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THE RESPONSES OF SINGLE SMOOTH MUSCLE CELLS OF GUINEA-PIG TAENIA COLI TO INTRACELLULARLY APPLIED CURRENTS, AND THEIR EFFECT ON THE SPONTANEOUS ELECTRICAL ACTIVITY

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When a polarizing current is applied to an excitable membrane, the amplitude of the action potential as well as that of junction potentials, e.g. end-plate and synaptic potential, are modified. The amplitude of the action potential is decreased or increased when the membrane is artificially depolarized or hyperpolarized by applying a polarizing current. This is explained by the fact that, during the action potential, the membrane resistance is so low that the effect of the polarizing current which shifts the resting membrane potential becomes negligible, and also by the fact that the ability of the membrane to undergo its increase in sodium permeability, on which the amplitude of the action potential depends, is influenced by the level of the membrane potential (Hodgkin & Huxley, 1952). The amplitude of junction potentials, e.g. end-plate and synaptic potential, is modified in the same direction as that of the action potential. This is explained by the reduction of the junctional membrane resistance which alters the IR drop across the membrane during the junction potential (Fatt & Katz, 1951).

Nagai & Prosser (1963) observed in the circular intestinal smooth muscle of the cat that the amplitude of the spike was reduced by both depolarization and hyperpolarization and that the membrane resistance was not changed during the spike. They concluded that 'the decrease of spike amplitude during hyperpolarization may result by anodal block from a reduction in the responsiveness to summating fields from converging cells', and that 'failure to record a resistance drop during the spike' could be due to 'the arrangement in three dimensions of many interconnected fibres'.

The first part of the present experiments was carried out to investigate the effect of polarizing currents on the spontaneous electrical activity of longitudinal intestinal muscle. We found that in the spontaneously active taenia coli of the guinea-pig, the amplitude of the spike was increased by

hyperpolarization and decreased by depolarization, as would be expected for an excitable membrane, though the effectiveness of polarizing currents differed from fibre to fibre.

Since the spontaneous electrical activity observed in single smooth-muscle cells of taenia coli seems to be influenced by some interaction between neighbouring cells (Bülbring, 1962), it is important to examine the electrical properties of the cell membrane. Nagai & Prosser (1963) have measured the electrical parameters in cat's circular intestinal muscle using intracellular stimulation. The object of the second part of the present work was to investigate the electrical response of single cells of taenia coli of the guinea-pig to intracellular electrical stimulation. The results obtained are similar to those of cat's intestinal muscle, and they indicate that the properties of mammalian smooth muscle are in many respects similar to those of crustacean muscle.

METHODS

The smooth muscle of isolated taenia coli of the guinea-pig was used as described by Bülbring (1954). Pieces of 4–6 mm length were mounted in an organ bath, through which solution flowed continuously at a constant temperature of 36° C. A modified Krebs's solution of the following composition was used (mM): Na^+ 137·4; K^+ 5·9, Ca^{2+} 2·5; Mg^{2+} 1·2; Cl^- 134; HCO_3^- 15·5; $H_2PO_4^-$ 1·2 and glucose 11·5; equilibrated with 97 % O_2 + 3 % CO_2. A single intracellular micro-electrode was used for electrical recording as well as for stimulating by means of the Wheatstone bridge method (Araki & Otani, 1955). The resistance of the micro-electrode was between 40 and 80 MΩ. In order to supply a constant current into the cell the resistance of one bridge arm, in series with the micro-electrode, was 1000 MΩ which was very high compared with that of the micro-electrode. The range of applied current was between 10^{-10} and 10^{-8} A. The current intensity was calculated from the division of the voltage applied to the bridge current by 1000 MΩ plus the electrode resistance. The error in this calculation introduced by a possible resistance change of the micro-electrode during the experiment was probably less than 10 % since the high resistance of one bridge arm was in series with that of the electrode. Before inserting the micro-electrode into a cell, the bridge was balanced so that the potential changes produced in the micro-electrode were zero when the current pulse passed through the electrode. However, since the resistance of the electrode was usually increased by its insertion into the cell and by passing a strong current through it, the potential shift observed by applying a current was too large and could therefore not be taken as the true measurement of the potential change across the membrane.

RESULTS

The effect of polarizing currents on the spontaneous electrical activity

In the taenia coli, the spontaneous electrical activity consists of slow potentials and spikes. The slow potential has been called the local potential, slow wave or generator potential (Bülbring, 1956, 1959; Holman, 1958; Bülbring & Kuriyama, 1963). Its amplitude and its shape varies not only from preparation to preparation, but also from cell to cell,

and it is sometimes difficult to distinguish clearly between the slow potential and the spike.

Figure 1 shows the effect of polarizing currents on the spontaneous electrical activity. The upper trace monitors the current, the shift of the trace being small due to the low gain. In both fibres a large slow potential with a small spike was seen in the normal condition, and the amplitude of both components fluctuated spontaneously. When a depolarizing current of gradually increasing intensity was applied through the micro-electrode (*a*), the spike components became smaller and disappeared. The amplitude

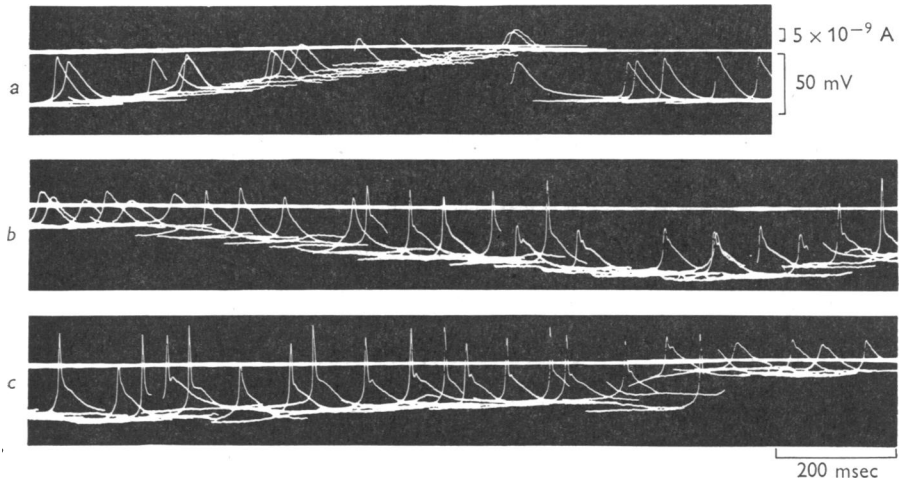

Fig. 1. Guinea-pig taenia coli, intracellular records (lower trace). Effects of depolarization (*a*) and hyperpolarization (*b* and *c*) on the spontaneous activity. Current intensity (upper trace) was gradually increased and suddenly returned to zero. Note block of spike components but little change of slow components by strong hyperpolarization (*b*). Records (*b*) and (*c*) are continuous records taken from the same fibre, record (*a*) from a different fibre in the same preparation. Records were taken by fast sweeps on a moving film. The time scale is the sum of both and refers to individual spikes, but not to spike frequency.

of the slow potential was relatively less reduced, but, if the current intensity was sufficiently strong, the slow component also disappeared. The rate of rise of the slow potential was reduced by depolarization, but the rate of fall was scarcely affected. On the other hand, a hyperpolarizing current of gradually increasing intensity increased the amplitude and the rates of rise and fall of the spike, while the slow component remained almost unchanged (*b* and *c*). These effects were completely reversible when the current was discontinued. When a strong current was applied causing hyperpolarization beyond a critical level, which was about 40 mV more than the normal membrane potential, most of the spike component was

blocked (*b*), while the slow component remained unaffected. The properties of the slow component were thus quite different from those of the spike component.

In some cells, the spike component was clearly distinguishable from the slow potential as shown in Fig. 2, and the polarizing currents changed only the amplitude of the spike. Sometimes the amplitude of the slow potential decreased spontaneously and then no spike was generated. This fluctuation was neither influenced by depolarization nor by hyperpolarization.

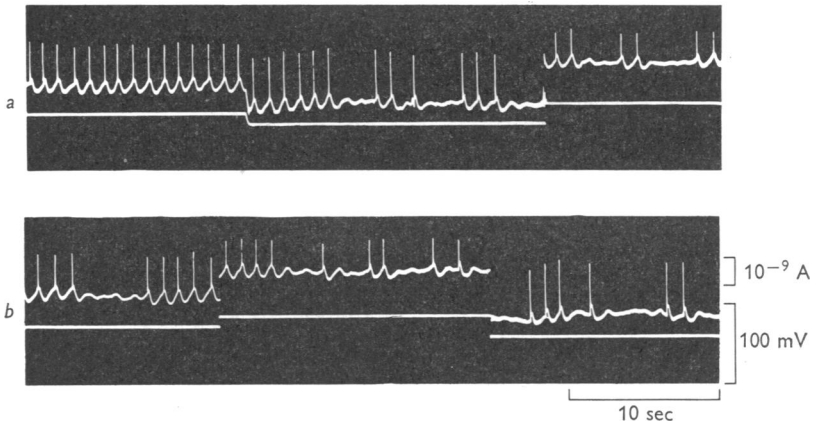

Fig. 2. (*a*) Effect of hyperpolarization followed by depolarization; (*b*) reversed order. Note that polarizing current changed spike amplitude but had no influence on the amplitude of the slow potentials nor on their occasional failure to generate a spike. Both records were taken from the same fibre.

If polarization is applied by external electrodes the spike frequency is easily modified (Bülbring, 1955). However, when polarization was applied intracellularly, a change in frequency of the electrical activity was never observed.

In a fibre in which the spontaneous action potentials consisted of large slow components and small fast components, as for example in Fig. 1, the amplitude and maximum rates of rise and fall were roughly proportional to the intensity of hyperpolarizing current. However, the depolarizing current was much less effective. This is probably due to the fact that in this type of cell the hyperpolarization affects the spike component only, while the depolarization affects both the spike and the slow component (probably due to a decrease in membrane resistance). If the electrical activity consisted mainly of spikes (as, for example, in Fig. 2), the relation between spike height and current intensity was linear in both directions.

The effect of polarization varied greatly in different cells and sometimes almost no effect was observed. The opposite effect, observed by Nagai &

Prosser (1963) in the circular intestinal muscle of the cat, i.e. the decrease of the spike amplitude by hyperpolarization, was never seen, though excessive hyperpolarization blocked the spike component (Fig. 1*b*). The maximum increase of spike amplitude was about 50 mV per nA. The biggest effects were observed in cells in which the amplitude and the rate of rise were low and the slow potential relatively big. This might be due to the polarizing current exerting its main effect on the inactivation process of the sodium carrier system (Hodgkin & Huxley, 1952).

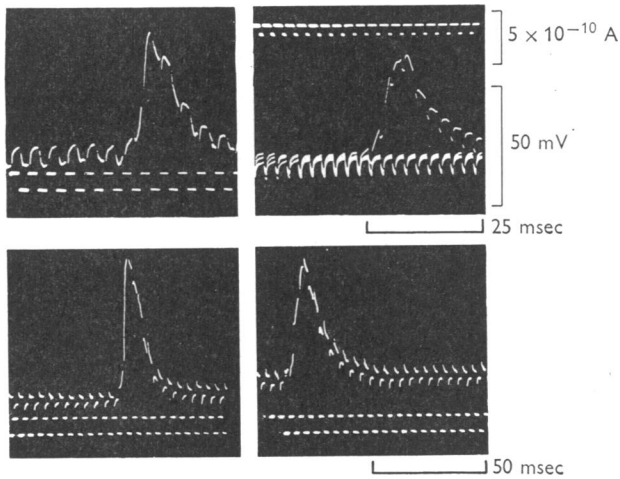

Fig. 3. Measurement of the resistance change during the spike by application of small current pulses. The reduction of amplitude and of time constant of the potential deflexion shows some decrease of the membrane resistance during the spike.

In order to measure the resistance change during the spike, small current pulses were applied to the membrane. In contrast to the observation by Nagai & Prosser (1963) on circular muscle, a decrease of the membrane resistance was observed, as shown by the reduction of the potential deflexions and the change in time constant of the electrotonic potential during the spike in Fig. 3. Spontaneous fluctuation of the spike amplitude made quantitative analysis difficult. The greatest reduction of the membrane resistance was observed during large spikes. If the muscle has ordinary cable properties the observed resistance change would be smaller than the actual change (Cole & Curtis, 1941), but if it has properties of a sheet-like membrane like heart muscle (Woodbury & Crill, 1961; Noble, 1962), or if it has a three-dimensional arrangement of interconnexions (Nagai & Prosser, 1963) the observed resistance change might be even smaller.

The membrane resistance in the resting state may be calculated from the relation between the applied current intensity and the change of the spike amplitude, if it is assumed that the membrane resistance becomes very low during the spike phase as in other excitable tissues (Coombs, Curtis & Eccles, 1959; Frank & Fuortes, 1956; Johnson & Tille, 1961). For the purpose of calculation, the fibres which had large spikes of about 60 mV were selected, not only because the resistance during the spike was lower in a large spike, but also because the effect of the polarizing current

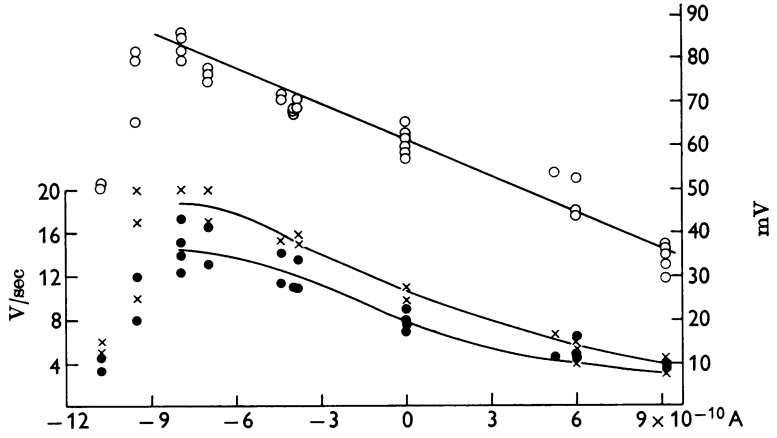

Fig. 4. Relation of amplitude (○) and maximum rates of rise (●) and fall (×) of the spike (ordinate) to depolarizing (abscissa, right side) and hyperpolarizing current (left side).

on the inactivation process of the sodium-carrier system of large spikes might be smaller. An example of the relation between the current and the spike amplitude used for the calculation of the membrane resistance is shown in Fig. 4. The highest value of the effective membrane resistance obtained by this method was about 30 MΩ (range 10–30 MΩ).

In some preparations, the changes of spike amplitude and of the rates of rise and fall produced by a constant polarizing current were gradual, reaching a steady state only after 5 sec or more, as shown in Fig. 5. This very gradual change in amplitude and rate of rise of the spike may be explained mainly by a very slow change of the inactivation process of the sodium carrier system according to the ionic theory (Hodgkin & Huxley, 1952).

When an action potential is propagated along a fibre of uniform structure bathed in a large volume of conducting fluid, the membrane current density is proportional to the maximum rate of rise of the action potential. In the squid giant axon, the inward current density is known to be related to the membrane potential (Hodgkin & Huxley, 1952). The rate of rise of

the action potential, which is indicative of the inward sodium current, should also be related to the membrane potential. As seen in Figs. 4 and 5, the maximum rate of rise was increased by hyperpolarization and decreased by depolarization, and this might correspond to the relation between the rate of rise and the membrane potential in Purkinje fibre (Weidman, 1955) and also to the inactivation curve in squid nerve fibre (Hodgkin & Huxley, 1952).

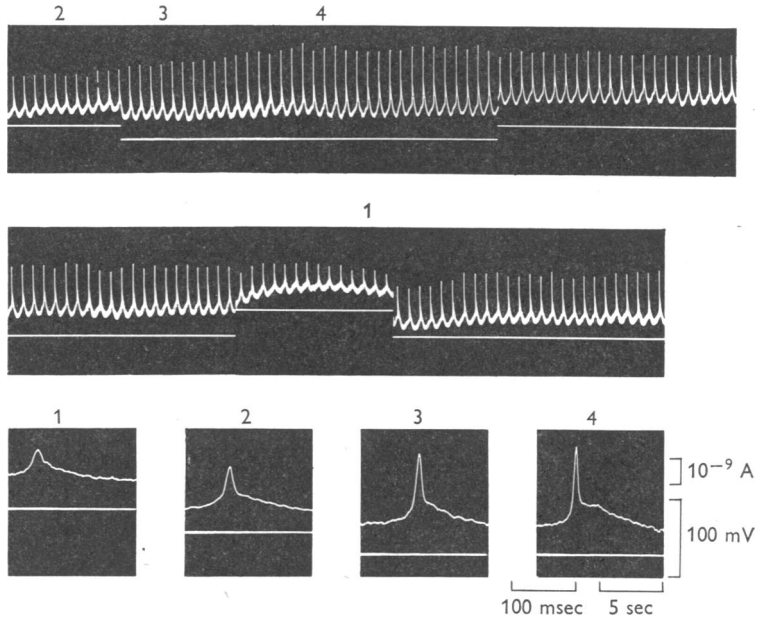

Fig. 5. Effects of hyperpolarization (top) and depolarization (middle) on spike amplitude. Note gradual change of the spike amplitude during polarization but constant frequency. Bottom records on faster time base: effects of polarization on spike shape. Each record corresponds to the spikes which have the same number in the upper records.

In taenia coli, the rate of fall was changed in the same manner as the rate of rise when a polarizing current was applied. However, the value of the rate of fall was very variable (Bülbring, 1959; Holman, 1958). In some fibres, the rate of rise was slower than the rate of fall; in other fibres the reverse was found.

The response to intracellular electrical stimulation

When a weak current pulse was applied to the membrane through the intracellular micro-electrode an electrotonic potential with a time constant of 2–4 msec in different fibres was produced. In most cells the voltage–current relation showed no rectification up to 30 mV polarization.

In some cells the membrane resistance seemed to be slightly decreased by depolarization, but, when a strong depolarizing current was applied, the resistance appeared often to be slightly increased. However, using the Wheatstone bridge method, a change of resistance of the micro-electrode could not be distinguished from a change of the membrane resistance, since the electrode resistance was usually increased by passing a strong depolarizing current through it.

The effective membrane resistance, calculated from the voltage–current relation, was for weak currents 20–70 MΩ (40 MΩ ± 12 S.E.).

In most cells no spike could be triggered by intracellular electrical stimulation even if the membrane was depolarized by more than 50 mV or the stimulating current was increased to more than 5×10^{-9} A. In such cells the effect of polarizing currents on the amplitude of the spontaneously generated spike was, however, clearly seen. Moreover, it is unlikely that this inexcitability was due to cell damage, because the membrane potential was high and the spontaneously generated spikes had a high amplitude and overshoot.

Neither depolarization nor hyperpolarization up to 30 mV produced instability or oscillation of the membrane potential as seen in the circular muscle of the cat by Nagai & Prosser (1963). Occasionally irregular deflexions in the potential tracing were observed with strong polarizing currents but they proved to be due to a resistance change of the micro-electrode since they were also observed when the electrode was taken out of the cell.

In some cells a graded response was recorded when the stimulating current intensity was increased. In a few cells spikes were triggered in an all-or-none fashion. This was easier if the preparation was bathed in a solution containing excess calcium (7·5 or 12·5 mM). Figure 6 shows the effects of conditioning depolarization (upper row) and of conditioning hyperpolarization (middle row) on the spikes triggered by intracellular stimulation. Conditioning hyperpolarization increased the evoked spike amplitude and its rate of rise and fall, while conditioning depolarization decreased them. The effect developed gradually and was completed in a few seconds after the application of the polarization (Fig. 6, bottom row). These effects were similar to those observed on the spontaneously generated spike (cf. Fig. 5).

The maximum amplitude of the evoked spike during conditioning hyperpolarization was 50 mV from the firing level which was usually −35 to −30 mV. The maximum rates of rise and fall were 18 and 20 V/sec respectively and the spike duration at half spike amplitude was 7 msec. These figures are similar to those of the spontaneously generated spike and also to those of crustacean muscle fibres recorded by Fatt & Katz (1953).

The latency of spike was very short (a few msec), except in one cell in which the maximum latency was about 50 msec. Repetitive firing was never observed. In a few cells damped oscillations following the spike were recorded during conditioning hyperpolarization. During conditioning depolarization a spike was evoked in some cells as a break response upon cessation of a sufficiently strong anodal pulse.

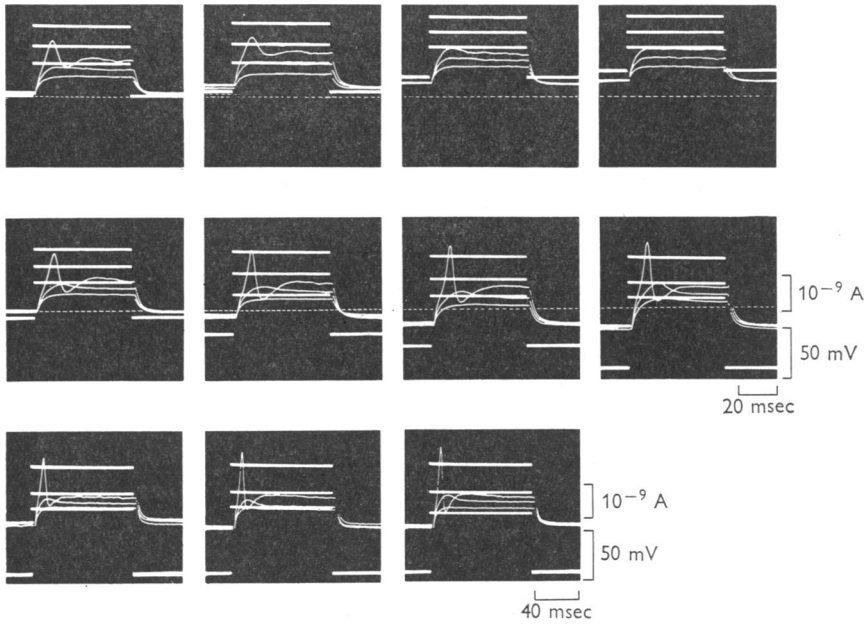

Fig. 6. Spikes triggered by intracellular stimulation. In each record three depolarizing pulses of increasing intensity are superimposed. Interrupted line = original membrane potential. Top: left to right: effect of increasing conditioning depolarization. Middle: effect of increasing conditioning hyperpolarization. Bottom: records taken after 1, 3 and 5 sec constant conditioning hyperpolarization.

Calculation of the specific membrane resistance and capacitance

Two different methods were used. It has to be pointed out, however, that the calculations are very rough because of the complicated morphological situation.

(i) Neglecting the internal longitudinal resistance of the myoplasm, the muscle fibre was assumed to be a double cone of 100 μ length and 5 μ maximum diameter (Nagai & Prosser, 1963; Sperelakis & Lehmkuhl, 1964). The specific membrane resistance (R_m) is calculated from the effective membrane resistance of 40 MΩ and the surface area of 8×10^{-6} cm^2. It is 320 Ω cm^2. From this and the time constant of the electrotonic potential (3 msec), the specific membrane capacitance (C_m) is calculated to be about 10 μF/cm^2. Using the same assumption Nagai & Prosser (1963) obtained the following values in the circular muscle of the cat intestinal muscle: R_m = 560 Ω cm^2; C_m = 56 μF/cm^2, from the effective membrane resistance of 70 MΩ and the time constant of the electrotonic potential of 31 msec.

(ii) The internal longitudinal resistance is probably so high, owing to the small diameter of the muscle fibre, that it must be taken into account. However, the ordinary cable equations assuming an infinitely long fibre cannot be applied to the smooth muscle fibre because the ratio of fibre length to diameter in smooth muscle is too small. Fatt & Ginsborg (1958) used a special equation to calculate the membrane characteristics of crustacean muscle fibre which also has a small length to diameter ratio. In their equation, the electrode is assumed to be inserted at one end of the fibre, while in the present experiment the electrode may be placed in the middle part of the fibre. In the second calculation, therefore, the equations used for the crustacean muscle fibre were employed with some modification.

$$\frac{2\lambda}{l}\coth\frac{l}{2\lambda}=\frac{2\pi d^2R}{R_i l} \tag{1}$$

$$R_m=\frac{\pi^2 d^3R^2}{R_i}\tanh\frac{l}{2\lambda} \tag{2}$$

where d is the fibre diameter, R_i is the internal specific resistance and l is the fibre length. If the internal specific resistance (R_i) is 250 Ω cm (Katz, 1948, frog skeletal muscle; 225 Ω cm, Nagai & Prosser, 1963, cat circular intestinal muscle), R_m is 1500 Ω cm^2 and λ is 250μ. From the R_m and the time constant of 3 msec C_m is calculated to be 2 μF/cm^2.

Hyperpolarizing response

When a weak current was applied to the membrane, the membrane behaved like a constant electrical resistance since the voltage–current relation was linear. In some cells, when the membrane was hyperpolarized beyond -90 to -100 mV, which was probably a little higher than the potassium equilibrium potential, the membrane resistance increased 3–8 times, the effective membrane resistance becoming more than 100 MΩ. Figure 7 shows an example of such a voltage–current relation. When such a cell was hyperpolarized by a strong current, the membrane potential did not stay at a steady level, but continued to increase slowly at first and then rapidly, while the current intensity remained constant (Fig. 7*b*). Still stronger currents made this process faster. This phenomenon is similar to the hyperpolarizing response observed in various other tissue (Stämpfli, 1958; Segal, 1958; Moore, 1959; Tasaki, 1959; Hagiwara, Kusano & Saito, 1961; Reuben, Werman & Grundfest, 1961; Tomita, Szaimi & Toida, 1961; Koketsu & Koyama, 1962). There was a limit of the potential difference across the membrane, at about -200 mV, beyond which the membrane potential could not be further increased, even if a strong current pulse was applied. It indicated that the dynamic resistance of the membrane was decreased. At this level the membrane potential fluctuated irregularly (Fig. 7*b*).

When a membrane was gradually hyperpolarized by conditioning current, the electrotonic potential produced by a constant depolarizing current pulse became bigger and slower (Fig. 8a_1). In the records of Fig. 8a_2, *b* and *c*, the strong conditioning hyperpolarization (more than 100 mV) was kept constant. When the intensity of the depolarizing

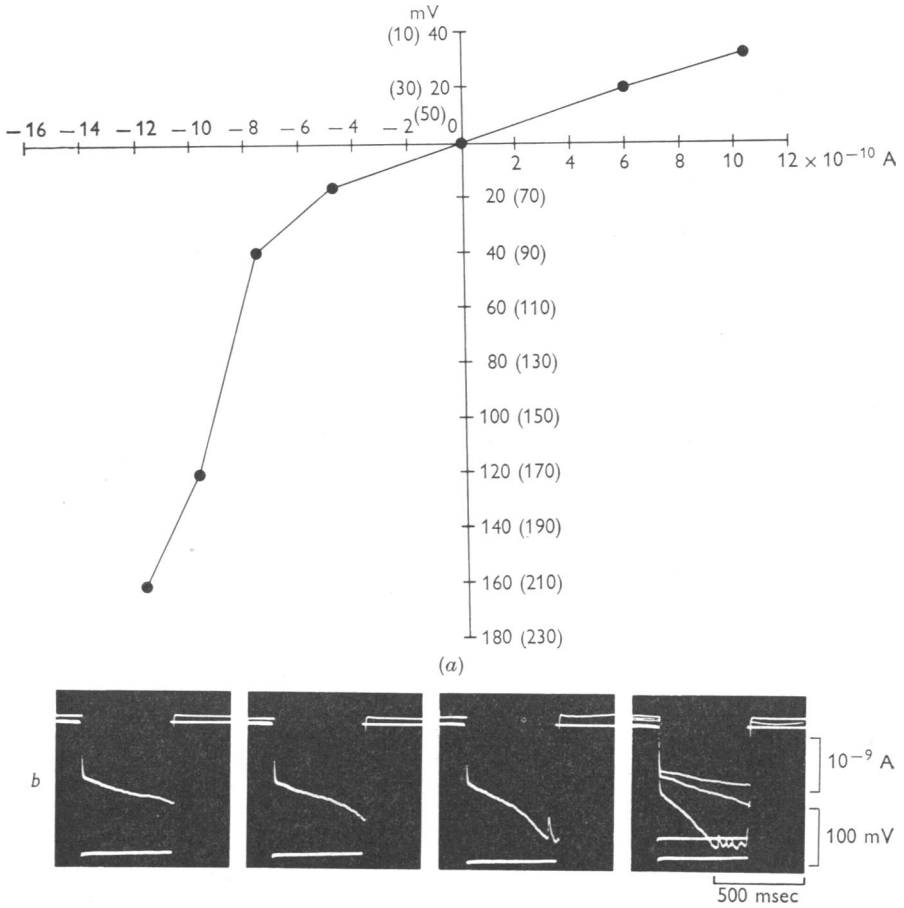

Fig. 7. (*a*) Voltage–current relation in a cell which showed a non-linear increase in the membrane resistance during application of strong hyperpolarizing current. Abscissa = polarizing current intensity. Ordinate = potential shift (actual values in brackets). (*b*) Responses to strong hyperpolarizing current pulses of increasing intensity. (For description see text.)

current pulse was increased, the rising phase of the electrotonic potential became faster and the time course of the falling phase became very different from that of the rising phase. This difference was clearly caused by some slow response. These pictures were taken from three different cells. In some cells, only a slow response appeared without spike (a_2 and b), but in other cells a spike and a slow response appeared (c).

Figure 9 shows examples of spike and slow response on a slow time base. It can be seen that the plateau of the slow response lasted for several seconds. The amplitude and duration of the plateau phase depended on

the degree of the conditioning hyperpolarization. If the membrane was less hyperpolarized, the plateau phase was smaller and longer (top records) than in the strongly hyperpolarized membrane (bottom records). Such a plateau occurring during strong conditioning hyperpolarization could be due to a change of the e.m.f. of the membrane, or to a change of the membrane resistance, or to a combination of both.

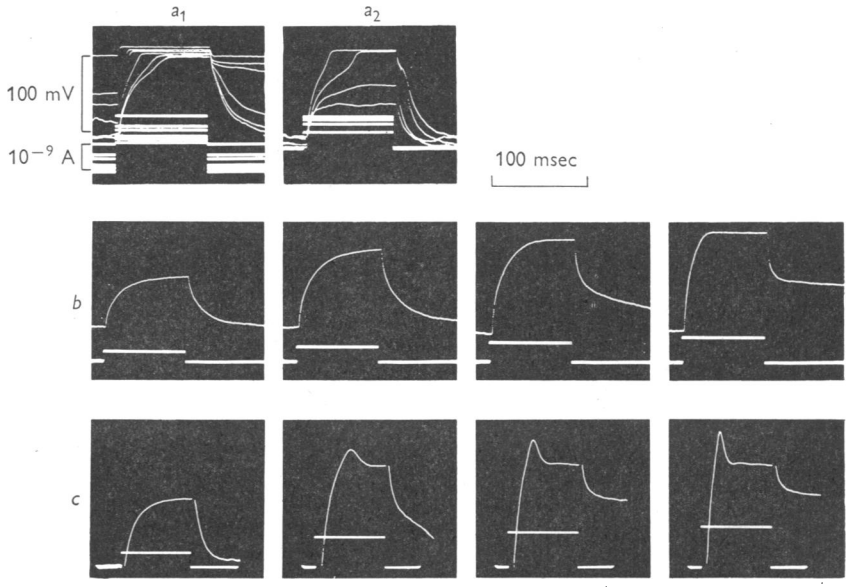

Fig. 8. (a_1): Effects of increasing the conditioning hyperpolarization on the electrotonic potential produced by a constant depolarizing current pulse. (a_2), (b) and (c): The intensity of the depolarizing current pulse was increased while the strong conditioning hyperpolarization was kept constant. Records (a), (b) and (c) were taken from three different fibres.

In order to measure the change of the membrane resistance during this slow response, small constant current pulses were applied from a third stimulator (Fig. 10). During the plateau the membrane resistance was at first decreased and then gradually returned to the initial high value with the time course of the plateau phase.

DISCUSSION

The effect of polarizing current on the spontaneous electrical activity

The slow potential changes and spikes, generated spontaneously in taenia coli, usually occur in close relation to each other but the spikes may also occur independently on any phase of the slow waves (Bülbring, 1959; Holman, 1958; Bülbring, Burnstock & Holman, 1958). The spikes and the

slow potential changes are also differently affected by changes in the ionic composition of the external solution (Bülbring & Kuriyama, 1963).

In the present experiments it was found that intracellularly applied polarizing currents strongly influenced the spike but had little effect on the slow potential. The differences in behaviour of the spike and the slow potential suggest that it may be generated in a different part of the membrane from that which is involved in the generation of the spikes.

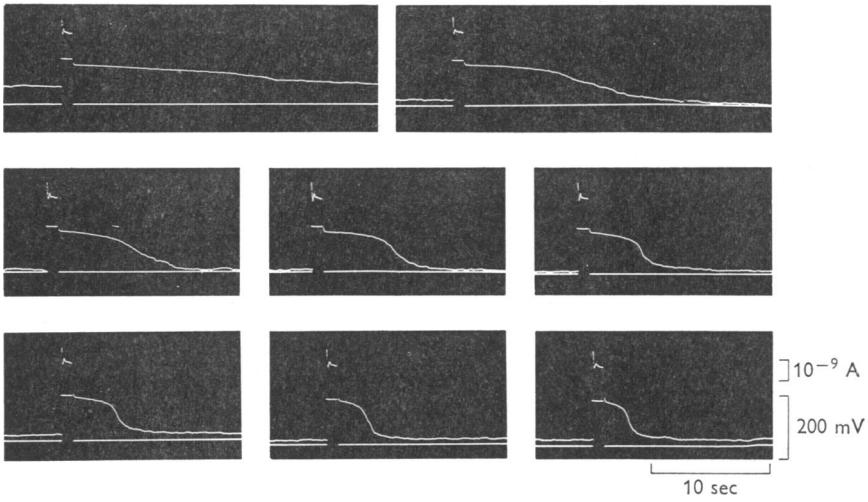

Fig. 9. Slow response observed during strong conditioning hyperpolarization. The hyperpolarizing current was gradually increased from the left top to the right bottom record; the stimulating current pulse was kept constant.

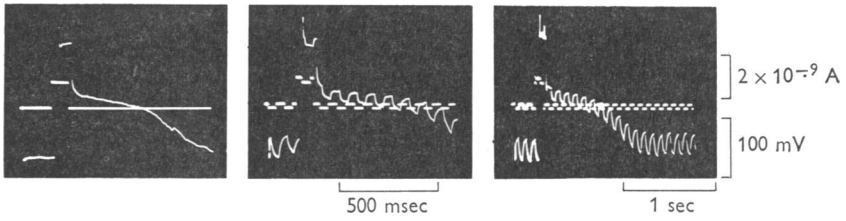

Fig. 10. The measurement of the membrane resistance change during the slow response by application of small constant current pulses.

Alternately, the slow potential may be produced by a different mechanism from that of the spike. However, in spite of all the evidence that the spike and the slow component are two different phenomena there is no doubt that they usually occur in close relation.

Prepotentials in cultured chick embryonic cardiac cells can be arbitrarily classified into small and large types (Sperelakis & Lehmkuhl, 1964). Neither depolarizing nor hyperpolarizing currents affected the size of the

small prepotentials, while hyperpolarizing currents increased and depolarizing currents decreased the large prepotentials. Both types may represent synaptic interaction between adjacent cells, and the difference in their magnitude may be a function of the distance between the synaptic junctions and the micro-electrode.

It may be that the slow potentials in taenia coli, like the small prepotentials in embryonic heart, are not affected by polarizing currents, because the distance between that part of the membrane where they are generated and that where the micro-electrode is inserted exceeds the space constant. It is also possible that the slow potential is produced not only in a different area of the membrane but in a special structure which is not influenced by polarizing currents. Vesicles located close to the membrane might be such a structure or areas of close apposition (Prosser, Burnstock & Kahn, 1960). Another possibility is that the slow potential is an electrotonic (ephaptic) potential from adjacent cells, and it should be unaffected by polarizing currents if the membrane resistance remains constant.

If the slow potential is a simple ephaptic potential produced by current coming from adjacent cells, it is not necessary to assume that it is produced far from the micro-electrode in order to explain its being unaffected by polarizing currents. However, it is still difficult to explain its failure to generate a spike during conditioning depolarization as seen in Fig. 2. Current coming from neighbouring cells, and the polarizing current coming from the micro-electrode should sum, the result being the sum or the difference according to the direction, so that spike generation should be affected. However, this was not seen (Fig. 2). For this reason it is more likely that the slow potential is produced far from the electrode.

In pace-maker cells of the heart polarizing currents influence the frequency of discharge, but in non-pace-maker cells the frequency is not affected even though the spike height may be drastically diminished or greatly enhanced (Trautwein & Kassebaum, 1961; Sperelakis & Lehmkuhl, 1964). All cells examined in the present experiments on taenia coli seemed to be non-pace-maker cells and polarizing currents had no effect on the frequency of the slow potential nor the spikes. It may well be that if the polarizing current were applied to a pace-maker cell in taenia coli, its spike frequency might be changed.

The effect of polarizing currents on the shape of spontaneous and evoked action potentials

The spikes of taenia coli show a relatively slow rate of rise (about 7 V/sec) and low sensitivity to a change of the external sodium concentration (Holman, 1958; Bülbring & Kuriyama, 1963). From these observations it has been postulated that the membrane of smooth muscle may

have not only a limited number of sodium carriers to increase the sodium influx during the rising phase of the spike (Daniel & Singh, 1958; Holman, 1958; Bülbring & Kuriyama, 1963) but also that fewer are available, because in normal solution the sodium carrier is partly inactivated, which may be related to the low resting potential. Excess calcium increases the rate of rise of the spike (Holman, 1958; Bülbring & Kuriyama, 1963). Conditioning hyperpolarization has the same effect. When the membrane is hyperpolarized to about -90 mV the rate of rise of the spike is increased from 7 to 20 V/sec. Even this increased rate of rise is still lower than that of many other excitable tissues, for example, in squid giant axon, 630 V/sec (Hodgkin & Katz, 1949); in skeletal muscle fibre, 450 V/sec (Nastuk & Hodgkin, 1950); in heart muscle, 570 V/sec (Weidman, 1955).

Intracellular stimulation produced a spike in an all-or-none manner only in a few cells. In some cells a graded response was observed but in the majority of cells no spike could be triggered. During conditioning hyperpolarization the evoked spike amplitude was, like that of the spontaneous spike, about 50 mV, and its maximum rate of rise about 20 V/sec. In crustacean muscle fibre, it is also difficult to trigger a spike. The spike amplitude is relatively small (mean 63 mV), the maximum rate of rise slow (20·5 V/sec), and spike duration at half-spike amplitude is 5·2 msec (Fatt & Katz, 1953). These figures are very similar to those of the smooth muscle of the taenia coli.

Membrane constants

In the smooth muscle the membrane resistance during the spike is not reduced as much as in other excitable tissues. Furthermore, the polarizing current has a marked effect on the rate of rise of the spike indicating a strong influence on the sodium carrier mechanism which controls the spike amplitude. The relatively small reduction of the membrane resistance during the spike, giving a lower value, and the effect on the sodium carrier, giving a higher value than the true membrane resistance, seem to cancel each other out. Thus the measurement of the effective membrane resistance from the relation between applied current and the change of spike amplitude gave a similar value (30 MΩ) to that obtained by the direct method (40 MΩ) in which the resistance is calculated from the applied current and the electrotonic potential.

We have tried to calculate the specific membrane resistance by two different methods. Neither of these presents the actual electrical situation in the smooth muscle fibre which is neither spherical nor cylindrical. The true value may well lie between those obtained by the first method and those obtained by the second method, i.e. $R_m = 300 - 1500\ \Omega\ cm^2$, $C_m = 2 - 10\ \mu F/cm^2$.

Since the muscle fibre has an elongated spindle shape, the internal resistance becomes higher at the two ends of the fibre owing to the small diameter. The current distribution cannot be uniform but must be more concentrated in the centre portion than at the two end portions, and thus the space constant becomes shorter at the two end portions. In addition, a high resistance path for current flow due to the narrow spaces between cells might also shorten the space constant. There is a large nucleus in the middle part of the fibre. If the nucleus membrane has a high electrical resistance, this should make the internal resistance of the thin layer of protoplasm high, and should change the electrical properties of the middle part of the membrane. If smooth muscle, like heart muscle, has the properties of a sheet membrane rather than cable properties (Woodbury & Crill, 1961; Noble, 1962), intracellularly applied current would be concentrated close to the electrode. Furthermore, if there is a low resistance connexion between cells, which was observed by Nagai & Prosser (1963), the current distribution will be even more complicated. The effective membrane resistance should be different depending on the place of the electrode insertion. This might be one of the reasons for the large scatter in the values of the membrane resistance (20–70 MΩ).

If we make use of the argument of Hodgkin & Katz (1949), the ionic inward current during the rising phase of the spike can be calculated from the rate of rise and membrane capacity. If the largest value (10 μF/cm^2) is used for calculation, it is 0·2 mA/cm^2 (20 V/sec $\times$ 10 μF/cm^2). The active inward current must be even greater because a passive outward current was presumably flowing through the depolarized membrane. If the passive outward current is taken to be 50 mV/400 Ω cm^2 = 0·12 mA/cm^2, the active inward current becomes 0·32 mA/cm^2 (0·2 + 0·12). This value is smaller than that of other excitable tissue (for example, in crustacean muscle fibre, 1·29 mA/cm^2, Fatt & Katz, 1953; in squid giant nerve fibre, 0·9 mA/cm^2, Hodgkin & Katz, 1949; in frog skeletal muscle fibre, 2 mA/cm^2, Nastuk & Hodgkin, 1950). This result is a further indication that the sodium carrier system in smooth muscle cells is poorly developed.

In order to explain the fact that a cell in which a spike cannot be triggered by intracellular electrical stimulation discharges spontaneously spikes with high amplitude, it seems necessary to postulate different membrane properties in different parts of the cell as concluded from the different response to conditioning polarization. If one assumes that the slow potential arises at the tapered end of the fibre, which has a high internal resistance and thus a small space constant, and further assumes that the micro-electrode is usually inserted in the middle part of the fibre with the largest diameter, it may well be that the electrode was always

located close to that part of the membrane which produced the spike, but far from that which produced the slow potential change.

The difficulty in triggering the spike may be explained by assuming that the middle portion of the cell membrane is electrically less excitable. According to this assumption, the spontaneous spike is generated at the membrane between the middle part which is less excitable and the end portion in which the slow potential change occurs. This situation would be rather similar to the motoneurone (Freygang & Frank, 1959) or the crustacean stretch receptor (Edwards & Ottoson, 1958). In these, the neurone soma is less excitable, the dendrite produces the slow potential and the axon hillock produces the spike.

Hyperpolarizing response

The non-linear increase of membrane resistance and the slow response during strong hyperpolarization is seen in nerve only when the membrane is depolarized by excess potassium (Stämpfli, 1958; Segal, 1958; Moore, 1959; Tasaki, 1959; Hagiwara *et al.* 1961). The spinal ganglion of the frog is also only capable of producing a hyperpolarizing response (Koketsu & Koyama, 1962) if the membrane is depolarized by Ca deficiency or excess K. In taenia coli the hyperpolarizing response is seen without prior depolarization, as has also been observed in lobster muscle fibre and in several kinds of electroplaques (Grundfest, 1961). These phenomena appear only in such membranes in which the potassium conductance is high. They may be explained by potassium-activation (increase of potassium conductance) and potassium-inactivation (decrease of potassium conductance) processes which are able to operate independently from, and are much slower than, the sodium carrier process (Grundfest, 1961; Wright & Tomita, 1961). The smooth muscle seems thus to be different from nerve and skeletal muscle in having a high potassium and sodium conductance. This may be the reason for the low membrane resistance and the production of the hyperpolarizing response. The electrical properties of the smooth muscle resemble most those of crustacean muscle.

SUMMARY

1. The experiments were performed on the spontaneously active intestinal smooth muscle of the isolated guinea-pig taenia coli. A single intracellular electrode was used for electrical recording as well as for stimulating with the Wheatstone bridge method.

2. Spontaneous electrical activity consisted of slow potential changes and spikes. Hyperpolarizing currents increased the amplitude and maximum rates of rise and fall of the spike. Hyperpolarization beyond a

critical level blocked the spike component. The size of the slow component remained almost unchanged even during strong hyperpolarization. Depolarizing currents reduced the spike amplitude and prolonged its duration; the spike and the slow component finally disappeared. The effects were completely reversible. Polarizing currents had no effect on the frequency of spontaneous spike generation.

3. An effective membrane resistance of about 40 MΩ was obtained from the voltage–current relation which was linear for weak currents. The time constant of the electrotonic potential was 3 msec. The effective membrane resistance obtained from the relation between applied current and the change of spike amplitude was 30 MΩ. The specific membrane resistance and capacitance were estimated as R_m = 300–1500 Ω cm² and C_m = 2–10 μF/cm².

4. In most cells a depolarizing current pulse failed to evoke a spike. In some cells, a graded response was recorded and occasionally an all-or-none spike could be triggered. Polarizing current had the same effect on the evoked spike as on the spontaneously generated spike.

5. In some cells, 'hyperpolarizing responses' were observed upon application of a strong hyperpolarizing current pulse, without prior depolarization. If these cells were strongly hyperpolarized (> -90 mV) their response to a stimulating current pulse consisted of a spike followed by a long plateau component lasting for several seconds.

6. The observations suggest that the membrane properties of taenia coli may be similar to those of the crustacean muscle fibre, and that, during spontaneous activity, the slow potential change may be generated in a different part of the membrane from that which is involved in the generation of the spike.

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