Patterns of electrical propagation in the intact pregnant guinea pig uterus

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Lammers WJ, Mirghani H, Stephen B, Dhanasekaran S, Wahab A, Sultan MA, Abazer F. Patterns of electrical propagation in the intact pregnant guinea pig uterus. Am J Physiol Regul Integr Comp Physiol 294: R919–R928, 2008.—Previous studies have reported on propagation of individual spikes in isolated segments of the pregnant uterus, but there is no information on patterns of spike propagation in the intact organ. There is also no information on propagation of myometrial burst. The aim of this study was to record, at high resolution, patterns of propagation of electrical activities in the pregnant uterus. Sixteen timed-pregnant guinea pigs were euthanized at term, and their uteruses isolated. Fetuses were removed and replaced by an equal amount of Tyrode. A 240-electrode array was positioned at various locations along the organ, all signals were recorded simultaneously, and the electrical propagations were reconstructed. In the intact pregnant uterus at term, spikes propagated with high velocity in longitudinal (6.8 ± 2.4 cm/s) and slower velocity in circular direction (2.8 ± 1.0 cm/s; P < 0.01). Direction of propagation and frequency of activity were highly variable but showed similar patterns at the ovary or cervical end and along the anterior, posterior, and antimesometrial borders. Along mesometrium, spike propagation was sparse and fractionated. Migration of burst (0.6 ± 0.4 cm/s) was significantly much slower than that of individual spikes (P < 0.001). Initial burst activity was located at variable locations along the ovarial end of the antimesometrial border, while the latest excitation occurred at the cervical end (1.2 ± 0.9 min). In conclusion, high resolution electrical mapping of the intact pregnant uterus reveals fundamental properties in spatial and temporal patterns of spike and burst propagation that determine the contraction of the organ.

spikes; myometrial burst migration

ELECTRICAL ACTIVITY in the pregnant myometrium is characterized by phasic bursts of action potentials (spikes), which could be based on cyclic changes in transmembrane potential (24). Cyclic changes in potential resemble in some respects slow waves in the intestines and presumably propagate through the myometrium, similar to the propagation of the intestinal slow waves. In both cases, the depolarization induced by the (slow) waves initiate the opening of L-calcium channels leading to the occurrence of spikes. In the intestines, it is possible to record both slow waves and spikes with extracellular electrodes, and this made it possible to reconstruct the pattern of propagation of both signals and to study the complex interaction between these two electrical waveforms (22).

In the myometrium, the basic electrical wave is too slow or its magnitude too small to be recorded extracellularly (9). The spikes, however, are visible in extracellular recordings and this led to several studies on their behavior. Miller et al. (25) analyzed spike propagation in pregnant uterine segments that were 3 × 1 cm in size and showed that velocity in the longitudinal direction was much faster than in the circumferential direction. Lammers et al. (20) found similar results in equally small segments (8 × 2 cm). Most of these studies were performed on isolated segments of myometrium, but a few studies have recorded from a limited number of electrodes in the whole intact myometrium in vivo. Harding et al. (15), with four implanted electrodes in the pregnant sheep, could not detect propagation and suggested that there did not seem to be a specific pacemaker area, while Parkington et al. (26), also in pregnant sheep, discovered that propagation could only be detected if the recording electrodes were closer to each other than 3 cm.

The goal of this study was to reconstruct, during spontaneous contractions, the propagation of the spikes in the intact guinea pig horn and to analyze the patterns of propagation of individual spikes and of the spike bursts in the whole organ.

METHODS

Sixteen timed-pregnant term guinea pigs (63.6 ± 3.9 days; range 55.5 to 69.5 days; 1.1 ± 0.2 kg body wt) were used in this study. The normal gestation period in the colony ranged from 65 to 70 days. The guinea pigs were euthanized by CO2 inhalation (institutional ethical approval number: AE/03/30). After a midabdominal incision, both pregnant horns together with the connecting cervix were rapidly removed and placed in a cold Tyrode solution. One of the horns was chosen for the experiment, and its cervical end was opened. The one to three fetuses (average 2.4 ± 0.5) were gently delivered with their placenta and their weight, (95.0 ± 31 gm), length (10.9 ± 2.0 cm), and volume noted. These values are compatible with term values from Draper (10). The open cervical end of the uterus was fixed to a modified stopper (Fig. 1) to close the uterine cavity, whereupon the uterus was gradually inflated with a volume of Tyrode equivalent to the size of the removed fetuses (218 ± 77 ml; range 110 to 350 ml). The closed and filled preparation was then positioned in a 1.6-liter tissue bath where it was superfused at a rate of 200 ml/min with a modified Tyrode solution (composition in mM): 130 NaCl, 4.5 KCl, 2.2 CaCl2, 0.6 MgCl2, 24.2 NaHCO3, 1.2 NaH2PO4, and 11 glucose saturated with carbogen (95% O2-5% CO2), while pH was kept constant at 7.35 ± 0.05 and temperature at 37 ± 0.5°C. Intrauterine pressure was recorded from an inlet in the stopper at the cervical end, while contraction was recorded with an isometric transducer attached to the ovarial mesometrium.

A 240-extracellular recording array (10 × 24; Teflon-coated silver wires; 0.3 mm diameter; 2 mm interelectrode distance) was positioned at various locations along the length and around the circumference of the horn. All 240 signals (amplification, ×4,000; band filter, 2–400 Hz; sampling, 1,000 Hz) were recorded simultaneously during burst activity (19, 20). In four separate experiments, five rows of 32
Electrodes each (interelectrode distance, 4 mm) were positioned around the circumference of the horn (Fig. 1). Selected 32-s recordings, recorded during the beginning, middle, or end of the bursts were analyzed. The signals were digitally filtered (20-point moving average) and displayed on-screen in sets of 20-24 electrograms at a time (Fig. 2). The digital filtering effectively removes ambient 50-Hz noise and reduces the frequency range to 2–40 Hz. The local activation time of a spike was identified by the moment of maximum negative slope (19, 20) and marked with a cursor. All local activation times are related to a common reference time, determined by the timing of the first detected spike in the mapped area (Fig. 2, electrode 19; t = 0). After all spikes were time marked, their activation times were displayed in the format of a grid of the original recording array (24 × 10) (Fig. 2C). Isochrones were drawn manually around areas activated in steps of 100 ms (Fig. 2C).

To measure spike conduction velocity (Fig. 2, D and E), two sites were selected between which conduction was homogeneous. The longitudinal distance between the two sites (28 mm) and the time difference (2,237–1,858 ms) were used to calculate the longitudinal velocity (7.4 cm/s; Fig. 2D). The circumferential velocity was measured in Fig. 2E (8 mm and 424-0 = 424 ms; conduction velocity = 1.9 cm/s).

All pooled data are given as averages and SDs. Significance was tested by Student’s t-test.

RESULTS

The propagation maps in Fig. 2 show several important characteristics of electrical propagation in the intact guinea pig pregnant uterus. Spikes are initiated at local sites and propagate in an anisotropic pattern at high velocity in the longitudinal and...
at lower velocity in the circumferential direction. Propagation of subsequent spikes is very variable, and, in this case, 2 s later (Fig. 2E), the same area was activated by a spike propagating in the opposite direction.

An analysis of bursts and of spike propagation was performed in 12 preparations, and the results of one experiment is presented in Figs. 3–6. As shown in the pressure and contraction records (Fig. 3), guinea pig contractions at term are characterized by a rapid onset, achieving maximal amplitudes in the first minute followed by an oscillatory pattern of diminishing contractions and relaxations until resting values were reached once more. The average duration of the contractions was 5.1 min (SD ± 2.0 min; range 1.3–9.6 min) and the average period in which they occurred was 13.1 min (SD ± 6.3 min; range 5.3–20.0 min). Spike propagation was analyzed at the beginning of the contraction (Fig. 4) when maximum contraction was reached (Fig. 5) and at the end of the contraction (Fig. 6).

The beginning of a burst (Fig. 4) was always (n = 22 bursts) characterized by a gradual increase in the excited area. First activities (spike a) were recorded at the ovarial end of the region, and subsequent spikes propagated further into the area often taking several seconds before exciting the whole area (spike c). Conduction was rapid in the longitudinal direction with a first wave propagating in a narrow corridor toward the cervix and another wave, about 1 s later, propagating adjacent to this region in the ovarial direction.

A few minutes later, at the height of contraction (Fig. 5), spiking activity is at its highest with anisotropic propagation and spontaneous variations in propagation from spike to spike.
including an occasional brief reentry of the impulse. At the end of the burst (Fig. 6), patterns of propagation remained highly variable.

Patterns of spike propagation were also determined at various sites along the guinea pig horn (n = 4 experiments). In Fig. 7, patterns were compared between areas located around the circumference of the horns. Except for the mesometrial side, where the fetal placentas had been located, variable patterns of spike propagations were found in all other areas. At the mesometrial side (Fig. 7B), recorded signals were weak and propagations were limited to small areas that were not connected with each other.

Spike conduction velocities were measured in six preparations (see METHODS). Propagation was 6.8 cm/s (SD ± 2.4 cm/s; range 3.5–11.3 cm/s) in the longitudinal direction and 2.8 cm/s (SD ± 1.0 cm/s; range 1.2–4.6 cm/s; P < 0.001) in the circumferential direction. There was no difference in velocity when the spike was traveling toward the cervix or toward the ovaries (6.9 ± 2.2 cm/s and 6.6 ± 2.6 cm/s, respectively).

As mentioned above, the first spike in a burst does not activate the whole mapped area. In fact, it took considerable time to excite an area of ~10 cm². An example of this is shown in Fig. 8 in which, instead of mapping the propagation of individual spikes, the timing of the first local excitation at the beginning of a burst was plotted at every site. As indicated in the electrograms in Fig. 8, the leading edge of the burst gradually migrated toward the cervical end. This migration was not uniform but activated distal areas after longer or shorter time periods. This is also shown in the spatial plot in the Fig. 8, illustrating this uneven process. It took ~8 s for the burst to migrate a distance of 5 cm. In four experiments, the beginnings of 22 bursts were mapped. The average time it took for the front to excite the whole mapped area was 22.9 s (SD ± 12.6 s; range 5.9–54.2 s). In 12 cases (60%), there was a gradual migration from the ovarial side to the cervical side of the mapped area, which took ~10 s (SD ± 6.1 s; range 4.0–22.1 s), resulting in an apparent propagation velocity of 0.6 ± 0.4 cm/s (range 0.2–1.5 cm/s). In eight other cases, the burst had started elsewhere in the mapped area, and migration occurred toward the cervix or toward the ovaries.

The origin and pattern of migration of the initiation of a burst was further studied by spanning rows of electrodes along the length and distributed circumferentially around the horn (Fig. 1F). Simultaneous recordings were performed from these five rows (5 × 32 = 160 sites). An example from one experiment is presented in Fig. 9 in which the local times of the start of the burst were plotted on a flattened map of the whole horn. This activity was recorded in several sequential bursts with the first two plotted in the figure. In all cases, first activity occurred along the ovarial end of the antimesometrial border. Migration from this area was rapid in the longitudinal direction and could occur at several separate sites within a second (red isochrone; burst 1). From these areas, migration was rapid along the antimesometrial border but progressed much slower in the circular direction. The last areas to be excited, after 25–30 s, occurred in areas located close to the cervix and the mesometrial border. In a series of four experiments, it took an average of 1.2 min (SD ± 0.9; range 0.4–2.2 min) for the horn to be activated.

**DISCUSSION**

This study has analyzed the pattern of propagation of individual spikes and that of the electrical burst in the pregnant guinea pig uterus at term. In general, the propagation pattern of individual spikes in the pregnant guinea pig was similar to that
seen earlier in isolated segments from the rat (20). Miller et al. (25) measured, in isolated preterm rat segments, a longitudinal velocity of 9.2 cm/s and a circumferential velocity of 2.3 cm/s, while Parkington et al. (26) measured in the conscious sheep at term a longitudinal velocity of 7.2 cm/s. These values are comparable to what was, in this study, measured in the isolated guinea pig horn (6.8 cm/s longitudinal and 2.8 cm/s circumferential). Thus, spikes propagated anisotropically and the anisotropic ratio in the rat (25) is similar to what was, in this study, measured in the guinea pig (4.0 and 2.4, respectively).

In addition, data from the present study show that, during the burst, spikes occur at many sites and propagate from these both in the ovarian and the cervical direction. There is a large degree of spontaneous variability in these patterns as illustrated in Figs. 4–6. We found relatively few reentries and circus movements in the guinea pig compared with that in the rat (18, 21). The example shown in Fig. 5 is only one of five discovered in the hundreds of maps constructed during this study.

With such a high-resolution mapping technology, it was possible, for the first time, and in the whole and intact organ, to determine whether there were any regional variations in patterns of spike propagation in the guinea pig horn. With the exception of the mesometrial border, we could not find large differences in patterns of spike propagations in other parts of the uterus. Spike propagation was similar at the ovarian and the cervical end and at the ventral and the dorsal parts of the horn. The fractionated propagation along the mesometrial border, where the placenta had been located,
correlates well with studies in the rat myometrium in which weak potentials, slow propagations, and a shorter length constant were found in microelectrode recordings (16). In the pregnant cat, extracellular recordings performed in vivo and in vitro showed that the placental region was less excitable and showed little or no spontaneous action potentials (6). In humans, a similar functional asymmetry was shown whereby weaker contractions occurred in the placental regions compared with other regions (17).

With the organ intact, it was also possible to determine the overall pattern of electrical activities in the pregnant uterus. There have been many attempts to elucidate the pattern of propagation of the electrical impulses and its resulting contraction in the pregnant uterus. Earlier contraction studies seemed to show a fundus or corneal dominance (2, 12, 27). In contrast to the relative ease of determining contraction propagation, the electrical pattern seemed much more variable and therefore much more difficult to determine (13, 15, 28, 31).

The initiation of the myometrial burst along the antimesometrial border has not been reported before. Crane and Martin (4), in their laparoscopic study of myometrial contractions in rats in estrus, mentioned that circular contractions often seemed to move along the antimesometrial border and that, after implantation of the embryos, circular contractions became more prominent than longitudinal ones (3). This origin and orientation is also present in the initiation and propagation of circular spikes in the canine small intestine (22). It would seem that this
is a property of some visceral organs and may have to do with the anchoring of the organs to the mesentery.

An important finding in this study is the fact that bursts migrate in the myometrium at a much slower speed than the velocities of individual spikes. This phenomenon explains an anomaly (26) as to why spikes that propagate quite fast (in our case 7 cm/s) can take so long to travel relatively short distances (Figs. 8 and 9). This is analogous to the situation in the small intestine where spike velocity is much faster than that of the underlying slow waves (22).

The limitations of this study must be clear. The pregnant horns were isolated and superfused in vitro. Especially the handling of the uterus, with the removal of the fetus and its deflation and subsequent inflation with intraluminal fluid, will certainly have had influence on its behavior. Indeed, several studies have shown the sensitivity of the myometrium to uterine volume (5) and to stretch (7, 30). Another potential complication is the fact that gap junctions that electrically connect myometrial cells may form spontaneously in vitro, which may improve electrical coupling and affect electrical propagation (14, 29). Finally, even if a stable pacemaker had been found, there is evidence to suggest that this may not be stable during longer recording periods (26). Nevertheless, this study provides information as to the electrical behavior of the
pregnant uterus at term. It suggests that spike propagation forms the basis for contraction and that the pattern of spike propagation and variability in origin and direction are normal features of the pregnant uterus, at least at term. In addition, by analyzing the initiation of the burst at local sites, some insight was obtained on the origin and direction of migration of the myometrial burst.

In contrast to other organs, we know very little about the normal, let alone about the abnormal, patterns of excitation and contraction in the uterus. The approach presented here could be helpful in elucidating patterns of propagation during different stages of gestation, during labor and after delivery. Abnormal delivery, premature labor, and other pathological situations could also be investigated. Fortunately, other techniques are also coming online, such as abdominal uterine electromyography (1, 23), magnetomyographic recordings (11), and even uterus transplants (8) that could lead to better understanding of uterine pathology. Increasing our knowledge of the basic function of the pregnant uterus could help in applying these technologies in the treatment of patients.

**Perspective and Significance**

There is surprisingly little information about the pattern of propagation of electrical impulses in the pregnant uterus. Most studies have used one to six extracellular electrodes, either in vivo or in vitro, to characterize myometrial pregnant activity, which essentially consists of cycles of spiking bursts. Our own group reconstructed the propagation of individual spikes in isolated segments of pregnant myometrium from the rat (20) and showed that a myometrial burst consists of many spikes that propagate mainly in the longitudinal direction, either toward the cervix or the ovaries and that this can change spontaneously from spike to spike. This pattern is present in all parts of the horn, along the anterior and posterior surface and along the antimesometrial border. In contrast, propagation along the mesometrial border where the placentas are located is very sparse and fragmented.

The present study has also, for the first time, studied the propagation of the myometrial burst. It was found that bursts originate most often from the ovarian end of the horn and propagate preferentially along the antimesometrial border. Significantly, the propagation of the burst is much slower than that of spikes and could be termed “migration” analogous to the migrating complex in the intestines. With these results, we now obtained a first glimpse of the patterns of electrical activities in the pregnant uterus. These propagation patterns are important as they determine the sequence of contraction, ultimately used in the process of labor.

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Fig. 9. Spatial plot of the migration of a burst along a guinea pig horn. The horn has been virtually cut open along the mesometrial border. Two migration maps are shown from 2 bursts indicated in the top tracing. The first isochrone (red) indicates excitation during the first second, while the other isochrones are drawn in steps of 5 s. The initiation of the 2 bursts occurred along the antimesometrial border, occasionally at several sites, within 1 s (red isochrone). Other areas were activated later, while the mesometrial border was excited 25–35 s after the initiation of the burst.