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EFFECTS OF CHANGES OF IONIC ENVIRONMENT ON THE NEGATIVE AFTER-POTENTIAL OF THE SPIKE IN RAT UTERINE MUSCLE

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SUMMARY

1. The spontaneous activity of the smooth muscle of rat uterus consists of bursts of spikes, each spike being followed by a negative after-potential. The effect of changes in ionic environment on the negative after-potential was investigated at various stages of pregnancy and in the non-pregnant condition.

2. The amplitude of the negative after-potential was the same in spontaneously generated and electrically evoked spikes. During repetitive discharge, whether spontaneous or in response to depolarizing current application, the amplitude of the after-potential was smallest in the first spike of a burst and it increased gradually with repetition of discharge.

3. The decay of the negative after-potential was slower than the passive return of the membrane potential to its resting level.

4. The amplitude of the negative after-potential was larger in non-pregnant uterus than during late pregnancy.

5. In pregnant uterus, the replacement of the Cl in the medium with benzene sulphonate transiently augmented the negative after-potential and then gradually reduced it. Eventually, the negative after-potential disappeared and, instead, a positive after-potential was observed. This conversion took place without a noticeable change in the resting potential or in the initial falling phase of the action potential itself. Replacement of Cl with NO_3 had no appreciable effect on the negative after-potential.

6. In non-pregnant uterus, the conversion of the negative to a positive after-potential was never observed. However, in Cl-deficient solution the size and duration of the negative after-potential were reduced.

7. In Cl-deficient solution (benzene sulphonate substitution), the decay of the electrotonic potential following the break of cathodal current became faster than that in normal solution. On the other hand the development of the anodic electrotonic potential became slower.

8. Replacement of the NaCl in the medium with sucrose converted the negative after-potential to a positive after-potential. On the other hand, reduction of Na only by replacement of NaCl with Tris-Cl had no noticeable effect on the negative after-potential.

9. It is concluded that the negative after-potential of the spike in rat uterine muscle is largely due to an increase of Cl conductance of the membrane.

INTRODUCTION

In rat uterine muscles the spontaneously generated or the electrically evoked spike is followed by a 'negative after-potential' throughout all stages of gestation and in the non-pregnant condition (Casteels & Kuriyama, 1965). Several interpretations for the negative after-potential of other excitable tissues have been put forward: (1) accumulation of K ions close to the excitable membrane as a result of its release during each spike (Shanes, 1949*a*, *b*, 1951; Frankenhaeuser & Hodgkin, 1956; Freygang, Goldstein & Hellam, 1964), (2) some changes in membrane permeability (Shanes, Grundfest & Freygang, 1953) and (3) termination of the rapidly declining phase of the action potential when the membrane is still slightly depolarized (Frank, 1957).

The present work was undertaken to study the effects of various environmental factors on the negative after-potential of the smooth muscle of rat uterus. The results of the experiments described below support the view that an increase in Cl conductance of the membrane accounts for a large part of the negative after-potential.

METHODS

The rat myometrium at various stages of pregnancy and in non-pregnant condition was used. Strips, about 2 mm wide and 15 mm long, were dissected and mounted in a bath with two compartments, one for stimulating and another for recording (Tomita, 1966; Abe & Tomita, 1968). The muscle strip was pulled through a hole in the partition, which served also as one large external stimulating electrode, the second electrode being placed on the end of the tissue at a distance of about 10 mm. The other end of the tissue in the recording compartment was fixed by a pin. Rectangular current pulses of known intensities were applied. The membrane potential was recorded, at a distance of 0·2–3·0 mm from the stimulating electrode, with glass micro-electrodes filled with 3 M-KCl solution. Their resistance varied from 40 to 90 MΩ.

The normal Krebs solution had the following composition (mM): Na 136·9, K 5·9, Ca 2·5, Mg 1·2, Cl 133·6, HCO_3 15·5, H_2PO_4 1·2 and glucose 11·5. It was oxygenated with a gas mixture of 97 % O_2 and 3 % CO_2 and maintained at 35° C. To this solution 292 mM sucrose was added to make it hypertonic, since this abolishes the contraction and, hence, the dislocation of the micro-electrode (Tomita, 1966). Changes of the ionic composition are described in the appropriate sections.

RESULTS

The action potential

All preparations were first equilibrated for 1 hr in normal Krebs solution before starting the experiment. Usually spontaneous activity was observed, consisting of bursts of spikes. Each spike, whether spontaneously generated or electrically evoked, was followed by a slow recovery of the resting

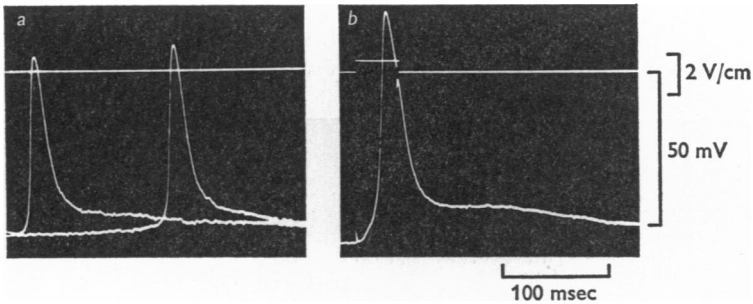

Fig. 1. Action potentials, (*a*) spontaneous and (*b*) evoked, recorded from a 13 days pregnant uterus. Upper trace: reference level (0 mV) and potential field (V/cm). Lower trace: intracellular record (mV). Normal Krebs solution. 35° C.

TABLE 1. The parameters of the action potential in three different hormonal conditions

	Non-pregnant	13 days pregnant	18 days pregnant
Membrane potential (mV)	45·9 ± 1·0	54·5 ± 0·9	49·8 ± 1·0
Spike amplitude (mV)	48·2 ± 1·9	66·0 ± 1·2	57·5 ± 1·9
Maximum rate of rise (V/sec)	2·5 ± 0·2	4·5 ± 0·3	3·4 ± 0·3
Maximum rate of fall (V/sec)	1·5 ± 0·1	1·8 ± 0·1	1·5 ± 0·1
Potential at which negative after-potential appears (mV)	12·8 ± 1·8	42·2 ± 0·7	33·1 ± 0·9

potential or a 'negative after-potential', as shown in Fig. 1. The parameters of the spike depended on the hormonal condition (Casteels & Kuriyama, 1965), but they also varied to some extent between spikes recorded on the same day of pregnancy. Table 1 shows the mean values for the parameters of the action potential obtained from ten spikes recorded from each of three preparations in each of three hormonal conditions, i.e. non-pregnant, 13 days and 18 days pregnant.

Some experiments to be described below were carried out in hypertonic

solution. Exposure to hypertonic solution caused no appreciable change of the membrane potential, but the number of spikes in a burst was markedly reduced and the interval between bursts was prolonged. The configuration of the action potential, however, remained substantially unaltered, though the time course was usually slightly prolonged.

The negative after-potential

The amplitude of the negative after-potential, measured as the difference in voltage from the resting potential, was always smallest in the first spike of a burst and it increased gradually with the repetition of discharge

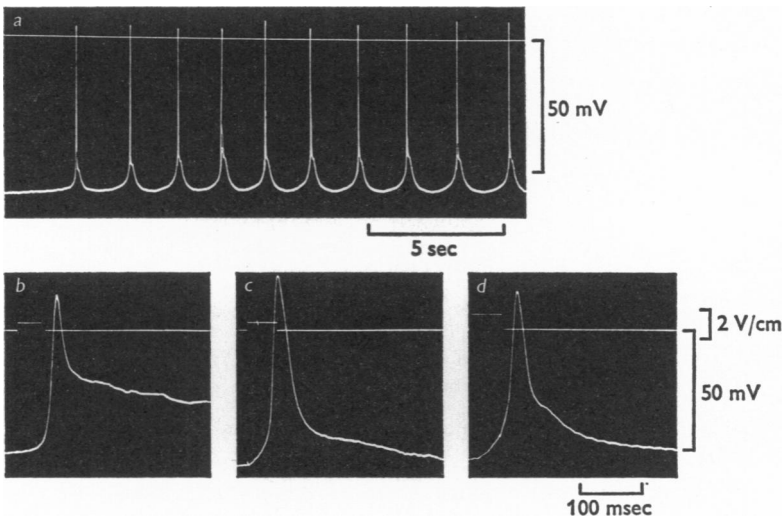

Fig. 2. Action potentials with negative after-potentials (*a*) following spontaneous repetitive spikes, recorded from a 18 days pregnant uterus, and (*b*) following a single evoked spike, recorded from a non-pregnant uterus, (*c*) 13 days pregnant and (*d*) 18 days pregnant uterus.

(Fig. 2*a*). A similar relationship was observed when a succession of spikes was evoked by repetitive stimulation. The amplitude of the negative after-potential was also related to the stage of gestation (Table 1 and Fig. 2*b*, *c*, *d*). Action potentials recorded from non-pregnant or early pregnant uterus (less than 1 week of pregnancy) were followed by large negative after-potentials which lasted more than 500 msec and, in some preparations, one or more small spike-like potential changes occurred on its peak. With the advance of pregnancy the negative after-potential became smaller and reached its minimum between the 13th and 16th day of pregnancy, but it could be seen throughout gestation, becoming slightly larger again near term.

The effect of exposure to Cl-deficient solutions

One possible interpretation for the negative after-potential is that G_{Cl} may be raised during the spike. If, as in the smooth muscle of rat uterus, $[Cl]_i$ is high and E_{Cl} much less negative than the resting potential (Casteels & Kuriyama, 1965) an increase in G_{Cl} will cause a depolarization towards the chloride equilibrium potential, E_{Cl}. Thus if G_{Cl} would be appreciably

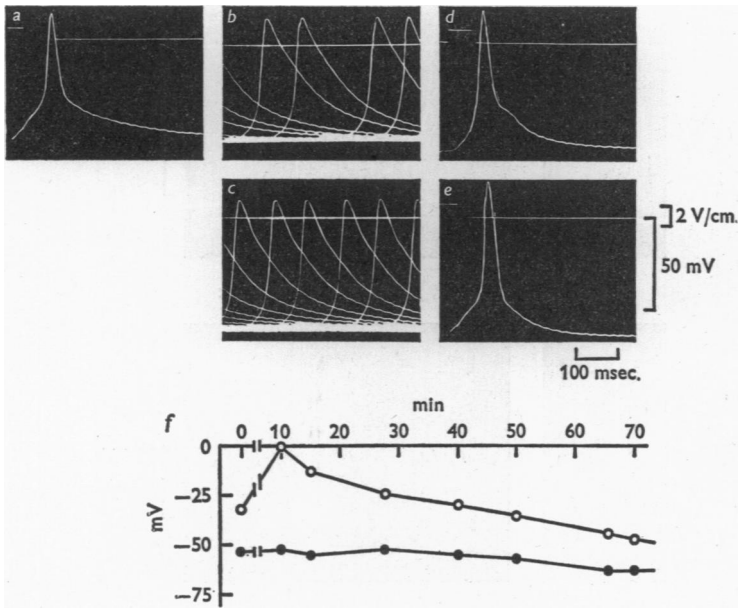

Fig. 3. Action potentials, generated spontaneously or evoked electrically, recorded from a 16 days pregnant uterus after different times of exposure to Cl-deficient solution (34 mM-Cl + 100 mM benzene sulphonate). *a*, control; *b*, 10 min; *c*, 15 min; *d*, 50 min and *e*, 70 min in Cl-deficient solution. The graph (*f*) shows the change of the negative after-potential and the resting potential (mV) with time of exposure (min) to the Cl-deficient solution taken from the same experiment.

elevated during the spike, the repolarization would proceed up to a value approximating E_{Cl}, and then, as G_{Cl} falls, the repolarization would gradually continue towards the original level of the resting potential.

Benzene sulphonate. When the normal Cl concentration was reduced from 134 mM to 67, 34 or 17 mM, by substitution with benzene sulphonate, the intermittent spontaneous activity became generally continuous or, in silent preparations, continuous activity appeared within 5–10 min. There was no noticeable change or only a slight increase in the resting potential. When continuous spontaneous activity was established, the rate of

repolarization was at first so much decreased that discrimination between the spike and the negative after-potential became difficult. After this stage, the action potential gradually increased in amplitude and then a notch or negative after-potential could be clearly seen. Gradually, the potential at which the notch appeared moved towards the resting potential. The interval between spikes became longer. Finally, after 40–60 min, the spontaneous activity ceased. At this time the evoked action potential was

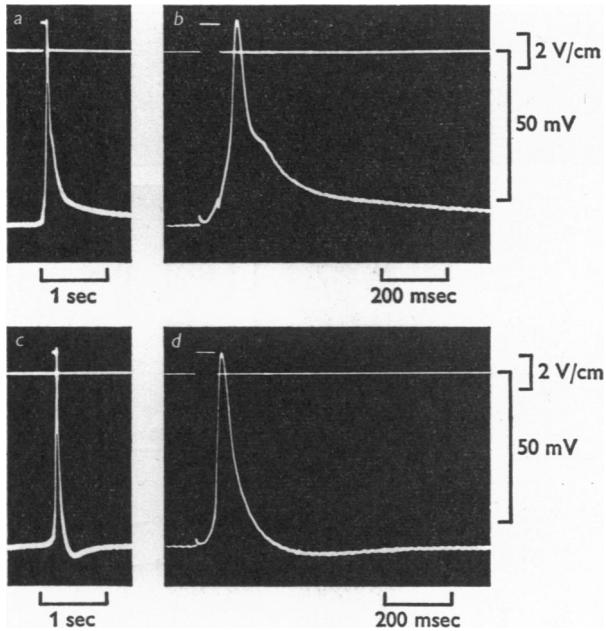

Fig. 4. A typical transition from a negative after-potential to a positive after-potential recorded at two different sweep speeds from 16 days pregnant uterus. *a* and *b*, control; *c* and *d*, after 40 min exposure to Cl-deficient solution (134 mM-Cl + 100 mM benzene sulphonate).

still followed by a small negative after-potential. This continued to decrease and, eventually, disappeared. Fig. 3 shows this sequence of events recorded from a 16 days pregnant uterus during exposure to Cl-deficient solution (34 mM-Cl + 100 mM benzene sulphonate).

If the resting potential was low, the negative after-potential was replaced by a 'positive after-potential', so that the spike potential terminated in a phase of hyperpolarization. A typical transition from a negative after-potential to a positive after-potential is shown in Fig. 4. Cl deficiency seems to have no effect on the mechanism determining the initial falling phase of the spike, since there was no detectable change in the initial rate of fall of the spike itself.

Responses to stimulation with a long current pulse

Stimulation with a long depolarizing current pulse usually induced repetitive responses, as shown in Fig. 5*b* and *e*. On rare occasions, a single spike was evoked by a current pulse of threshold intensity (Fig. 5*a* and *d*). In normal solution a positive after-potential could be seen only as a slight dip in the electrotonic potential (Fig. 5*a*), but in Cl-deficient solution,

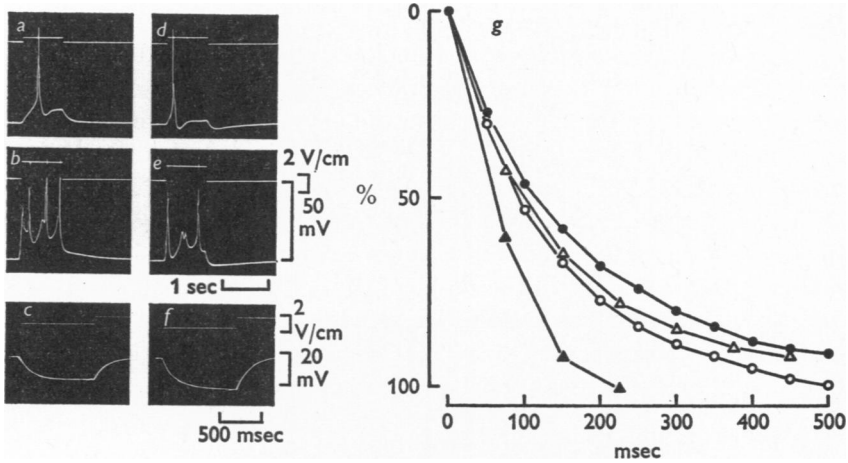

Fig. 5. Typical responses to cathodal and anodal current pulses of 900 msec duration, recorded from a 14 days pregnant uterus in hypertonic solution. All records from the same cell. *a*, *b* and *c* in normal solution; *d*, *e* and *f* after 30 min exposure to Cl-deficient solution (17 mM-Cl + 117 mM benzene sulphonate); *g* graph showing the difference in the time course of the decay of the cathodic electronic potential (△: normal solution; ▲: Cl-deficient solution) and of the development of the anodic electrotonic potential (○: normal solution; ●: Cl-deficient solution). Ordinate: the increase in membrane potential after break of cathodal current and make of anodal current, expressed as percentage of the total electrotonic potential reached at the end of current application. Abscissa: time (msec). See text.

an undershoot beyond the resting potential took place (Fig. 5*d*). The number of repetitive spikes during prolonged application of depolarizing current was reduced in Cl-deficient solution probably because the increased positive after-potential of the preceding spike suppressed the next spike. This does not mean that the refractory period was prolonged, since spikes could be evoked without any decrease in amplitude at a frequency up to 1/sec. In contrast, when repetitive stimulation was applied at a frequency exceeding 0·5/sec in normal solution, a sustained depolarization developed and the spike amplitude was markedly reduced, as previously reported by Casteels & Kuriyama (1965).

Another observation shown in Fig. 5 is a significant difference in the time course of the decay of the electrotonic potential, on which a spike was superimposed, following the break of the cathodal current. Not only the decay was faster in Cl-deficient than in the normal solution, but there was also a transient hyperpolarization before the return to the resting potential. On the other hand, the time course of the development of the anodic electrotonic potential was slower in Cl-deficient solution than in normal solution. In Fig. 5*g*, the increase of membrane potential following the break of the cathodal current in *a* and *d*, and the increase of membrane potential following the make of the anodal current in *c* and *f* are plotted against time, in order to show the difference in time course. The results suggest that, during the recovery from the depolarization, the K conductance was increased, but that this was masked in normal solution by the relatively high Cl conductance.

NO_3. Replacement of Cl with NO_3 at first slightly increased the membrane potential and then reduced it again to about its normal level. There was no noticeable change in the shape of the action potential although the frequency of spontaneous activity was slightly increased. It may be that the membrane permeability to NO_3 is close to that to Cl and therefore NO_3 is much less effective in changing the negative after-potential.

Effects of Na on the negative after-potential

An additional possibility for the negative after-potential, and not necessarily exclusive of the preceding one, is that Na is involved, since it is the predominant cation in the external solution. For the experiments carried out to test this possibility, solution of normal tonicity was employed.

Replacement of the NaCl in the medium with the corresponding amount of sucrose very effectively suppressed the negative after-potential which was eventually converted to a positive after-potential. Na deficiency led to a slight hyperpolarization of the membrane (2–8 mV), but this change was always small compared with the change in the amplitude of the negative after-potential. Fig. 6 shows records of the action potential recorded in normal solution and in a solution containing only 16 mM-Na.

It should be noted that, in substituting NaCl with sucrose, Na as well as Cl in the medium was decreased, so that the effect on the after-potential may not solely be due to Na deficiency. In order to test this point, the NaCl in the medium was replaced with Tris-Cl. The replacement gave rise to a decrease in the membrane potential (from 53 to 46 mV) and in the amplitude of the action potential (from 71 to 55 mV). In contrast to the condition after sucrose substitution, the negative after-potential was not appreciably affected although, when measured as mV above resting

potential, the size was reduced since the membrane was depolarized. A typical record is shown in Fig. 7. Electrotonic hyperpolarization of the membrane to the normal potential restored the normal size of the negative after-potential but not the amplitude of the action potential. The decay of the negative after-potential was slightly enhanced. The results suggest that

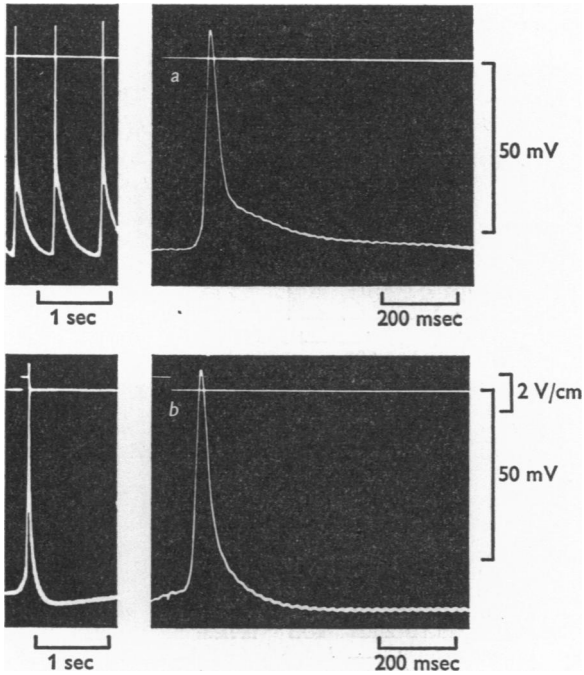

Fig. 6. Action potentials recorded from a 16 days pregnant uterus (*a*) in normal solution and (*b*) after 40 min exposure to Na-deficient solution (16 mM-Na, sucrose substitution). See text.

Cl deficiency rather than Na deficiency accounts for the decrease in the negative after-potential and for the transition to the positive after-potential.

It should be mentioned that the conversion of the negative after-potential to a positive after-potential was never observed when non-pregnant uterus was exposed to Cl-deficient solution, which only reduced the size and duration of the negative after-potential.

DISCUSSION

The present results suggest that the negative after-potential of the rat uterine muscle is largely due to an increase in Cl conductance of the membrane. The supporting findings are that (1) in pregnant uterus,

replacement of Cl with a less permeant anion converts the negative after-potential into a positive after-potential, (2) the initial falling phase of the spike itself is not affected by Cl deficiency, (3) the negative after-potential is insensitive to changes of the external Na concentration, and (4) the decay of the negative after-potential is slower than the passive return of the membrane to its resting level. Since the intracellular Cl content is high and E_{Cl} is probably much lower than E_m (Kuriyama & Casteels, 1965), an increase

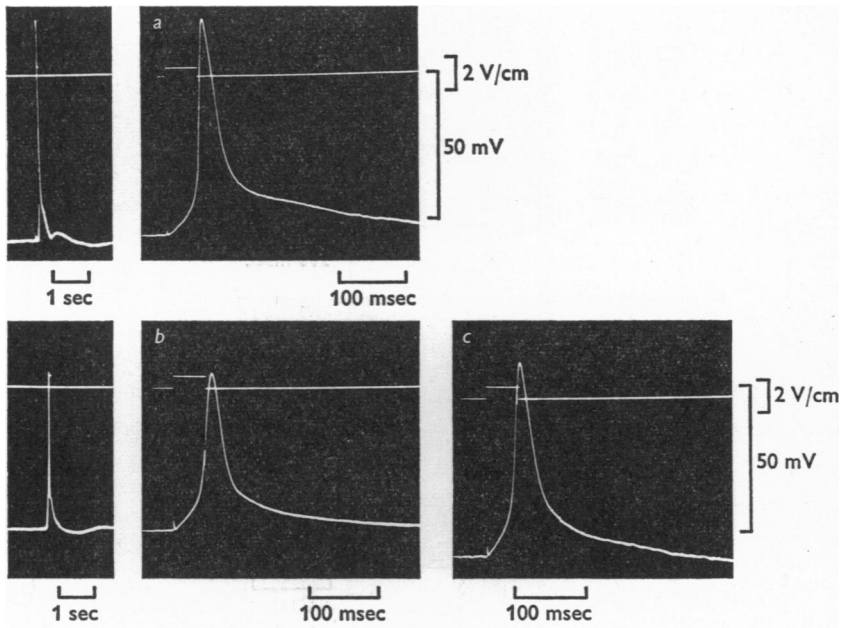

Fig. 7. Action potentials recorded from a 13 days pregnant uterus preparation (*a*) in normal solution and (*b* and *c*) after 40 min exposure to 16 mM-Na solution (Tris-Cl substitution), (*c*) during conditioning hyperpolarization. See text.

of Cl conductance would be expected to cause depolarization or a delay of repolarization, i.e. a negative after-potential. The replacement of extracellular Cl with benzene sulphonate, a less permeant anion (Abe, 1970), should augment the size of the negative after-potential because, when Cl permeability is increased, anion influx will be smaller than efflux. In fact, a larger negative after-potential is observed during the initial stage of exposure to benzene sulphonate solution. However, this augmentation is not maintained and the size of the negative after-potential gradually declines until, eventually, it is converted to a positive after-potential. This may be explained by assuming that, in normal conditions, an active Cl uptake mechanism accumulates Cl inside the cell (Casteels, 1965) and

that the loss of intracellular Cl in Cl-deficient solution appears to be balanced by some other intracellular anion to which the cell membrane is impermeable (Casteels, 1964). Consequently, the negative after-potential disappears with time of exposure to Cl-deficient solution as E_{Cl} moves towards E_m. In addition, the replacement of Cl with benzene sulphonate increases the membrane resistance (Abe, 1970) probably by a decrease in Cl conductance as in the taenia coli (Bülbring & Tomita, 1969; Ohashi, 1970). Therefore, the rise in K conductance during the falling phase of the action potential, which is normally opposed by the relatively high Cl conductance, will become more effective and result in the transition of the negative to a positive after-potential. This assumption is supported by the observation that the decay of a sufficiently large electrotonic potential following the break of cathodal current is much faster in Cl-deficient than in normal solution, whereas the time course of the development of the anodal electrotonic potential is slower in Cl-deficient solution.

If the tissue consists of many functional bundles like the taenia coli (Tomita, 1967) and spikes are evoked in one region by the present stimulating method, they may be conducted at different velocities and, depending on the time lag between the arrival of spikes, a notch or a negative after-potential may result. However, this is unlikely to be the main explanation for the negative after-potential which remained more or less unchanged at different distances (0·2–3·0 mm) from the stimulating electrode.

The present results do not rule out the possibility that the negative after-potential may be due to potassium accumulation around the muscle fibres caused by its release during each spike, as shown for crab nerve (Shanes, 1949*b*, 1951) and for squid axon (Frankenhaeuser & Hodgkin, 1956; Shanes, 1949*a*). However, the facts that the negative after-potential in smooth muscle is seen following a single spike, and that replacement of external Cl with benzene sulphonate eliminates the negative after-potential following both a single spike and repetitive stimulation, are not in favour of this explanation.

It has been suggested that the production of repetitive firing in myelinated nerve fibres by veratrine (Tasaki & Mizuguchi, 1948) and in muscle fibres by tetraethylammonium (Hagiwara & Watanabe, 1955) is due to restimulation by the negative after-potential. The same mechanism may determine the pattern of spontaneous activity in rat uterine muscle which consists of bursts of activity.

The present results also show that the amplitude and time course of the negative after-potential are changed by factors related to pregnancy. Casteels & Kuriyama (1965) suggested an increase of P_K with the advance of pregnancy, since the membrane potential rises without any appreciable change in the intracellular ion content and the maximum change of the

membrane potential produced by a tenfold change of the external K concentration increases during pregnancy. Alternatively this observation can be explained by assuming a decrease in P_{Cl} during pregnancy. If the Cl equilibrium potential remains constant throughout gestation (Casteels & Kuriyama, 1965), the potential at which the negative after-potential appears should remain almost unchanged, so that, when the membrane potential is raised the absolute value of the negative after-potential would be larger. In fact, however, it becomes smaller, since the potential at which the after-potential appears moves closer to the membrane potential which is increased in late pregnancy. This can be explained by assuming that in late pregnancy, when P_{Cl} may be low, and P_K is high, any increase of P_{Cl} interferes less with the falling phase of the spike than in non-pregnant uterus. The possibility that the difference in the size of cells (Reynolds, 1965), i.e. the change in the surface: volume ratio, may contribute to the variable amplitude of the negative after-potentials during pregnancy requires examination.

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