally. These data strongly suggest that the mechanisms controlling this process are able to exert their influence via the systemic circulation.

In addition to measurements of spontaneous activity (an indicator of stimulation) we also examined two responses more closely related to activation, i.e. oxytocin responsiveness (in ACTH-induced labour) and the electrical stimulation threshold (in spontaneous labour). Both of these responses changed, in the explants, consistent with myometrial activation, although in each case significance was only achieved on the day of labour itself. It is likely that the increase in oxytocin response reflects an increase in myometrial receptors, as has been reported for the rabbit (Reimer

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**Fig. 4.** Mean maternal plasma concentrations of oestradiol-17β, progeserone and PGFM during the 3 days before start of the ACTH infusion, and on the 6 days leading to parturition.
et al., 1986). The decrease in electrical stimulation threshold implies an increase in the sensitivity of the myometrium to electrical stimulation. We suggest that this reflects a relative depolarization of the myometrial cells at this time as has been reported to occur in the rat (Sims et al., 1983).

We consider it highly significant that measures of both stimulation and activation were temporally and qualitatively related in the explants and the uterine myometrium. Furthermore, these physiological changes in the myometrium were associated with a significant increase in the concentration of maternal plasma oestradiol (and in the oestradiol:progesterone ratio). Oestrogen domination is associated with a variety of changes within the myometrium that would aid increased contractility, including increased responsiveness to agonists (Liggins et al., 1973; Windmoller et al., 1983) and increased propagation (Lye et al., 1983b). These effects are believed to be mediated by a direct action of the steroid on the myometrium, including increased agonist binding (Soloff & Pearlmutter, 1979), change in membrane potential (Kao, 1977), increases in intracellular calcium levels (Pietras & Szegó, 1976), gap junctions (Garfield et al., 1979), contractile protein synthesis (Michael & Schofield, 1969), calmodulin content and myosin light chain kinase activity (Matsui et al., 1983). The results of the present study would suggest that this effect of oestrogen is mediated through the systemic circulation.

In addition, the rise in plasma oestradiol concentrations during ACTH is associated with a marked increase in prostaglandin synthesis by intrauterine tissue and an elevation in the plasma levels of this myometrial stimulant (Olson et al., 1985). While the plasma half-life of prostaglandins is very short, the massive increase in production together with the increased myometrial responsiveness may result in significant systemic stimulation by these eicosanoids (Thorburn & Challis, 1979) as is suggested by the present study.

We therefore suggest that the combined effect of oestrogen is to activate the myometrium and also to increase the production of prostaglandins or release of oxytocin (Roberts & Share, 1969) and thereby exert a systemic control on the process of labour in the ewe.

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References


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