Co-ordination of electrical activity in the myometrium of pregnant ewes

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Summary. The co-ordination of spontaneous and evoked electromyographic (EMG) activity was examined in the uterus of ewes from Day 100 of pregnancy and during labour. During pregnancy the onset of bursts of EMG activity was synchronous to within 2–3 min when recorded by electrodes at any recording site. Individual EMG spikes, whether occurring spontaneously or evoked by electrical stimulation of the myometrium, appeared to propagate between recording electrodes that were 1–3 cm apart, in the longitudinal or circular orientation of the uterus, but not when the electrodes were separated by more than 3 cm. Apparent conduction velocity in the longitudinal orientation was 7 cm sec^{-1} during pregnancy and increased to 13 cm sec^{-1} during labour. No consistent pattern could be distinguished in the sites at which EMG activity was first observed at any stage of pregnancy studied. The nature of propagation of EMG activity remains unresolved but it is clear that it is not initiated at a single site in the myometrium.

Keywords: propagation; myometrium; pregnancy; parturition; sheep

Introduction

Uterine muscle undergoes periodic contractions during pregnancy in a number of species (pigs: Taverne *et al.*, 1979a, b; monkeys: Germain *et al.*, 1982; rabbits: Demianczuk *et al.*, 1984; sheep: Harding *et al.*, 1982; goat: Taverne & Scheerboom, 1985; human: Bell, 1983). In sheep, extracellular recording electrodes, attached at multiple sites on the pregnant uterus, record bursts of electromyographic (EMG) activity that appear synchronous (Harding *et al.*, 1982). Although the concept of the myometrium as an electrical syncytium at the time of parturition has been emphasized (Csapo, 1969) and the importance of impulse propagation from a single pacemaker site in the co-ordination of uterine contractions has been proposed (Garfield *et al.*, 1982), experimental data on the extent of the co-ordination of myometrial activity in the intact animal are scanty. In the present investigation an attempt was made to assess the extent to which EMG activity might spread throughout the uterus in the intact ewe by comparing spontaneous EMG signals in arrays of recording electrodes and EMG activity evoked by electrical stimulation of the myometrium.

Materials and Methods

Pregnant ewes of known mating dates were surgically prepared 100–127 days after mating. The surgical preparation for recording uterine EMG and intrauterine pressure (IUP) has been described previously (Harding *et al.*, 1982). Briefly, the 19 ewes were anaesthetized with pentobarbitone sodium and maintained with 1.5–2% halothane in O_2 and N_2O . The uterus was exposed via a midline abdominal incision and pairs of Teflon-insulated stainless-steel wires (0.07 mm in diameter with 0.5–1 mm of the wire bare of insulation) were sewn into the myometrium (see Harding *et al.*, 1982), approximately 3 mm apart, for recording EMG activity. Pairs of EMG leads were connected to differential pre-amplifiers (Grass EKG tachograph, model 7P4). The limitations of this method of recording electrical activity in smooth muscle have been discussed by Bortoff (1975). Since no special emphasis was placed on the amplitude or

Fig. 1. Positions of recording electrodes on the uterus of ewes. In (a) pairs of electrodes were placed 4 cm (R_1 – R_2 and R_3 – R_4) or 8 cm (R_2 – R_3) apart along the longitudinal orientation of the uterine horn. In (b) pairs of electrodes were placed 1 cm apart along the longitudinal orientation of the uterine horn (R_1 – R_2 – R_3) and 1 cm from a pair of stimulating electrodes (S.E.) (100–150 mm²). Pairs of recording electrodes were also placed circumferentially, 1 cm from the stimulating electrodes and 1 cm apart (R_4 – R_5).

configuration of the waveforms recorded, and the conduction velocity was calculated only from evoked responses, the arrangement of electrodes in a recording pair was not critical. IUP was detected via a saline-filled catheter inserted into the amniotic sac and connected to a Statham transducer (P23 ID). All EMG and pressure signals were recorded on a Grass polygraph (Model 7D).

EMG activity was studied with either 4 pairs of recording electrodes arranged in a line along the greater curvature of the uterine horn following the line of the longitudinal myometrium (Fig. 1a), or with 5 pairs of the electrodes arranged in an L-shaped configuration; 3 pairs were orientated along the greater curvature and 2 pairs were at right angles, following the orientation of the circular myometrium (Fig. 1b). The separation between pairs of electrodes was: 1, 2, 3, 4, 8 or 16 cm (see Fig. 1, legend). Stimulating electrodes consisted of a pair of discs of silver (5 ewes), stainless steel (5 ewes) or platinum (6 ewes), 12 mm in diameter and insulated on one side with silicone rubber. One disc of each pair was sutured to the uterus such that the bare side lay in contact with the myometrium while the other was placed inside the uterus, with the bare surface in contact with the endometrium, subjacent to the other of the pair, and fixed in place with three sutures. The stimulating electrodes were placed such that they lay in the angle of the L made by the array of recording electrodes, 1 cm from the closest pairs (Fig. 1b). Square pulses were applied from a Grass S44 stimulator via an isolation unit (Grass SIU 5).

Data are presented as means $\pm$ one standard deviation of n observations (the number of animals studied). Differences between means were tested using Student's t test (paired or unpaired as appropriate). Values of P less than 0.05 were taken as significant.

Results

Spontaneous electrical activity during pregnancy

EMG activity, recorded differentially between each pair of electrodes, consisted of spikes that were essentially biphasic in nature. The most striking arrangement of spikes was in bursts that occurred at a frequency of 1.6 ± 0.13 h and lasted for 7.5 ± 1.5 min ($n = 10$) (Fig. 2).

Within a burst spikes occurred continuously for a variable period at first but this was interrupted by quiescent periods of 7.8 ± 0.5 sec separating 9.6 ± 0.6 sec of spike activity ($n = 10$) and appeared as groups of spikes within the burst at fast chart speeds (see Fig. 3, for example) while the phenomenon was not discerned at slower chart speeds (Fig. 2). Spikes occurred at 1.2 ± 0.05 Hz in the groups within the burst ($n = 10$) (Fig. 3). These bursts were associated with rises in IUP of some 5 mmHg (Fig. 4).

Besides bursts, spikes also occurred alone or in small isolated groups of less than 2 min duration. These were not associated with any detectable change in IUP (Figs 2 and 4). The frequency of spikes in these small groups was not different from that occurring in major bursts.

Fig. 2. Bursts of EMG activity recorded from pairs of electrodes placed (as in Fig. 1b) 1 cm apart in the longitudinal orientation (a–c) and circumferentially (d and e).

Fig. 3. There is a close correspondence between spikes recorded via electrodes placed (as in Fig. 1b) 1 cm apart in the longitudinal orientation (a–c). This relationship is less precise when recorded via electrodes placed circumferentially (d and e).

Co-ordination of EMG activity during pregnancy

Throughout pregnancy, excluding labour, bursts of EMG activity recorded from pairs of electrodes placed 1–3 cm apart were synchronous (Fig. 2). When the data were examined at a faster chart speed it was clear that there was a one-to-one correspondence between the occurrence of individual spikes recorded by the electrodes that followed the line of the longitudinal myometrium (Figs 3a–c). The occurrence of individual spikes at different pairs of electrodes in the longitudinal orientation remained constant within a burst (see below). This contrasted with the pattern of activity recorded via the electrodes that followed the orientation of the circular myometrium where the relationship between individual spikes was less precise (Figs 3d and e). However, spike activity in the circular orientation always began within 3 sec of activity in longitudinally placed electrodes.

Fig. 4. Intrauterine pressure (IUP) and EMG activity (a–d), recorded via electrodes placed (as in Fig. 1a) 4 and 8 cm apart.

Fig. 5. The duration of bursts of EMG activity and the interval between them at 18, 8 and 0.5 h before delivery. Data from 5 ewes.

The results were different when the separation between pairs of electrodes was greater than 3 cm. The precise timing of the start and finish of the EMG bursts varied between the different recording sites along the linear array and could differ by many minutes (start, 2.3 ± 0.6 min, $n = 10$) (Fig. 4). When the activity within a burst was examined on an expanded time scale there was no correspondence between spikes recorded by any pair of electrodes. At times, one region of the uterus could remain electrically quiescent during activity in the others (for example, Fig. 4d). Despite the approximate nature of the synchrony between bursts of EMG activity, each event, consisting of a burst of EMG activity occurring at most pairs of electrodes, was associated with a detectable rise in IUP (see above and Fig. 4).

Spontaneous electrical activity during labour

The onset of labour was recognized by an increase in the frequency and decrease in duration of major bursts. This is summarized in Fig. 5, which shows that the duration of a burst and interval between bursts decreased from 4–5 min 18 h before delivery to <1 min just before delivery. Each burst was associated with a rise in IUP that reached 25–30 mmHg approximately 8 h before delivery. The frequency of spikes within the burst increased significantly during labour to reach a maximum of 1.8 ± 0.06 Hz ($n = 5$) 8–12 h before delivery.

Fig. 6. Spike activity between electrodes placed (as in Fig. 1b) 1 cm apart in the longitudinal orientation (a–c) and circumferentially (d, e) during labour.

Co-ordination of EMG activity during labour

Recordings were made from 7 ewes at the onset of labour at which time bursts of EMG activity recorded from electrodes separated by 1–3 cm seemed more disorganized than those recorded from the same animals before labour. This can be appreciated if the recording from a ewe during labour (Fig. 6) is compared with a record made 10 days earlier in the same animal (Fig. 3): before labour the start and finish of each group of spikes recorded by electrodes in each orientation (longitudinal and circular) were synchronous to within 1–2 sec (Fig. 3). This contrasted with observations during labour when some synchrony existed between spikes but 'extra' spikes and apparent asynchrony were more common.

In an attempt to quantify the apparent loss of order during labour the intervals between 100 spikes, recorded by each of 2 electrodes during labour, were compared with the intervals between 100 spikes recorded by the same 2 electrodes 48 h previously. These measurements were made in 3 animals. The mean interval between spikes was 939 ± 189 msec (for 300 intervals) during pregnancy and 609 ± 266 msec (for 300 intervals) in labour. The interval between spikes was significantly reduced during labour and, in addition, the variance during labour was significantly greater than that observed during pregnancy (the mean variance ratio, *F*, for the 3 animals was 1.98 and this was significantly different from 1; *t* = 6.09, d.f. = 2).

During labour the starts of bursts of EMG activity recorded from electrodes that were greater than 3 cm apart were significantly closer (0.6 ± 0.2 min, *n* = 5) than those observed during pregnancy before labour in the same animals (2.3 ± 0.6 min) (compare Fig. 7, in labour with Fig. 4 from the same animal 3 days previously). This may result from the decrease in the interval between bursts during labour.

Evoked EMG activity

Stimulating electrodes were used in an attempt to elicit propagated EMG activity in 15 ewes. When pairs of recording electrodes were 1 cm apart, and formed the arms of an 'L' with respect to the stimulating electrodes (see Fig. 1b), pulses of 50 msec duration and 70–90 V consistently produced a single spike in each electrode placed 1, 2 or 3 cm along the longitudinal axis but only in the proximal pair of electrodes that were 1 cm from the recording electrodes in the circumferential orientation (Fig. 8). From responses evoked during the quiescent period in the 5–15 min following

Fig. 7. EMG activity recorded during labour via electrodes placed (as in Fig. 1a) and 4 and 8 cm apart.

Fig. 8. The uterus was stimulated with square pulses, 50 msec duration and 70 V in amplitude, and spikes were recorded by all 3 pairs of electrodes (placed as in Fig. 1b) in the longitudinal orientation (a–c) but by only one electrode in the circular orientation (d and e).

a major burst of spontaneous activity, the apparent conduction velocity was calculated at 7.2 ± 0.3 cm/sec ($n = 5$) in the longitudinal orientation. This increased significantly to 13.3 ± 0.7 cm/sec ($n = 5$) during labour. Since an evoked response could be recorded in only one pair of electrodes in the circular orientation no attempt was made to calculate conduction velocity. Evoked responses were not observed from recording electrodes that were at distances greater than 3 cm from the stimulating electrodes.

Site of initiation of contraction

There was no site on the uterus at which bursts of EMG activity consistently occurred before appearing at other sites. However, in individual animals there were occasions when the majority of EMG bursts occurred initially at the same recording site but, over a period of days, there was a shift in the site at which most bursts started. For instance, in one animal on Day 130, 77% of bursts were observed first in electrode 1, while on Day 141 87% started 16 cm away at electrode 4. Similarly, in another ewe 63% of bursts occurred first at electrode 4 on Day 128, whereas 4 days later, 87% started at electrodes 1 or 2. Overall, no consistent pattern could be distinguished in the site at which

bursts seemed to be initiated. This applied even during labour. There was no evidence for a static pacemaker region at any stage of pregnancy (Fig. 4) or in labour (Fig. 7).

The temporal relationship between spikes, recorded from pairs of electrodes at 1–3 cm separation, in the longitudinal orientation, remained constant throughout the duration of 84% of bursts during pregnancy (Figs 3a–c); for example, the pattern of the 'progression' of spikes from one pair of electrodes to another did not change during a burst but the pattern did not necessarily remain constant between bursts. However, this relationship of spike 'progression' held for only 16% of bursts when activity was examined in electrodes placed circularly (for example, Figs 3d and e). During labour the constant 'progression' of spikes between pairs of electrodes during a burst did not hold and the site at which spikes first appeared fluctuated even in the longitudinal orientation. Figure 6 shows that spikes were recorded in the order; (c), then (b) and then (a) initially but 30 sec later the order was reversed.

Discussion

It is clear from our recordings of spontaneous EMG activity at multiple sites on the uterus that individual spikes, or groups of spikes, whether spontaneous or electrically evoked, rarely propagate over distances greater than 3 cm during pregnancy. In longitudinal myometrium isolated from oestrogen-treated rats evoked spikes traversed only 3 cm in 80% of trials (Melton & Saldivar, 1964) and contractions elicited by mechanical stimulation of the sheep uterus when exposed at surgery are usually confined to an area with a radius of about 5 cm (unpublished observations). The values of apparent conduction velocity in the uterus of intact ewes are close to values observed in isolated smooth muscle preparations (~ 6 cm/sec; Abe, 1970), including isolated longitudinal myometrium of pregnant (Miller *et al.*, 1986) and oestrogen-treated (Melton & Saldivar, 1964) rats. Conduction velocity increased approximately 2-fold when ewes were in labour (present study) and also in longitudinal myometrium obtained from rats in labour (Miller *et al.*, 1986).

The results in the present study indicate that propagation may occur more readily in the longitudinal orientation rather than circumferentially and the present and previous studies failed to report a consistent direction of propagation during pregnancy or at parturition in a variety of species (sheep: Naaktegeboren *et al.*, 1975; Harding *et al.*, 1982; Toutain *et al.*, 1983; cows: Hanzen, 1981; sows: Taverne *et al.*, 1979a, b; women: Wolfs & Rottinghuis, 1970; Sakaguchi & Nakajima, 1973). In contrast, when Fuchs (1969) inserted two balloons into the uterus of pregnant rats, co-ordinated contractions were observed during labour and these always originated at the ovarian end of the uterus and progressed to the cervical end.

Despite the observation in this study that EMG activity rarely propagated over distances that were greater than 3 cm, bursts of spikes are recorded within 1–2 min by electrodes placed at any location on either horn (pregnant or non-pregnant) of the uterus. That this represents generalized activity throughout the uterus is indicated by the observation that each burst of EMG activity, across the uterus, is associated with a rise in IUP. Thus it remains to be explained how activity travelling at 7 cm sec^{-1} can take up to 3 min to travel 4 cm. Another apparently contradictory result emerged from this study. Even at close electrode separation, the synchrony of recordings made from animals during pregnancy contrasted strongly with the asynchronous pattern of activity that occurred along arrays of electrodes during labour, and electrical activity in the uterus seems to become more chaotic at a time when greater co-ordination might be expected. This apparent disorganization seems surprising in view of some of the changes that take place as the ewe goes into labour: the increase in apparent conduction velocity at labour (present study), the increase in the space constant of the myometrium (an index of current spread; Parkington, 1985), the additional gap junctions that appear during labour (Garfield *et al.*, 1979), the increase in amplitude of EMG activity (Harding *et al.*, 1982) and the rise in the pressure generated by individual contractions, the last two indicating activation of an increased proportion of myometrial cells

during each period of contractile activity. Some of these apparent paradoxes might be reconciled by considering the propagation of spikes in a syncytium.

The anatomical characteristics of the myometrial syncytium in the ewe have been studied (H. C. Parkington, unpublished observations): the bundles of smooth muscle cells in the outer, longitudinal layer of the myometrium are discrete, with relatively few interconnecting strands between bundles. In contrast, discrete bundles of cells are less obvious in the inner circular myometrium which bears a greater resemblance to a thick sheet. Finally, the two layers are connected by strands of smooth muscle that pass between the blood vessels and connective tissue that separate them. The morphological complexities of the preparation, together with the large area (0.5–1 mm length and 0.07 mm diameter) of bare wire, precluded any special emphasis being placed on the amplitude or configuration of the waveforms that were recorded. Propagation of spikes is constant in a uniform cable (Goldstein & Rall, 1974). Since lack of homogeneity in the myometrium includes changes in the diameter of the cable (the bundle in the myometrium), branching, and regional variations in axial resistivity that could arise from changes in cell-to-cell coupling and differences in excitability, then regional variations in conduction are to be expected. The effects of inhomogeneities on spike propagation have been analysed extensively in relation to the cardiac syncytium and variations in conduction velocity, conduction block and changes in direction of propagation can all be explained in terms of cell geometry (including intercellular coupling) and arrangement (Joyner, 1982; Spach & Kootsey, 1983; Joyner *et al.*, 1984). Since spikes in one layer of the myometrium invade the other layer (Osa & Katase, 1975; Parkington, 1983), spikes originating in one small area of the myometrium might eventually spread to all areas via a circuitous pathway, arriving at any given point (under a recording electrode) at a variable and unpredictable time after activity first began. This situation would be complicated further in the event of the existence of multiple pacemakers.

The precise nature of the propagation of EMG activity throughout the uterus during pregnancy therefore remains unresolved. It may be that contraction of the muscle in one area initiates pressure waves in intrauterine fluid which would increase circumferential stress in the wall of the uterus, stretching and depolarizing the smooth muscle cells and resulting in contraction. Such a mechanism is suggested by the experiments of Takeda (1965). In the present study we made the simplifying assumption of an exclusively myogenic origin for the bursts of activity that are observed. However, it is perhaps worthy of consideration in future experiments that some chemical factor(s) generate the bursts, accounting for their approximate synchrony.

We thank Ms J. Norman and Ms J. Buttress for skilled assistance in surgical preparation and animal care; and Professor M. E. Holman, Dr H. A. Coleman and Dr G. Jenkin for useful criticism of the manuscript. This work was supported by an NH&MRC Program Grant to Professor G. D. Thorburn. H.C.P. was supported by NH&MRC Grant No. 860865.

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Received 23 July 1987