The Effect of Changes in NaCl Concentration on the Action Potential of Rat Uterine Smooth Muscle

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The effect of changes in ionic environment on the smooth muscle cell membrane activity has been widely investigated and it has been discussed whether or not the sodium theory might be applicable to the smooth muscle cells. It has come to be considered generally that the smooth muscle cell accept the sodium theory basically, though there should exist some differences between the smooth muscle and the other excitable tissues concerning the ionic transportation through the cell membrane.

It has been stressed that the activities of smooth muscle cells after prolonged exposure to extremely low Na-ion concentration or to a Na-free medium are different from that of other excitable tissues. (Burnstock, G. et al. 1958; Holman, M. 1958; Axelsson, J. 1961.)

In the present experiments, the spontaneous activities of pregnant rat uterine smooth muscle were studied by intracellular microelectrode technique together with the effects of lowering the enternal Na-ion concentration upon them.

METHODS

The smooth muscle used in all experiments was taken from pregnant rat uterus near or at term. Muscle strips (0.5 × 1.0cm.) from nonplacental region were mounted isometrically in a bath of 3ml. capacity which was irrigated continuously by oxgen saturated modified Krebs's solution (36°C) at a rate of about 2 ml./min. The composition of the fluid was similar to that of Holman; NaCl 134, KCl 4.7, $MgCl_2$ 0.1, NaH_2PO_4 1.1, $NaHCO_3$ 16.3, $CaCl_2$ 2.5, and glucose 7.8(mM).

For reducing the external sodium concentration, the sodium ion in K-buffer normal solution was replaced by choline chloride. The floating microelectrode technique was used to record the membrane and action potential. The resistance of the microelectrode (filled with 3M KCl) were 30–70MΩ (usually 35–50MΩ).

RESULTS

Normal activity

During spontaneous activity, the membrane potential of pregnant rat uterus was very unstable and varied from 40–50 mV. The amplitude of spikes accompanied by spontaneous contractions ranged from 50 to 67 mV. The typical recordings are illustrated in Fig. 1. The overshoot reached usually 5–17 mV. The configuration of spikes was similar to that recorded from taenia coli. The spike arose steeply from the resting level or from the prepotential to reach the peak in about 7 msec. later. Generally repolarization proceeded somewhat slowly in comparison with depolarization (Fig. 1, e). The maximum rate of rise and decay were 9 and 5 V/sec. respectively.

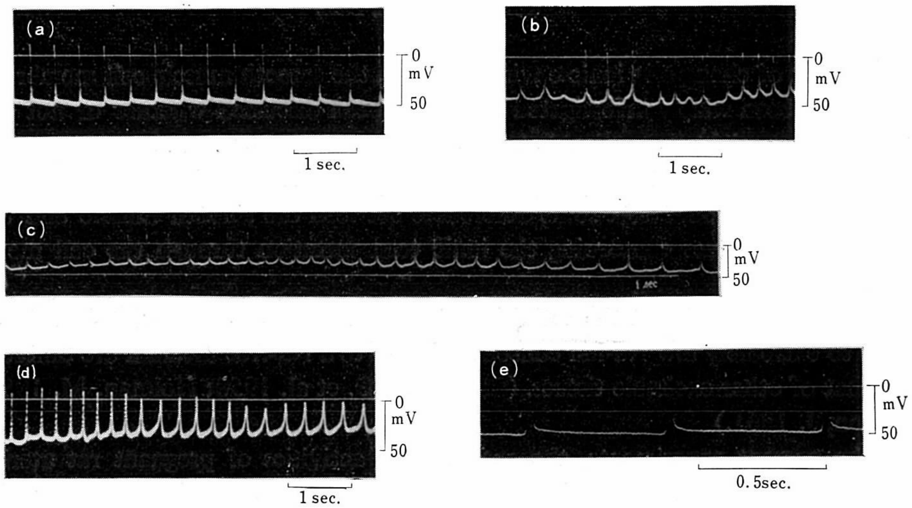

Fig. 1. Variations of spontaneous activity of pregnant rat uterus. (a), (b), (c) and (d) illustrate the range of activity accompanied by spontaneous contration. Resting potential varied from 40 to 50 mV and action potential ranged from 50 to 67mV. (b), (c) and (d) illustrate the three types of slow fluctuation of membrane potential. (b) local potential, (c) long term fluctuation accompanying the burst of spikes, (d) pace maker potential and illustrate that the pace maker activity shifted easily from one cell to another. (e) faster sweep of (a).

There could be seen several kinds of slow fluctuation of membrane potential in the spontaneous activities of pregnant rat uterus. One was a long term fluctuation accompanied by bursts of spike discharges which corresponded to each tetanic contraction (Fig. 1, c). Another was slow rhythmic fluctuation of the resting potential appearing in the same interval as that spikes, although the action potential sometimes dropped so that only a slow fluctuation remained (Fig. 1, b).

There seemed to be a close correlation between the initiation of spike and the appearance of slow rhythmic fluctuation. On some occasions, there was some delay between the appearance of spikes and the slow fluctuations so that the spikes arose in the various phases of the slow fluctuation. Further was it found that there were some omission of spikes during the burst of spike discharges and only the slow fluctuation of membrane potential could be recorded there. In some other instances a spike discharge continued smoothly from the rising phase of the slow fluctuation which resembled just what was recorded in the pace maker area of heart. On pregnant rat uterus near or at term the pace maker potentials were occasionally recorded at random along the whole uterine strip and it seemed not to be localized in any definite area.

Furthermore was it found that the pace maker potential might suddenly disappear while the repetitive discharges of normal action potential occured, presumably indicating that the pace maker activity shifts easily from one cell to another.

Low sodium chloride concentration: (replacement with choline chloride)

The effects of low sodium chloride concentration were tested on pregnant rat uterus. In order to reduce the sodium ion concentration in the external medium, the NaCl was replaced with isosmotic amount of choline chloride. However, even if all the NaCl had been replaced, the modified Krebs's solution still contained 17 mM Na as the Na-buffer, so the K-buffer normal solution was used in all the Na-replacement experiments.

Reduction in external sodium concentration to 20mM had slight effect upon the amplitude of the spike potential and its frequency. In Fig. 2 the first record (a) was taken during a spontaneous activity in normal solution. The second record (b) was

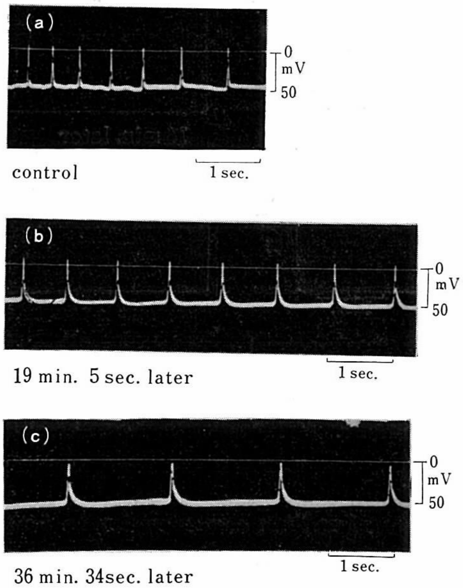

Fig. 2. In this experiment the Na^+ ion concentration was reduced to 20mM. The first record (a) was taken in normal solution. The next records (b) and (c) were taken after about 19 min and 36.5 min exposure to the 20 mM Na^+ solution respectively.

taken about 19 min. after the exposure to 20mM Na solution. Except the slight decrease in frequency and the slight increase in duration of spike discharge, it could not be distinguished from normal ones. The third record (c) was taken 36 min. 34 min. later. The frequency decreased further but the amplitude decreased a little. There were no more changes discernible in the spike amplitude and frequency after very prolonged exposure to any higher NaCl concentration than 20mM.

When the sodium chloride was reduced to 10mM, there appeared nearly the same tendency in the spike height and frequency to change as was seen in the experiment with 20mM Na solution (Fig. 3).

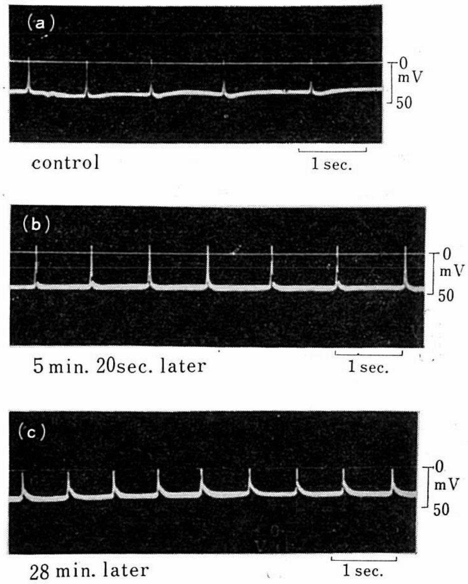

Fig. 3. The first record (a) was taken in normal solution. (b) was taken after 5 min 20 sec exposure to the 10mM Na^{+} solution and (c) was taken 28 min later.

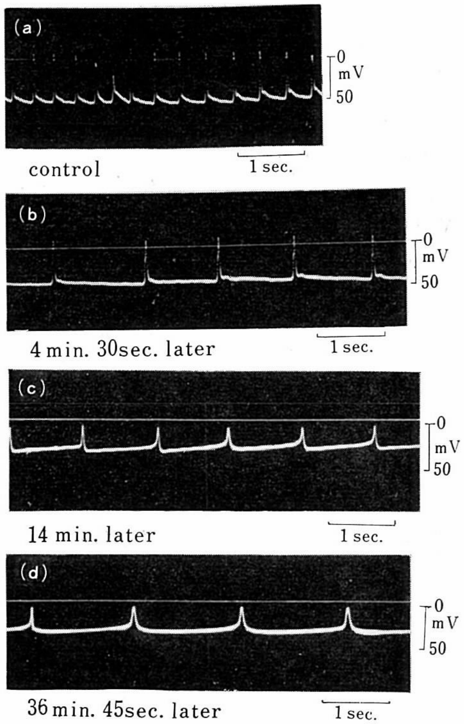

Fig. 4. The first record was taken in normal solution (a), The next records (b), (c), and (d) were taken after 4 min 30 sec, 14 min and 36 min 45 sec exposure to the 5mM Na^{+} solution.

Further reduction in external sodium concentration to 5mM gave rise to similar but somewhat stronger changes in spike height, frequency and configuration than in 10mM-Na. In Fig. 4 the first record (a) was taken in normal solution. The second (b) was taken 4 min. 30 sec. after the exposure to 5mM Na solution. Here the marked decrease in spike frequency was noted but there was little change in spike height and configuration. After 36 min. 45 sec. the spike height decreased markedly and the half duration of spikes increased. These deformed spikes appeared in more prolonged intervals than those in the first and second records.

When the total sodium ions were replaced with choline chloride (K-buffer), no change was detected in spike height and configuration during the first 6–7 min. but later the height began to decrease, the duration to increase and the configuration to be deformed gradually into a small peaked negativity (Fig. 5). The peaked negativity did not easily disappear but continued to appear though in long intervals for 22 min. or more.

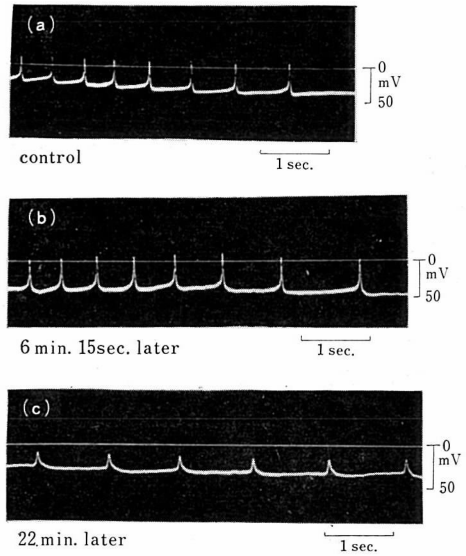

Fig. 5. The first record (a) was taken in normal solution. The next records (b) and (c) were taken after 6 min 15 sec and 22 min exposure to the Na^+-free solution.

DISCUSSION

The muscle strips from pregnant rat uterus nearer at term exhibited the spontaneous contractions in intervals of several minutes. Accompanying a spontaneous contraction a series of action potentials of 50–70mV were recorded which started at the initial membrane potential of 40 to 50mV, attaining the overshoot of 10 to 17mV. Recently it was reported that the membrane activity was usually restrained in pregnancy; thus action potential appeared asynchronously and the overshoot were seldom found (Kuriyama et al 1961, Marshall 1959). This restrained membrane activity was considered by them to be caused by progesterone dominance in the pregnant uterus. In our experiments the muscle strips obtained from pregnant rat uterus near or at term frequently exhibited considerably regular membrane activities, but it could not be ascertained whether the well integrated activities were virtually occuring in the pregnant uteri near or at term, or not, because we did not record electrical and mechanical activities simultaneously. That the dropping of the spike could sometimes be seen (Fig. 1, b) might indicate the presence of asynchronous activity of muscle cells. Nevertheless the general occurrence of regular membrane activity might suggest that the uterine muscle at the preliminary stage for parturition is already under the influence of estrogen or other activating substances resulting in the increased excitability.

In spontaneous activities there could be seen three types of fluctuation of membrane potential in late pregnancy of rat uterus which are in agreement with the results of other previous investigators (Bülbring et al 1958, Kuriyama et al 1961, Bülbring 1955). They are the local potential having the same frequency as the spike, a long standing fluctuation accompanying the burst of spikes and the pace maker potential. In pregnant rat uterus near term the pace maker potentials were very often recorded by chance along the whole surface of the uterine horns and it seemed probable that they were not localized in any definite portion. The presence of pace maker area under various hormonal conditions of the uterus has been reported (Marshall 1959). It was then shown that in progesterone-dominated uterus pace maker potentials were recorded not consistently in any particular area but over the whole uterus. In this point our findings are in agreement with that of Marshall. However, once the uterus in late pregnancy begins to develope labor, the uterine contractions are converted into the exquisitely co-ordinated efficient contractions. In our experiment with extracellular technique (unpublished) the parturient uterus seemed to initiate the contractions at both the upper and the lower pole of the fetal sack alternately. This evidence suggests that there could be found typical pace maker depolarization at both ends of fetal sack, though we have not yet record it with the intracellular electrode. Further investigation concerning the localization of pace maker area during parturition is in progress now.

It has been well discussed that the electrical activity of the smooth muscle is affected by changing the external sodium concentration, especially when the concentration is very low. Holman (1958) reported that the smooth muscle of guinea pig taenia coli was able to maintain almost indefinitely its spontaneous activity discharging the spike potentials at normal frequency when the external Na ion concentration was reduced to 20mM, and also that the activity continued in 5mM Na medium but was abolished in 2mM Na medium after 35 minutes. She also reported that when the muscle was exposed to 10mM Na medium for over 100 min. and then the solution was changed to one having O Na, the spikes changed rapidly to slow fluctuations and all the activities were abolished in 13 minutes. Daniel et al (1958) reported that in pregnant cat uterus the total replacement of external sodium ions did not block the spontaneous contraction but only affected the amplitude and the rate of rise of the action potential. In our experiments with pregnant rat uteri there were also little detectable changes in spike height and configuration when the external Na ions were reduced to 20mM. However, in prolonged exposure to 10mM or less Na^+ solution some changes appeared in spike height. When the muscle strips were bathed in a completely Na-free solution which flowed continuously at a rate of about 2ml/min., the spikes gradually decreased in amplitude until they became small peaked negativities which remained for over 25 min.

It should be emphasized that the smooth muscle cells are able to maintain its spontaneous activity in Na-free solution for such a longer time than at the other excitable tissues. To explain this phenomenon it has been assumed by Holman (1958) that the Na carriers in smooth muscle cell membrane might be saturated more easily by very small amount of Na ions, or that the Na ions in the carrier might be replaced by some other substitutional ions in the solution of low sodium concentrations. In our experiments, however, there was no evidence to explain the prolonged activity of smooth muscle on exposure to a solution of very low Na^+ concentrations.

SUMMARY

1. The electrical activities of pregnant rat uterus near or at term were investigated by intracellular microelectrode.

2. During spontaneous activity the membrane potential was very unstable and varied from 40 to 50mV. The spikes accompanied by spontaneous contraction ranged from 50 to 67mV. The overshoot recorded was usually 5 to 17mV.

3. Several types of slow fluctuation of membrane potentials were recorded during spontaneous activities which were similar to that previously reported by Bülbring et al.

4. The pace maker potential was found by chance all over the whole uterus, which seemingly was not localized in any particular area.

5. The behavior of the smooth muscle in the solution of low sodium concentration was examined. Little changes were detectable in spike height and configuration when the external sodium concentration was reduced to 20mM but further reduction to 10mM or less affected the spike height and configuration gradually.

6. The spontaneous activity was maintained in Na free solution for 25 min. or more though the spikes degenerated into small peaked negativities. The findings in our experiments seem to be important for explaining the electrical activity of the uterine muscle cell membrane from the stand point of the sodium theory.

LITERATURES

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