REFRACTORY PERIOD AND CONDUCTION OF EXCITATION IN THE UTERINE MUSCLE CELLS OF THE MOUSE*

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Since the applications of microelectrode techniques to the field of smooth muscle physiology (GREVEN, 1953; BÜLBRING, 1954; WOODBURY & McINTYRE, 1954), a considerable number of reports bearing fruitful results have been published on the intracellular electrical activity of smooth muscles. However, most of the reports are concerned mainly with spontaneous discharges of the muscle fiber and little is known on the electrical activity in response to stimulation.

BÜLBRING, BURNSTOCK & HOLMAN (1958), giving electrical stimulation with extracellular and intracellular electrodes to the smooth muscle of the guinea-pig's taenia coli, have shown that activity in one cell caused by the stimulation is quickly followed by electrical response in an adjacent cell. These observations seem to have important meanings suggesting an existence of direct electrical transmission of excitation between the smooth muscle fibers.

In connection with this, the present study, on the transmembrane action potential in response to electrical stimulation of the uterine smooth muscle of the mouse, has been undertaken to elucidate further the mode of excitation transmission in the muscle, particularly from a point of view of the intercellular functional connection or interaction.

METHODS

Isolated uterine smooth muscles taken from dd-progeny mice on the 16-20th day of pregnancy were used in all experiments. Approximately 1 cm of the horn was removed and cut longitudinally in order to allow the tissue to be spread flat. The uterine strip was mounted on a paraffin block by pinning it with its serosal surface upward. The tissue, thus partly immobilized, was immersed in a chamber filled with oxygenated solution. The solution contained (mM): NaCl 134.0, KCl 4.6, CaCl$_2$ 2.5, MgCl$_2$ 0.1, NaHCO$_3$ 16.3, NaH$_2$PO$_4$ 1.1, glucose 7.8. The temperature of the fluid within the chamber was 35°C (±0.5) for all experiments.

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The intracellular potentials of the muscle were recorded with Ling-Gerard ultramicroelectrode mounted flexibly on a fine straight silver wire, measuring 2.5 cm in length and 50 μ in diameter and suspended perpendicularly. The input stage for the microelectrode consisted of a negative capacity, feed-back preamplifier (Nihonkoden, MZ-3A), and connecting this to a dual-beam cathoderay oscilloscope (Nihonkoden, VC-6), the potential change was observed and recorded by a camera.

For the electrical stimulation, enamel-corted Ag-AgCl electrodes with naked tips were attached on a part of the muscle strip, and negative square pulses of 5 msec. duration were given by an electronic stimulator through an isolation transformer.

RESULTS

I. General features of the transmembrane potentials.

In the latter half period of the pregnancy, transmembrane resting potential of the myometrial cell varied markedly in size between 30 and 80 mV (Fig. 1). Single electrical stimulation applied to the muscle, in this period, frequently produced an action potential in the penetrated cell and even a series of discharges in some cases. The action potential which appeared overshooting the resting membrane potential was not rare, but no overshoot was calculated in the means, showing a value of 38.2±0.38 mV for action potential and 51.3±0.35 mV for resting potential respectively. In case of the typically overshot action potential, no particular difference was noticed between driven and spontaneous ones, both showing a marked similarity in time-course with that of the skeletal muscle if the time-scale was neglected. The time-course of the driven action potential, however, was found variable depending on strength and frequency of the stimuli, and on distance between the stimulating and recording electrodes. As the details will be mentioned in the following sections, a peculiar slow potential appeared often superimposed on the rising and falling phase of the spike and caused a

![Graph](image-url)

**Fig. 1.** Frequency distribution of resting potentials (RP), action potentials (AP) and the durations at 50% or 10% level of spike height. Ordinate: Number of specimens (SP). Abscissa: Membrane potential (mV) and duration of AP (msec).
marked deviation in shape of the action potential as a whole.

Because of the existence of the slow potential and of the after-potential variations, duration of the action potential altered in wide range. The mean value was 25±0.14 msec. in 50% level of spike height and 69±0.40 msec. in 10% level. The standard deviation of the means was larger in the case of the 10% level, suggesting the more labile character of the slow potential component than of the spike component in general sense.

II. Configuration of the action potentials and strength of stimulation.

When an electrical stimulus close enough to the threshold was applied, a peculiar slow potential was frequently elicited in the penetrated cell after a time intervals. Gradual strengthening of the intensity of the stimulation caused generally a growth in size of the slow potential which would finally evoke a spike potential. In some cases, however, two or three slow potentials were produced for a single electrical shock and elicited spikes when the slow potentials exceeded some threshold level, or evoked a single spike after summation of the slow potentials synchronized by further strengthening of the stimulation (FIG. 2).

![Images](image-url)

**FIG. 2.** Transmembrane potentials of the uterine smooth muscle of the pregnant mouse. A) Single spike elicited by extracellular electrical stimulation. B-F) Superimposed records of the action potentials in response to the stimuli of different intensities. The intensities and time of stimulation are marked as dots in the upper trace of each record. Note appearance and disappearance of small potentials with increasing intensity of stimuli in records C-F. Calibrations in the lower right-hand corner are 20 mV and 200 msec.

These findings will bespeak that the intracellular action potential of the uterine smooth muscle is composed of two different component potentials, i.e. the slow potential and spike potential, and that the former is a generator of the latter. The phenomenon that two or three intracellular slow potentials were produced despite an extracellular electrical stimulation applied to a remote area would be a special character of visceral smooth muscles which is discriminated from that of normal skeletal muscle fibers or nerve fibers.
III. Effect of the distance between stimulating and recording electrodes on the action potential.

Another important factor which affected the time-course of the action potential was the distance between stimulating and recording electrodes. Fig. 3 illustrates a series of the records obtained with various electrode-distances from 1 to 6 mm. As seen in the left-hand records, when the distance was small, particularly when a strong stimulus was applied, the action potential used to appear peculiarly deformed and complicated by humps. Generally, the spike decreased in height and increased in duration and became abortive for too strong stimulation.

![Fig. 3](image)

**Fig. 3.** Relations between the electrode position, strength of stimulation and intracellular action potential. Distances between the stimulating and recording electrodes are denoted on top of the figure. Strengthening of stimulation causes a shortening of latency but produces variable effects on the spike height and configuration depending on the electrode-distance. Further explanation is in text. Calibrations in the lower right-hand corner are 20 mV and 200 msec.

On the other hand, when the stimulating electrode was located more than 3-4 mm apart from the recording electrode, strengthening of electrical stimulation usually caused an increase of spike height and a decrease of spike duration. And, in consequence, a brisk smooth action potential was produced especially after the summation or unification of slow potential and spike.

The reason why these two different tendencies, increase or decrease in spike height for strengthening of stimulation, were produced, remains in question. However, in the case of small electrode-distance, almost synchronous excitation of numerous cells around the microelectrode and their developed tension seem to play an important role for the change in spike configuration. In the case of large electrode-distance, the growth of spike due to strengthened stimuli might be associated with a physiological change from local response to full spike.

IV. The latency and conduction velocity.

Generally, the stronger the electrical stimulation and the smaller the distance
between stimulating and recording electrodes were, the shorter became the latency of the response (Fig. 3). However, since there were numerous cells between the two electrodes, the latency looked outwardly might consist of four components at least; i.e. 1) the true latency of a stimulated cell, 2) that of a penetrated cell, 3) conduction time from the stimulated cell to the penetrated cell, and 4) effect of the electrotonic spread of stimulation when the intensity is strong.

Among these components, the third factor is presumed to play an overwhelming role, because this will be a total sum of latency and conduction time in each of the numerous cells and further of probable transmission delay between the cells. The presumption might be supported by the fact that almost linear relationship was observed between the latency in wide sense and the electrode-distance if the strength of stimulation was fixed at a level slightly above the threshold.

The conduction velocity of excitation in the uterine muscle tissue calculated from this relationship was about 10.2±0.41 cm per second. Almost similar values were directly estimated by inserting two microelectrodes into two cells in the same bundle of the smooth muscle, but much smaller and variable values were measured in case of double penetrations of the different bundles (Fig. 4).

V. The refractory period.

Excitability of the uterine smooth muscle varied more or less spontaneously
and changed markedly depending on the antecedents such as preceding excitation and stimulation. Even a subthreshold stimulus, if applied two or three times successively, usually caused a considerable change in the threshold. Under these conditions, determination of the relative refractory period was extremely difficult. The absolute refractory period, on the other hand, was decidable if the shortest time-interval between the first threshold and the second effective stimuli was taken. The value thus obtained was about 0.1 second. And the time which was necessary for an appearance of the second full spike was about 0.2 second in case of the strong second stimulus (Fig. 5). The latter value, however, does not mean the duration of relative refractory period, because the second stimulus was not equal in intensity to the first threshold stimulus.

**Fig. 5.** Superimposed records of intracellular action potentials educed by successive stimulations. I) With constant stimulus strength, time-interval between two stimuli was varied. II) With constant time-interval, the strength of the second stimulus was varied. The first and second stimulation in I and the former in II were strengthened from A to C. The absolute refractory period shows a tendency to increase with increasing strength of the first stimulus in both series of records. Calibrations are 20 mV and 200 msec.

On the other hand, these values obtained are considerably larger than those estimated from appearance of double spikes or maximum discharge frequency during spontaneous contractions. The reason might be reduced to the fact that in spontaneous discharges merely the refractory period of a penetrated cell is involved, while in the case of driven action potentials the refractory periods of the numerous cells in the conduction pathway between the two electrodes would participate in the observed refractoriness.
DISCUSSION

Recent studies concerning conduction of excitation in visceral smooth muscles support strongly a view that the cells behave as a kind of syncytium and almost no possibility of spread of excitation via nervous element exists (Bozler, 1948; Greven, 1955; Prosser, Smith & Melton, 1955. Prosser & Sperelakis, 1956; Bülbring, 1955, 1956). On the other hand, studies on electrical activity of the smooth muscle cells with ultramicroelectrode have elucidated that a peculiar slow potential frequently makes its appearance in between the intracellular action potentials and shows a character closely resembled to that of ephaptic potential or synaptic potential (Bülbring, Burnstock & Holman, 1958; Goto & Woodbury, 1958; Thiersch, Landa & West, 1959; Goto, Kuriyama & Abe, 1960).

Thus, when these series of investigations are summarized, the peculiar slow potential must be noted as a special potential at the third type of cell union, the muscle-muscle junction, next to the nerve-nerve junction and nerve-muscle junction. On this point of view, authors' special attention was focused on the mode of appearance and disappearance of the slow potential.

As was stated in the first section of Results, gradual strengthening of the electrical stimulation applied to an extracellular remote area caused first an appearance of the slow potential and next a spike potential when the former exceeded the threshold level. Such an intracellular slow potential might be ascribable to 1) an electrotonic spread of the stimulation, 2) depolarization of the cell membrane by tension development, 3) an artificial product educed by movement of the microelectrode, 4) a synaptic (or ephaptic) potential chemically produced, or 5) an electrotonic spread of excitation of the adjacent fiber.

Since duration of the slow potential was more than 50 msec. and hence approximately hundred times longer than that of the electrical stimulation (0.5 msec.), the first possibility will be easily rejected. The second possibility, on the other hand, is likely since close parallelism between the membrane potential level and tension has been known (Bülbring, 1955; Goto & Woodbury, 1958). It is, however, considerably difficult to explain the appearance of two or three slow potentials for a single electrical shock and also the fact that the slow potential was relatively consistent in shape as far as one penetration is concerned. Furthermore, the fact that the slow wave was always positive (depolarization), instead of negative (hyperpolarization) which might have been expected from contraction of parallel-running fibers, may allow to reject the second possibility.

The third possibility is also unlikely from the same reason. This possibility was disproved also by the fact that the slow potential tended to appear at a threshold stimulation and to disappear with strengthening of the intensity despite an increase in mechanical contraction of the muscle.

In contrast, all the characters of the slow potential mentioned seem to support the last two possibilities. Thus, the slow potential would be a special
potential which concerns excitation transmission process of low safety factor from an adjoining fiber to the penetrated one. But whether or not a special chemical substance is participating in appearance of the slow potential still remains in question. However, the fact that the spike height tended to increase instead of decrease in case of simultaneous appearance of the slow potential and spike might support the possibility of the electrical transmission of excitation between the fibers.

Bülbring, Holman & Lüllmann (1956) had studied the behavior of the frog’s striated muscle in calcium-deficient medium which showed remarkable similarity with that of the normal smooth muscle, and elucidated that peculiar slow waves appeared which grew in size until they gave rise to the action potential. Tamai, Abe & Goto (1961), performing double penetrations of adjoining two fibers in the same preparation, observed not only an intimate correlation between the slow potential and the adjacent fiber activity, but also the slow potential with negative or diphasic sign. However, since such a negative or diphasic slow potentials have never been demonstrated in visceral smooth muscle, mechanism of excitation conduction in the smooth muscle might be slightly different from that of the striated muscle in calcium-deficient solution, probably due to some structural difference in the junctional region between fibers. Thus, the syncytial nature of the smooth muscle cells might be emphasized.

**SUMMARY**

1. The transmembrane action potentials driven by extracellular electrical stimulations were recorded with flexibly mounted microelectrode in the isolated uterine muscle of the mouse at latter half period of pregnancy.
2. Single action potential was usually produced by an electrical shock, but a series of discharges on some occasions. Magnitude of action potential was 38.2±0.38 mV on an average, while that of resting potential was 51.3±0.35 mV at this stage of pregnancy.
3. Duration of the action potential at 10% level of spike height was 69±0.40 msec. on an average but varied markedly in individual cases, mostly because of variable magnitude of the after-potential and of the peculiar slow potential superposed on the rising or falling phase of the spike.
4. With gradual increase in the intensity of extracellular stimulation, a peculiar slow potential first made its appearance and then a spike when the slow potential exceeded a critical depolarization. It was also at the threshold stimulus that two or three slow potentials were frequently produced.
5. Further increase in the intensity, however, usually caused a shortening of latency of both slow and spike potentials and finally set up a simple typical action potential.
6. When too strong stimulus was applied to a portion near the recording
electrode, the action potential appeared mostly flattened and markedly deformed showing double peaks in some instances.

7. The refractory period was about 0.1 sec. for producing an effective second response and 0.2 sec. for producing the second full spike.

8. The conduction velocity of excitation was 10.2±0.41 cm per second in the same muscle bundle, but much slower and variable between different muscle bundles.

9. Discussions were made on the nature of the peculiar slow potential.

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REFERENCES


