J. Physiol. (1957) 135, 412–425

CHANGES IN CONFIGURATION OF SPONTANEOUSLY DISCHARGED SPIKE POTENTIALS FROM SMOOTH MUSCLE OF THE GUINEA-PIG'S TAENIA COLI. THE EFFECT OF ELECTROTONIC CURRENTS AND OF ADRENALINE, ACETYLCHOLINE AND HISTAMINE

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(*Received* 27 *September* 1956)

Autorhythmicity can be produced in many excitable tissues by introducing conditions in which the membrane potential becomes unstable. The most extensive studies have been carried out on nerve and on striated muscle, both of which normally have a very stable membrane but which in a calcium-deficient medium become rhythmically active. In this condition the behaviour of striated muscle resembles in some respects that of a continuously discharging sensory organ. The smooth muscle of the longitudinal layer of the intestine shows the same behaviour normally. Its membrane potential is very unstable, there is a spontaneous rhythm of spike potentials, and it responds to a number of stimuli by varying the frequency of its spontaneous discharge of impulses. The tension which the muscle produces in isometric conditions is directly proportional to the spike frequency, and the rate of discharge depends on the state of polarization of the membrane. A good correlation exists between the three variables: tension, membrane potential and spike frequency, but little is known about the size and the configuration of the spike potentials. A study has therefore been made to see whether the duration of the individual spike bears any relation to the tension produced, and whether the spike configuration undergoes changes which can be related to stimulation or inhibition of activity. A short report of the results obtained was given to the XXth International Physiological Congress in Brussels (Bülbring, 1956).

METHODS

All experiments were done on isolated smooth muscle strips taken from the taenia coli of the guinea-pig, in most experiments not exceeding 3 mm length *in situ*. The volume of the bath was 2 ml., and bathing solutions flowed continuously at a rate of 2 ml./min. Thus any substances

added were washed away. This method and that of taking intracellular records was the same as those described earlier by Bülbring & Hooton (1954), and Bülbring (1954). The method of recording the tension and of applying electrotonic currents was described by Bülbring (1955).

RESULTS

Spike potentials during spontaneous activity

All preparations were set up under 3–5 g initial tension and showed spontaneous activity. The action potentials which were recorded varied in size from less than 1 mV to 35 mV. They never caused a reversal of the membrane potential, and the percentage depolarization from the starting level, produced by the spike, was not proportional to the absolute membrane potential. However, the spikes arising from a low membrane potential were usually smaller than those arising from a high potential.

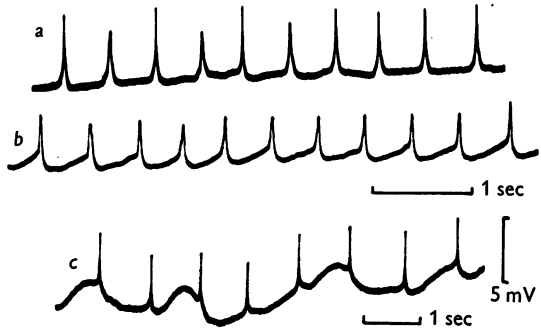

Fig. 1. Taenia coli. Intracellular records: spontaneous discharge of spike potentials (*a*) and (*b*) from the same preparation; (*c*) from another muscle.

The duration of the spike potentials and the slope of the rising and falling phase were equally variable. While the rise time to an average peak of 10 mV was between 7 and 35 msec the return to the base line lasted from 7 to 300 msec. The rate of repolarization could be either greater or less than the rate of rise. If it was fast it led to a phase of after-hyperpolarization lasting several hundred msec.

As a rule the spikes were preceded by a slow depolarization which varied in slope and in degree from being scarcely perceptible to a well-defined prepotential (Fig. 1*a*, *b*). Slow waves of depolarization of the membrane were of three kinds. The fastest changes occurred at a rate of about 1/sec and they usually led to the discharge of a spike potential, i.e. they were the prepotentials. The second type of slow potential changes was rarely seen; in this the waves were not regular, they occurred at intervals of 2–10 sec and had apparently no relation to the spike discharge (Fig. 1*c*). The third type of slow fluctuations of the membrane potential was associated with the pendular activity, the full cycle lasting 1–3 min (see Fig. 3). These waves were definitely correlated with the rate and configuration of the spikes.

The most frequently observed form of discharge consisted of single spikes like those in the upper record of Fig. 2, but they greatly varied in height. In many preparations, however, especially when the membrane potential was low, double spikes were recorded, the second spike occurring before the repolarization of the first was complete, thus arising from a plateau (see Fig. 8).

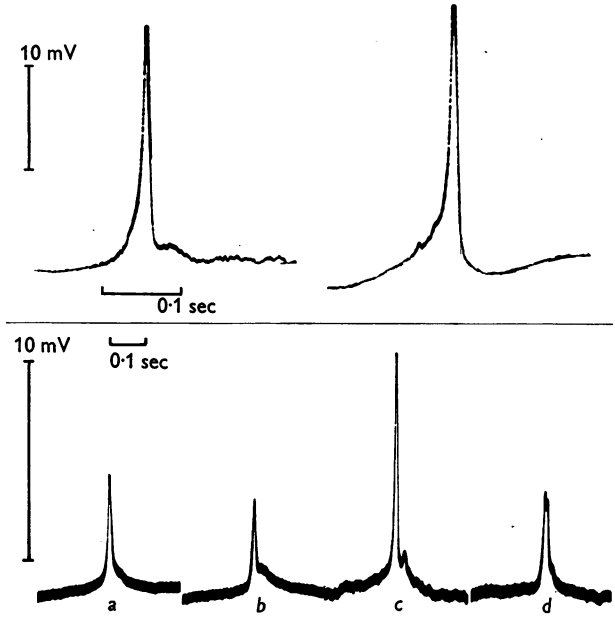

Fig. 2. Varying spike configuration; upper record, in the same fibre of one preparation; lower record, in different fibres of another preparation; frequency of spike discharge in (*a*) 18·8, in (*b*) 20·8, in (*c*) 16·5, in (*d*) 17·8/10 sec; these changes occurred within 5 min.

A real plateau like that seen in heart muscle was rarely observed. If it was seen it appeared to be simulated by an abortive second spike which then, in an adjoining discharge, could be discerned more clearly. Examples are shown in the lower record of Fig. 2 and in Fig. 13. Occasionally more than one additional spike arose from the plateau. Fig. 3 shows in the upper record the phase of rising tension, and in the lower record the phase of falling tension during spontaneous pendular activity. Spike potentials at first were less than 4 mV; each was followed by a small increment in tension. Gradually the hyperpolarization following each spike which at first amounted to 0·5 mV increased, and after the 8th spike on the upper record a conspicuous change took place. The potential rose, the hyperpolarization following the 9th spike now amounted to 2 mV and the spike size was doubled. The interval between spikes increased. The tension which had at first increased in steps following each spike reached its peak after the 9th spike, remained there for 3 sec, and then declined steeply.

During the relaxation shown in the lower record, which is the direct continuation of the upper part, the spikes were more widely spaced and not every one was followed by an increase in tension which thus no longer summated.

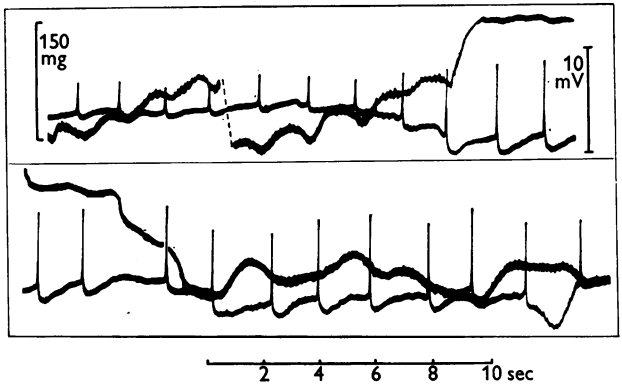

Fig. 3. Record of tension and electrical activity (intracellular electrode). Upper record rising phase, lower record falling phase of pendular cycle: the two records are continuous; for description see text. (Broken line in upper record when tension record was reset to bottom of screen.)

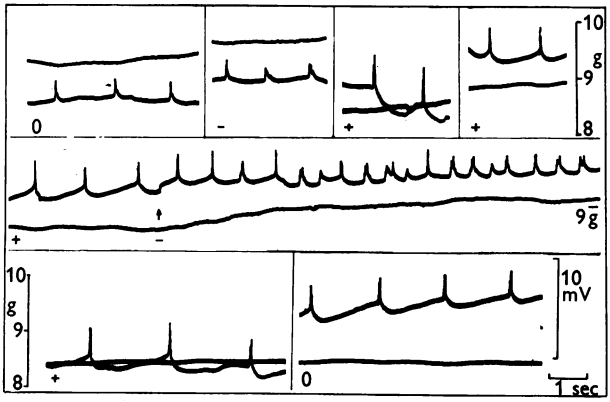

Fig. 4. Effect of electrotonic currents (100 μA) on spike frequency and configuration in relation to the tension. Changes of polarity every 20 to 60 sec: upper; (1) normal, (2) 20 sec −, (3) 30 sec +; middle; 60 sec + and reversal of polarity; lower; (1) 30 sec +, (2) 5 sec off.

The effect of changes in polarization of the membrane

When the muscle was exposed to electrotonic currents the size and configuration of the spike potentials underwent similar changes as during spontaneous fluctuation of the membrane potential. In the experiment from which records were taken for Fig. 4 weak electrotonic currents were applied. An attempt was made to imitate the spontaneous fluctuations by reversing the polarity every 20–60 sec. The change in the appearance of the spikes was a decrease in

size and a slowing of the rate of repolarization when the membrane was depolarized; this was associated with a rise in tension. On the other hand, when the membrane was polarized, the spike height increased and the rate of repolarization became faster; each spike was followed by a phase of hyperpolarization; this was associated with a fall in tension. The tendency to discharge multiple spikes was increased during cathodal stimulation. The

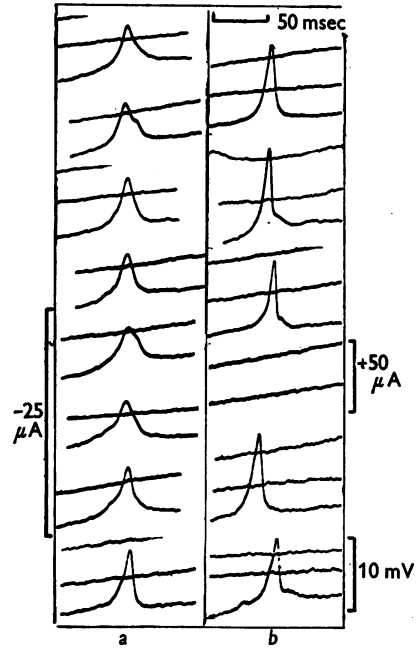

Fig. 5. Spike potentials recorded at 20 sec intervals (read from below upwards). In (*a*) $-25\ \mu A$ was applied for 60 sec; in (*b*), 15 min later, $+50\ \mu A$ was applied for 60 sec (two records without spikes omitted).

spikes changed to plateaus of depolarization lasting up to 500 msec on top of which there were double or triple spikes. The delay in repolarization resulting from application of cathodal currents as compared with the faster rate of repolarization as a result of anodal currents is shown on a faster time base in Fig. 5.

Strong anodal currents stopped the spike discharge. Preceding their reappearance slow waves of potential changes were sometimes observed (see Fig. 6). These were presumably prepotentials due to electrotonic spread from neighbouring already active fibres, as will be discussed below.

The effect of adrenaline

Adrenaline slowed or stopped the spike discharge. The height of the spike potentials was reduced until they finally disappeared, but this effect was some-

times preceded and often followed by a phase in which the spikes were increased in size. Such an effect is shown in Fig. 7. The relation between the rate of spike discharge, the change in membrane potential and spike size is shown in Fig. 7 (*a*) in which the points (a to m) on the graph correspond to the spike potentials shown in (*b*). During the first rise in membrane potential the spike discharge ceased; during the secondary rise, which occurred as the adrenaline was washed away, the spikes were double the size of those before or after. As with anodal polarization, slow waves of depolarization often preceded the reappearance of the spikes, and later gave rise to spike potentials as seen in the centre record of Fig. 7*b*.

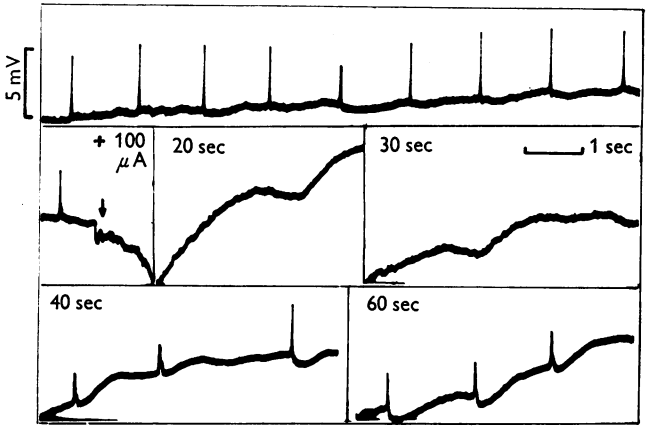

Fig. 6. Strong anodic polarization (+100 μA) for 1 min stopping spike discharge. Return of spikes, while still polarized, was preceded by slow waves of potential changes.

Adrenaline shortened spike duration. When paired spikes were discharged, as in the experiment illustrated in Fig. 8, the first effect of adrenaline was to suppress the second spike, then the first spike also disappeared. One minute later slow waves were noticed; when spikes reappeared they were at first single and when they formed pairs the spikes were sharper than before. The effect of adrenaline increasing the rate of repolarization is illustrated in Fig. 9*a*, to be compared with Fig. 9*b* showing the action of acetylcholine which was given about 15 min later.

The effect of acetylcholine and histamine

Both these substances, like cathodic polarization, increased the rate of spike discharge, decreased their height and prolonged their duration chiefly by slowing the rate of repolarization. This is shown for acetylcholine in Fig. 9*b*, and for histamine in Fig. 10. In the latter experiment the preparation was very active and, in relation to its varying spike frequency, produced varying spike shapes, the two extremes being shown in the first two records of Fig. 10.

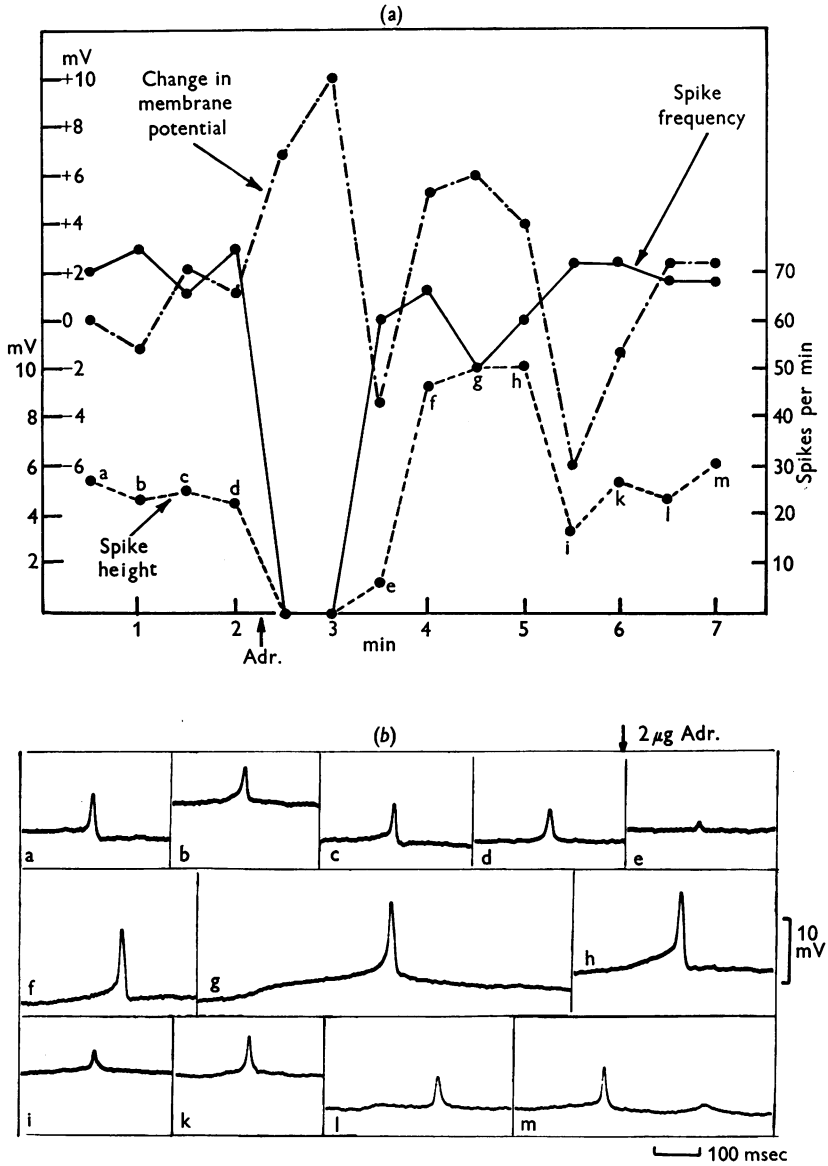

Fig. 7. The effect of adrenaline, initial concentration 1×10^{-6}, (*a*) on membrane potential, spike frequency and spike size, (*b*) on spike configuration at the corresponding points on the graph (no discharge omitted).

After the administration of histamine the gradually progressing delay in repolarization is shown in five successive spikes, changing the configuration to one not normally seen. As the histamine effect passed off and spike discharge slowed (third spike in Fig. 10*b*) the duration became distinctly shorter. This was followed by a period when double spikes were discharged. Only 17 min after histamine was given the preparation reverted to single spikes, which were briefer than initially.

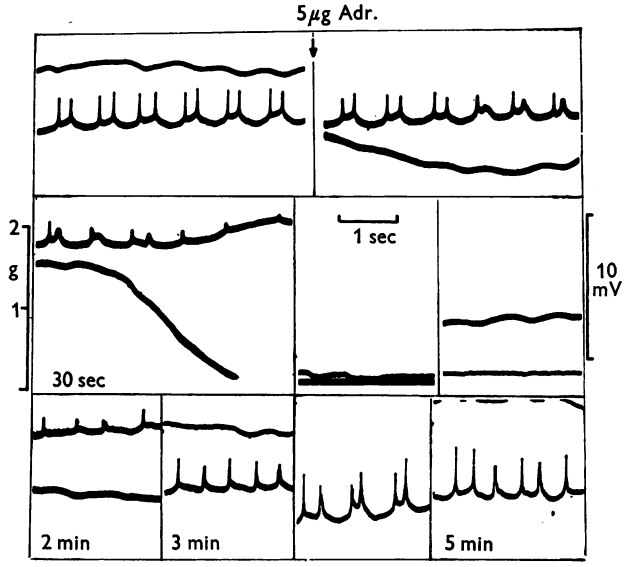

Fig. 8. Records of electrical activity and tension. The effect of adrenaline (initial concentration $2{\cdot}25 \times 10^{-6}$) abolishing spike potentials and shortening their duration when they reappear; this is preceded by slow waves. Same experiment as Fig. 11.

The striking similarity between the effect of acetylcholine and that of cathodic polarization is seen by comparing Fig. 4 with Fig. 11. The depolarization and increase in spike frequency often led to irregular discharges, the duration of depolarization was prolonged to form a plateau which sometimes lasted as long as 1 sec and on which multiple spikes occurred. While adrenaline changed the condition of a preparation from producing paired spikes to firing single ones, acetylcholine had the opposite effect and changed single spikes to pairs. Repolarization was delayed and the next spike arose before the membrane potential had returned to its previous level. In Fig. 12 the administration of acetylcholine led to depolarization and increased frequency of spike discharge. While the tension increased, the rate of repolarization of the spike potentials was slowed. Plateaus were seen carrying two or more spikes (tension off screen). At the peak of the tension (adjusted, to be visible on screen) the spike frequency already diminished; repetitive firing ceased and after-hyperpolarization appeared as the muscle relaxed. There followed a short

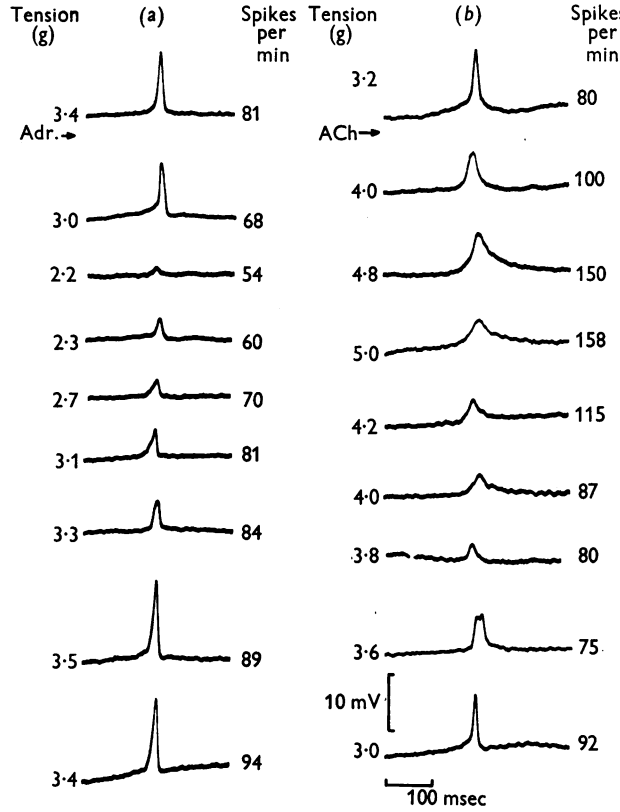

Fig. 9. The effect of (*a*) adrenaline, initial concentration 1×10^{-6}, increasing the rate of repolarization and (*b*) that of acetylcholine, initial concentration 2×10^{-6}, slowing the rate of repolarization.

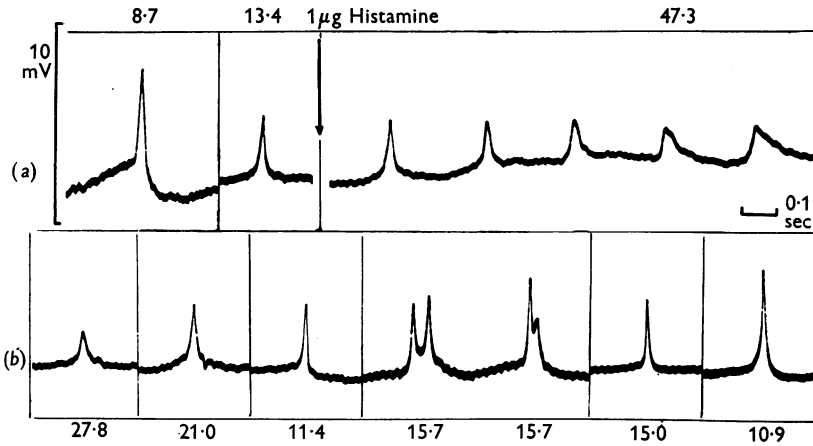

Fig. 10. The effect of histamine, initial concentration 5×10^{-7}, increasing the rate of spike discharge (number per 10 sec is given with each record) and delaying repolarization. Spikes in lower record were recorded 40 sec, 1 min, 2½ min, two successive 4 min, 17 min and 18 min after histamine was given.

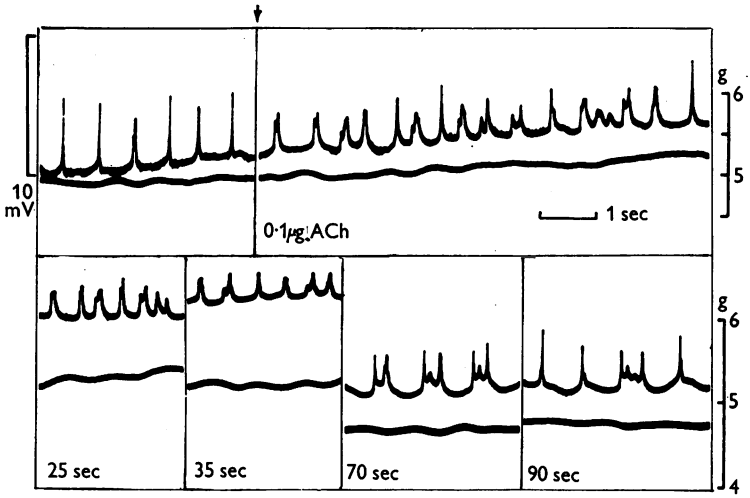

Fig. 11. Records of electrical activity and tension. The effect of acetylcholine, initial concentration 5×10^{-8}, prolonging the spike duration to a plateau carrying several spikes. Same experiment as Fig. 8.

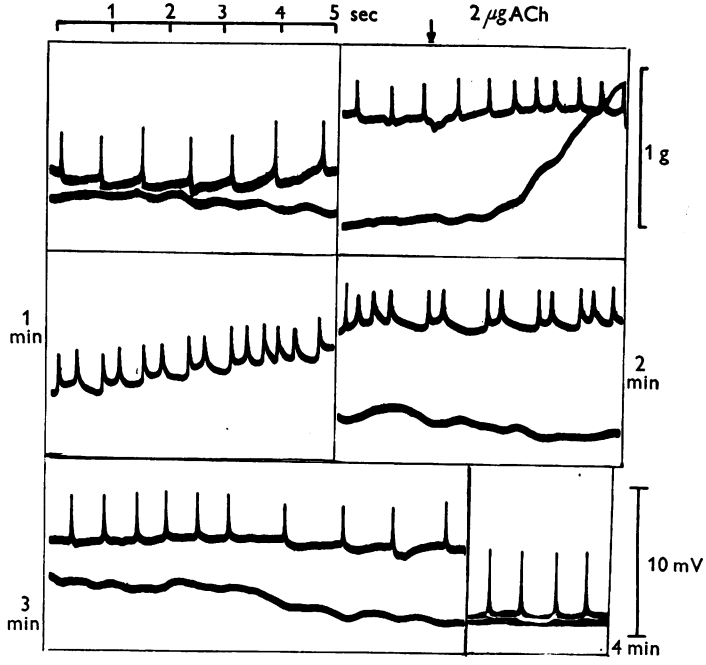

Fig. 12. The effect of acetylcholine on electrical activity and tension. 1 min after the injection of ACh tension record off the screen; it was readjusted so that the record shown at 2 min is 1 g higher than the corresponding levels on the other records.

period during the 4th minute when the size of the spikes was increased. This period of increased spike height was usually of short duration; it happened during the 3rd–5th minutes and, probably because of its transience, was not seen every time. In Fig. 13 the increase in spike height took place already during the period of repetitive firing. Spike potentials varied between 3 and 6 mV before acetylcholine was given. While the frequency increased they became very small and of long duration, producing irregular plateaus. During the 3rd minute they grew in size while the frequency was still fast, and finally, during the 5th minute, as the impulse discharge slowed, the spike height was 16 mV. It then declined rapidly.

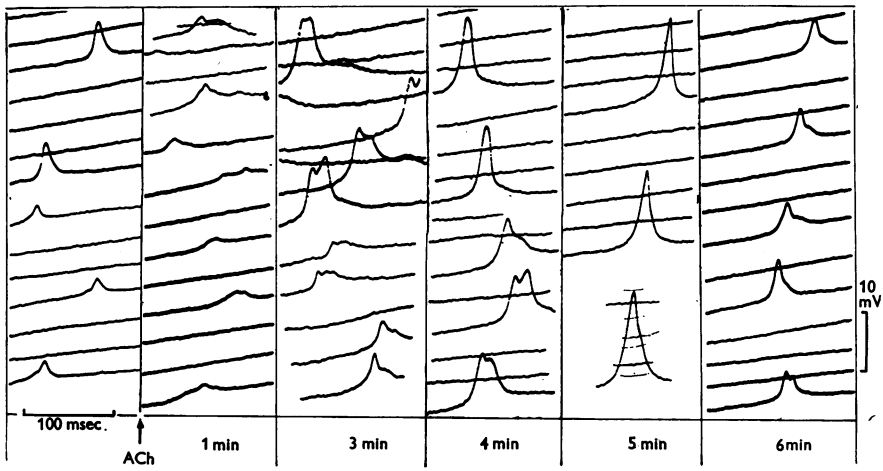

Fig. 13. The change in spike configuration caused by acetylcholine, initial concentration 8×10^{-7}: read from below upwards. Note reduced spike size, increased duration and repetitive firing, followed by a large increase in spike height.

DISCUSSION

The spike potentials which intestinal smooth muscle discharges spontaneously resemble those of other tissues with autorhythmicity in that they are preceded by a phase of slow depolarization of the membrane. If the prepotential is the characteristic of a pacemaker every smooth muscle cell appears to have this quality.

Bozler (1948) has pointed out that most visceral muscles behave like single muscular units, and this view has recently been once more substantiated for the dog's stomach (Ichikawa & Bozler, 1955). According to Prosser, Sperelakis & Bergman (1955) intestinal smooth muscle does not constitute a syncytium and intercellular bridges shown in electron-micrographs have double cell membranes and no fibrillar continuity. Nevertheless, muscular conduction proceeds in plexus-free circular intestinal muscle of the cat at 5 cm/sec, and in

the ureter of the rat at 3·4 cm/sec, nervous conduction being ten times faster (Prosser, Smith & Melton, 1955). Brune & Kotowski (1956), who recorded from guinea-pig colon and taenia *in situ*, found a conduction velocity of only 4 mm/sec. Ephaptic conduction of electrically induced as well as spontaneous action potentials was recently demonstrated by Prosser (1956). In analogy with observations made in calcium-deficient striated muscle (Adrian & Gelfan, 1933; Bülbring, Holman & Lüllmann, 1956) one might assume that slow waves of potential changes spread across sites of low resistance from cell to cell, affecting large numbers of cells simultaneously and initiating local spike potentials in each individual cell. This mechanism would be essentially different from that in the heart where the slow depolarization normally develops only in the pacemaker cells and initiates the propagated response which is conducted along a syncytium.

The slow potential changes occurring at the rate of, and leading up to, the spike deflexion are to be distinguished from the much slower changes proceeding over a wider range and taking 1–3 min for the full cycle. The origin of these 'pendular' fluctuations is unknown, but their effect on the rate of discharge and on the configuration of spike potentials appears to be similar to that produced by opposite electrotonic currents and by antagonistic pharmacologically active substances.

The mechanism by which changes in tension are linked to the membrane changes is unknown. Each spike is followed by a small production of tension. The total change in tension is a function of spike frequency and is built up by summation or, at high frequencies, by fusion of single contractions. The tetanic nature of smooth muscle contraction has been shown not only for the taenia coli but recently also for pregnant uterus (Jung, 1955) and for mytilus (Hoyle & Lowy, 1956).

With the changes in frequency the spikes undergo changes in configuration. A slower rate of repolarization of each spike prolonging its duration is associated with the production of tension. As the rate of discharge is fast and the rate of repolarization is slow the next spike may arise before the membrane potential has returned to its previous level. The tension rises in steps following each spike. As they arise in quick succession the steps summate because relaxation is incomplete before the next increment occurs. Under the influence of stimulating substances or cathodic currents repolarization may be so much delayed that plateaus develop carrying varying numbers of spikes. Similar configurations to those recorded from smooth muscle have recently been described by Arvanitaki & Chalazonitis (1955) in rhythmically discharging giant ganglion cells of *Aplysia* under the influence of depolarization.

On the other hand, a fast rate of repolarization, shortening the spike duration and merging with a prolonged phase of after-hyperpolarization, is associated with relaxation. The recovery from the after-hyperpolarization may be

continuous with the slow depolarization leading up to the next spike. The greater the after-hyperpolarization the more the next spike will be delayed and thus the rate is slowed. Each spike may still be followed by a production of tension, but this disappears before the next one arrives, consequently the tension does not build up. The degree of relaxation is a function of the intervals between spikes. In heart muscle the changes in configuration of the action potential produced by stimulation of autonomic nerves (Hutter & Trautwein, 1955) or by transmitter substances (Webb & Hollander, 1956) are in the opposite direction to those in smooth muscle. Acetylcholine and vagal stimulation which depress the contractions of the heart muscle decrease the duration of the action potential and accelerate repolarization, while adrenaline, in general, has the opposite effect and slows repolarization as it increases muscular contraction.

It is tempting to speculate that recovery processes concerned with active ion transport are already in progress during the falling phase of the action potential. Studies of the rate of uptake of ^{42}K (Born & Bülbring, 1956) have shown that this is reduced in those conditions when the rate of repolarization is slowed, and that potassium influx is increased in those conditions in which the rate of repolarization is fast and the spike is followed by prolonged hyperpolarization. As the mechanical change appears also to be related to the rate of repolarization of the spike potentials, it may be that the metabolic processes involved in active ion transport are also playing a part in the linkage between the membrane and the mechanical manifestations.

SUMMARY

1. The configuration of the spike potentials discharged by the isolated taenia coli of the guinea-pig has been studied using intracellular electrodes.

2. During spontaneous activity the spikes were preceded by a slow membrane depolarization (prepotentials) varying in slope and in degree. They were often followed by a prolonged period of after-hyperpolarization.

3. Spike size and configuration depended on the state of polarization of the membrane.

4. The spontaneous fluctuations of the membrane potential, the application of opposite electronic circuits, and the administration of antagonistic pharmacological substances produced qualitatively similar effects.

5. A delay in the repolarization of the spike potential occurred during spontaneous phases of depolarization as well as during cathodic current stimulation and after the administration of acetylcholine or histamine. The longer duration of the spike potentials was associated with a faster rate of discharge and with the development of tension.

6. A fast rate of repolarization and the appearance of an after-hyperpolarization following each spike occurred during spontaneous phases of rising

membrane potential, as a result of anodic polarization and after the administration of adrenaline. The shorter duration of the spike potentials was associated with a slow rate of discharge and with muscular relaxation.

I wish to thank Mr O. B. Saxby and Mr D. Groves for their most careful technical assistance.

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