MEMBRANE PROPERTIES OF THE SOMATIC MUSCLE (OBLIQUELY STRIATED MUSCLE) OF THE EARTHWORM

BY T. HIDAKA, Y. ITO AND H. KURIYAMA

Department of Physiology, Faculty of Medicine, Kyushu University, Fukuoka, Japan

(Received 10 July 1968)

INTRODUCTION

An extensive monograph of electrophysiological and morphological studies of the invertebrate nervous system has been written by Bullock & Horridge (1965). According to this monograph much work has been carried out on the nervous system, but electrophysiological studies of the somatic muscle fibres of annelids appear to have been very rare. Horridge & Robertes (1960) studied the relationship between the motor innervation and the muscle response of Nereis and Harmothoe (Annelida). They used the bridge technique to isolate nerve fibres and observed that at the first stimulus above a single sharp threshold the nerve-muscle preparation gave a maximum electrical response, which was independent of the stimulus strength. A reduced response persisted for many repetitions at low frequency. Prosser & Melton (1954) observed the conduction of excitation in the Phascolosoma proboscis retractors and the propagation of excitation between the smooth muscle cells, and attributed them to nervous elements.

In 1967, del Castillo, de Mello & Morales observed the membrane activity of the somatic muscle of Ascaris lumbricoides and reported that rhythmic depolarization occurs at the syncytium and gives origin to action potentials in the terminal portion of the muscle-cell arms. On the leech somatic muscle Washizu (1967) and Higuchi (1967) studied the electrical properties in the resting and active states of the membrane.

Recently, due to progress in electron-microscopic techniques, the histological features of the somatic muscle system in annelids have been clarified. (Kawaguti & Ikemoto, 1958; Hanson, 1951; Hanson & Lowy, 1960; Lowy & Hanson, 1962; Ikemoto, 1963; Rosenbluth, 1963, 1964, 1965; de Bell, 1965; Nishihara, 1967).

Our experiments were carried out to investigate the membrane properties of the longitudinal muscle of the earthworm in the resting and active states of the membrane using intracellular and extracellular stimulating methods. The results are discussed in the relation to the structural specificity of the tissue.

METHODS

Longitudinal muscle from the earthworm, Pheretima communissima, 5–8 cm. in length, was used. The earthworm was pinned on the plate and dissected from the dorsal side along its length. The alimentary tract was carefully dissected from the body wall and removed. A 1–1.5 cm. length of the excised tissue was fixed on a rubber plate with pins. The tissue was immersed in an organ bath through which solution at room temperature (20–25°C) flowed continuously.
The normal Ringer's solution for this tissue had the following composition (mM): Na, 140; K, 2.7; Ca 1.8; Mg, 1.0; Cl 148.3; and pH was adjusted to 7.3-7.5.

A single intracellular microelectrode was used for making the electrical recording as well as for stimulating by means of the Wheatstone-bridge method (Araki & Otani, 1955; Kuriyama & Tomita, 1965). The resistance of the microelectrode was between 30 and 60 MΩ and a modified floating method was used (Woodbury & Brady, 1956). In order to supply a constant current to the cell the resistance of one bridge arm, in series with the microelectrode, was 1000 MΩ. The range of the applied current was between $10^{-10}$ and $5 \times 10^{-9}$ A.

Sodium-free tris solution was prepared with tris-(hydroxymethyl)-aminomethane (C₄H₁₁NO₃) titrated with high concentration of HCl, and the pH adjusted to 7.4. Chloride-deficient solution was prepared using D-glutamate and pH adjusted to 7.5.

Fig. 1. Typical patterns of membrane activity recorded from the longitudinal muscle cells of earthworm. (a) The initial discharges with high frequency was generated by mechanical stimulation and then spikes of low frequency with slow decay of the amplitude appear spontaneously. (b) The termination of the spontaneous discharges and when a mechanical stimulus was given by knocking the table on which the organ bath was placed; the artificial shock to the tissue generated discharges of high frequency with rapid decay of the damped oscillation. (c) The spontaneous discharges. (d) The maximum rate of rise and fall of the spike recorded by integrated circuit method.

RESULTS

Spontaneous activity and spikes elicited by extracellular stimulation

The membrane potential of the longitudinal muscle of earthworm was $-35.4$ mV. ($n = 219, \text{s.d.} = \pm 3.6, \text{s.e.} = \pm 0.25$). The membrane was very sensitive to mechanical...
Membrane properties of the earthworm somatic muscle

stimulation, i.e. when the microelectrode was inserted into a cell rather roughly, trains of spike discharges appeared as a damped oscillation. Even gentle insertion of the microelectrode very frequently caused spontaneous discharges to appear and these discharges also showed damped oscillation. Differences between the discharges generated by mechanical stimulation and those spontaneously generated could be seen in the frequency of the discharges and the rate of decay of the damped oscillation. The mechanically elicited discharges were of higher frequency than the others and lasted for a shorter time.

Figure 1 shows typical patterns of membrane activity recorded from the longitudinal muscle cells. In (a) the initial discharges were due to mechanical stimulation, when spikes of low frequency but high amplitude appeared spontaneously. In (b) the termi-

![Figure 1. Typical patterns of membrane activity recorded from the longitudinal muscle cells.](image)

Fig. 1. Typical patterns of membrane activity recorded from the longitudinal muscle cells. (a) Initial discharges due to mechanical stimulation. (b) Initially discharges due to spontaneous activity.

nation of the spontaneous discharges can be seen; the frequency and the amplitude of the spikes gradually decreased to zero. When a mechanical stimulus was given to the organ bath (by knocking the table on which the organ bath was placed), the artificial shock to the tissue generated discharges of high frequency with rapid decay of the damped oscillation.

The membrane activity was composed of an overshoot potential and after-hyperpolarization. The overshoot potential and after-hyperpolarization gradually decreased in amplitude during the course of the damped oscillation. The maximum

![Figure 2. Effects of tetrodotoxin (10⁻⁷ g./ml.) on the spontaneous discharges of spikes in the longitudinal muscle and in the nerve.](image)

Fig. 2. Effects of tetrodotoxin (10⁻⁷ g./ml.) on the spontaneous discharges of spikes in the longitudinal muscle and in the nerve. (a) Control for the muscle membrane activity. (b) After treatment with tetrodotoxin. (c) Control for the nerve trunk activity recorded by sucrose-gap method. (d) After treatment with tetrodotoxin.
amplitude of the overshoot from the first spike in a train of discharges sometimes exceeded +25 mV. and the mean amplitude was +18 mV. \((n = 30)\) at a membrane potential of −35 mV. The after-hyperpolarization, however, never exceeded −60 mV. The mean value of the maximum rate of rise of the spike measured from the first spike in a train of discharges was \(10^{-5} \text{V./sec.}\) \((\text{S.D.} = ±2.1, n = 20)\) and the mean value of the maximum rate of fall was \(9.8 \text{V./sec.}\) \((\text{S.D.} = ±1.8, n = 20)\). However, the maximum rate of fall of the spike sometimes exceeded the maximum rate of rise. Figure 1. Analogous features have been observed in the taenia coli muscle (Holman, 1958; Büllbring & Kuriyama, 1963) and of the oesophagus muscle of Ascaris (del Castillo & Morales, 1967).

To investigate whether these spike discharges were due to myogenic responses or to nervous elements the effect of tetrodotoxin was observed. Figure 2 shows the effects of tetrodotoxin \((10^{-7} \text{g./ml.})\) on the activity of the nerve and of the muscle. Tetrodotoxin had no effect on either the membrane potential or the membrane activity of the muscle but the nervous activity, recorded by the conventional sucrose-gap method, was completely blocked. These results might indicate that the membrane activity of this muscle is a completely myogenic response as in barnacle muscle (Hagiwara & Nakajima, 1965) and in mammalian smooth muscle (Nonomura, Hotta & Ohashi, 1966; Kuriyama, Osa & Toida, 1966). Further supporting evidence was provided by the intracellular polarization of the spontaneously generated membrane discharges. Figure 3 shows the effects of intracellular polarization on the spontaneous discharges. During the train discharges depolarizing currents (outward) reduced the spike amplitude but increased the spike frequency. Hyperpolarizing currents (inward) enhanced the spike amplitude but lowered the spike frequency or stopped spike generation. This phenomena differed from that observed in the taenia coli muscle of the guinea-pig. In the taenia coli intracellular polarization modified the spike amplitude but changes
Membrane properties of the earthworm somatic muscle

in spike frequency were not observed (Kuriyama & Tomita, 1965). These modifications of the spike frequency might also indicate that spike generation was myogenic.

The membrane resistance in the resting state may be calculated from the relation between the applied current intensity and the change in spike amplitude, if it is assumed that the membrane resistance become very low during the spike phase as in other excitable tissues (Coombs, Curtis & Eccles, 1959; Johnson & Tille, 1961; Kuriyama & Tomita, 1965). In the longitudinal muscle of the earthworm, during the train discharges, the spike amplitude, membrane potential and the critical membrane potential gradually changed in the same way as the damped oscillation. It was therefore difficult to measure the membrane resistance by the above method. However, in some cells the damped oscillation was slow (Fig. 3a). The membrane resistance calculated from these discharges was about 25 MΩ.

![Graph of membrane resistance calculation](image-url)

Fig. 4. Spontaneous discharges of the longitudinal muscle cell (a) and spike elicited from the same cell by intracellular depolarizing current (b).

In general, the spike amplitudes in the train discharges decreased smoothly from the onset to the termination, however, as shown in Fig. 3d. The inward current blocked spike generation, and after the cessation of stimulation the spike amplitude of the train discharges was higher than the amplitude expected from observations made before application of the inward current. This phenomenon might be due to either anodal restoration of the membrane or a silent period caused by a hyperpolarizing block of spike generation. Further details will be described later.

**Spikes elicited by intracellular stimulation**

The overshoot potential and after-hyperpolarization were also observed with intracellular depolarizing currents. Figure 4 shows the spontaneous activity of the mem-
brane and the spikes elicited by an intracellular depolarizing current. The mean effective resistance calculated from the voltage-current relation obtained by applying a weak hyperpolarizing current was 38 MΩ (20–68 MΩ; n = 24). The time-constant was 50 msec. (20–80 msec.; n = 24) at a 64% level of the electrotonic potential (1 − e^−t) and 84 msec. (50–129 msec.) at an 84% level of the electrotonic potential (erf t). Various parameters of the passive membrane property were calculated as for a leaky condenser, infinite cable and limited cable because of the short length (1 mm.) and narrow diameter (5–10 μ) of cell; the calculations will be described in more detail later.

![Fig. 5. Effects of conditioning polarizations on the spike elicited by test pulse. I and II were recorded from two different cells, a and a' were control. The membranes were steeply hyperpolarized by conditioning hyperpolarizing currents. Detailed explanation is in text.](image)

The spike elicited by intracellular polarization showed an overshoot potential and after-hyperpolarization as observed in the spontaneous discharges. Increase of the depolarizing current elicited repetitive spike discharges (see also Fig. 8d). Further increased stimulus intensities produced oscillatory potentials and caused a depolarization block of spike generation. The spontaneous discharges recorded from the muscle membrane showed the changes in the critical membrane potential required to elicit a spike as shown in Fig. 4 (also see Fig. 1). These changes in the critical membrane potential might be thought to be due in part to dislocation of the microelectrode caused by contraction of the cells. To eliminate the above doubt, conditioning pulses were applied during test stimulation of the cells.
Figure 5 shows the effects of conditioning polarizations on the spike elicited by test stimulation. The figure shows two series of experiments on two different cells. The conditioning hyperpolarization increased the maximum rates of rise and fall of the spike. In series I, at a membrane potential of $-35 \text{ mV}$, the critical membrane potential was $-25 \text{ mV}$. When the membrane was hyperpolarized by the conditioning pulse to $-4 \text{ mV}$ or to $-15 \text{ mV}$, the critical membrane potential increased to $-27 \text{ mV}$, respectively. In series II effects were observed similar to those in series I, and in this cell the anodal break excitation elicited by the conditioning pulses appeared at a lower threshold than did the spike elicited by the test stimulation. Further increased intensity of the conditioning pulse reduced the latency to that which elicited anodal break excitation $(\text{II}c')$. These results might indicate that the critical membrane potential required to elicit the spike was not constant at different membrane potentials, and changes of the threshold during the train discharges were therefore not due to a movement artifact.

Figure 6 shows the inactivation and activation process of the spike-generating mechanism in longitudinal muscle. A stronger polarizing current to the cell elicited repetitive spikes. Successive stimulation gradually reduced the spike amplitude and prolonged the duration of the spikes without changing the membrane potential, then only generated abortive spikes and finally no spikes at all were generated. When hyperpolarizing currents were applied instead of depolarizing currents, the membrane activities were restored and the depolarizing current again elicited repetitive spikes.
In the taenia coli muscle the amplitude of the spikes elicited by intracellular stimulation was gradually modified by conditioning polarization and took about 10 sec. to reach a steady condition. Similar tendencies were also observed in this tissue.

**Components of the falling phase of the spike**

When spikes were generated repetitively the shape of the spikes, especially their falling phase, gradually changed. The falling phase consisted of three components: a rapid decay of the potential from the peak of the spike, then a slightly lowered velocity of the falling phase, and finally a rather rapid velocity of the falling phase which lasted until the peak of the after-hyperpolarization. Spontaneous repetitive discharges, or repetitive spikes elicited by extracellular stimulation, reduced the amplitude of the first component by lowering the spike amplitude, prolonged the second phase and produced a plateau, and finally reduced the rate of fall of the third component and the amplitude of the after-hyperpolarization. Figure 7 shows the shape of the spontaneous train discharges and the shape of the spikes elicited by repetitive stimulation. Series (b) was taken at a stimulus frequency of 0.5/sec. at 20 sec. intervals. When the stimulus frequency was 0.5/sec., the changes of spike shape, especially in the second component of the falling phase, did not appear significant. However, when the stimulus frequency was increased up to 2/sec., plateau formation became dominant. The records of series (c) were taken at a stimulation frequency of 2/sec. under similar conditions to series (b). The three components of the falling phase were also observed in the spike elicited by intracellular polarization.

Figure 8 shows the effects of polarization on the shape of spikes elicited by intra-
cellular polarization. In (a) the falling phase of the spike consisted of three clear components, and when a further strong depolarizing current was applied to the same cell a second spike was elicited with a higher threshold and lower amplitude than the first one, and was followed by a plateau phase. (c)–(f) were recorded from a different cell. Spikes were elicited from this cell membrane in response to an intracellular depolarizing current (d). The falling phase also consisted of the three components.

![Graphical representation of membrane properties](image)

**Fig. 8.** Effects of conditioning polarization of the shape of spike elicited by intracellular polarization. (a) and (b) are recorded from the same cell and (c)–(f) are recorded from the same cell. (c) Conditioning depolarization. (e) and (f), conditioning hyperpolarization. Detailed explanation is in text.

When a conditioning depolarizing pulse was applied to the membrane, the test stimulus elicited a spike with a very dominant second component of the falling phase (c). However, when the sequence was reversed the spike amplitude was enhanced and the second component of the falling phase was reduced in duration and finally ceased.
as shown in (e) and (f). These results might indicate that the falling phase, especially the second component, was very sensitive to the changes of the membrane potential level.

Cellular interactions and chronaxie

Interaction between the cells was investigated by the insertion of two microelectrodes within a distance of 100 μ. From fifteen successful penetrations of the two microelectrodes, no influence of the excitation of one cell upon another could be observed. Figure 9 shows the records from the cells located within distance of 100 μ. The two microelectrodes had penetrated the cells well, and neither spontaneous discharges nor spikes elicited by intracellular polarization influenced the other cell (a). During the quiescent condition of the cells the spikes elicited from one cell had no influence on another cell (b). Further increased intensities of stimulation elicited repetitive spikes from a cell but nothing was observed in the other cell (c and d). These phenomena were analogous to those observed in the smooth muscle cells of the taenia coli of the guinea-pig on inserting two microelectrodes within a distance of 50 μ (H. Kuriyama & T. Tomita, unpublished observation).

The intensity–duration relationship for generation of the spike was studied by the intracellular polarizing method. Figure 10 shows the intensity and duration relationship when the spikes were elicited by intracellular stimulation. The duration of the

Fig. 9. Membrane activities recorded from two different cells separated by 100 μ. (a) Upper trace shows spontaneous discharges and lower trace shows spike elicited by intracellular polarization. (b–d) lower trace: spikes are elicited intracellularly by various intensities of outward current; upper trace: membrane potential of the cell which showed spontaneous discharges (upper trace of a). Detailed explanation is in text.
stimulus was varied from 10 to 480 msec. and the intensities used to elicit the spike ranged from $3 \times 10^{-10}$ to $5 \times 10^{-9}$ A. The chronaxie calculated from this figure, summarized from five experiments, was 55 msec. This is much longer than in skeletal muscle but similar to the chronaxie of the various types of vertebrate smooth muscle (Bozler, 1948; Tomita, 1966; Kuriyama, Osa & Toida, 1967a, b).

Fig. 10. Intensity and duration relationship of the longitudinal muscle when the spikes are elicited by intracellular stimulation. The stimulus durations were varied from 10 to 480 msec. and intensities from $3 \times 10^{-10}$ to $5 \times 10^{-9}$ A.

Na-free (Tris). tetrodotoxin ($10^{-7}$ g./ml.), 0.5 msec. pulse.

Fig. 11. Spikes recorded at various distance from the extracellular stimulating electrode ($0.5$ msec. pulse, 1 c./sec.). The solution had no sodium ion (tris-Cl) and tetrodotoxin ($10^{-7}$ g./ml.) was added. The spikes were recorded at 1, 2, 2.5 and 3.5 mm. distance from the stimulating electrode.

The propagation of excitation in the longitudinal muscle was observed by the extracellular stimulating method using electrodes of 100 μ diameter in sodium-free (tris-substituted) solution and under treatment with tetrodotoxin ($10^{-7}$ g./ml.). Tetrodotoxin was used to block nervous activity and to eliminate the nervous factors influencing membrane activity and the sodium-free solution was used to prevent the repetitive generations of spikes which modified the excitability of the membrane, although the spike could be triggered by intra- and extracellular stimulation. The stimulating electrode was placed on the tissue and another different electrode was placed far away from the tissue in the organ bath. The tip diameter of the stimulating
electrode was 100 μ and the electrode was coated with silicon cement except for its tip. Figure 11 shows the spikes recorded at various distances from the stimulating electrode. The spikes could be triggered in response to short pulse stimulation (0.5 msec.) of maximum intensity. When the spikes were recorded at 1, 2 and 2.5 mm. distances from the stimulating electrode, the rising phases of the spikes were superimposed upon one another during the course of the stimulus artifact. However, when the microelectrode was placed 3.5 mm. away, the rising phase of the spike started from the resting membrane potential level. In each record in the figure three to five spikes, elicited by a stimulus frequency of 0.5 c./sec., are superimposed. However, we could not record the spikes more than 3.5 mm. from the stimulating electrode under these experimental conditions.

**Calculation of the various membrane characteristic constants**

The cells were assumed to have a maximum diameter of 10 μ and a length of 1 mm. (Ikemoto, 1963). It was difficult to calculate accurate values for the specific membrane resistance and capacitance due to the complicated morphological situation and also due to various assumptions made in the calculations. Furthermore, the Wheatstone-bridge method might only be reliable when the applied current is kept below 2 x 10^-6 A. and when the balance of the bridge remained nearly the same after insertion of the micro-electrode as it had been before. Therefore, our calculations used only results in which the above conditions were satisfied. Even so it has to be pointed out that these calculations give only a simple indication and are very rough.

(i) As a leaky condenser: the specific membrane resistance \( R_m \) and capacitance \( C_m \) can be calculated from the following equations.

\[
R_m = 2\pi a R_{eff}, \quad C_m = \frac{c_m}{2\pi a l}, \quad \tau = R_m C_m,
\]

where \( a \) is the radius of the cell, \( l \) is the length of the cell, \( R_{eff} \) is the effective membrane resistance, \( R_m \) is the membrane resistance per unit length, \( c_m \) is the membrane capacitance per unit length, \( \tau \) is the time constant at \( 1 - e^{-1} \) (the time taken to reach a 64% level of the electrotonic potential after reaching equilibrium). The calculated \( R_m \) was 12 x 10^3 Ω cm. and \( C_m \) was 5 x 10^-6 F./cm.².

(ii) as an infinite cable:

\[
R_m = (R_{eff}) \frac{S_a^2}{R_i}, \quad \tau(84\%) = R_m C_m, \quad \lambda = \sqrt{\frac{a}{2}} \sqrt{\frac{R_m}{R_i}}
\]

where \( R_i \) is the internal resistance of the cell, which was assumed to be 250 Ω cm.²; \( \lambda \) is the time-constant at erf 1 (84% level of the electrotonic potential), \( \lambda \) is a space constant. The calculated \( R_m \) was 56 x 10³ Ω cm.², the \( C_m \) was 1.5 x 10^-6 μF./cm.² and \( \lambda \) was 2.3 mm.

(iii) As a limited cable: assuming that both ends of the fibre are closed with a protoplasmic membrane but not closed electrically.

(a) A stimulating and recording electrode is assumed to be inserted at one end of the cell,

\[
\frac{\lambda}{l} \coth \frac{l}{\lambda} = \frac{\pi a R_{eff}}{R_i l},
\]
Membrane properties of the earthworm somatic muscle

\[ \lambda = \sqrt[3]{a \frac{R_m}{R_i}}, \quad (2) \]

from (1) and (2)

\[ R_m = \frac{2\pi^2 R_{\text{eff}} \tanh \frac{l}{\lambda}}{4R_i}, \quad (3) \]

\[ \tanh^2 \frac{l}{\lambda} \equiv \left( \frac{l}{\lambda} \right)^2 - \frac{2}{3} \left( \frac{l}{\lambda} \right)^4 + \ldots, \quad (4) \]

when the primary approximation of \( \tanh^2 \frac{l}{\lambda} = (\frac{l}{\lambda})^2 \) is used under the limitation of \( \frac{l}{\lambda} < \frac{1}{3} \pi \), then

\[ R_m = 2\pi a R_{\text{eff}} l. \quad (5) \]

(b) A stimulating and recording electrode is assumed to be inserted in the central part of the cell,

\[ R_{\text{eff}} = \frac{R_i \lambda}{2\pi^2 \coth \frac{l'}{2\lambda}}, \quad (1') \]

where \( l' \) is the distance from the centre to either end of the cell.

\[ \frac{4R_{\text{eff}} a}{R_i l} = \frac{2}{l} \coth \frac{l'}{2}, \quad (2') \]

\[ \tanh \frac{l'}{2} = \frac{\lambda R_i}{2\pi^2 R_{\text{eff}}}, \quad (3') \]

when the primary approximation of \( \tanh \left( \frac{l'}{2\lambda} \right) = \frac{l'}{2\lambda} \) is used under limitation, \( \frac{l'}{\lambda} < \frac{\pi}{2} \).

\[ \frac{l'}{2\lambda} = \frac{R_i}{2\pi^2 R_{\text{eff}}'}, \quad (4') \]

\[ \lambda = \sqrt{a \frac{R_m}{R_i'}}, \quad (5') \]

\[ R_m = 2\pi a R_{\text{eff}} l'. \quad (6') \]

The specific membrane resistance is the same whether calculated from (iiiia) or (iiib).

The calculated \( R_m \) was \( 12 \times 10^8 \Omega \text{ cm}^2 \), the \( C_m \) was \( 7 \times 10^{-8} \mu\text{F./cm}^2 \) and \( \lambda \) was \( 1.0 \text{ mm} \).

**DISCUSSION**

Recently, owing to progress in electron-microscopic techniques, the histological features of the muscle system in the earthworm have been clarified (Hanson & Lowy, 1960; Kawaguti & Ikemoto, 1958; Ikemoto, 1963; Nishihara, 1967). According to the results obtained by electron-microscopic examination the longitudinal somatic muscles of the earthworm are comparable with the striated muscle of vertebrates in that they contain interdigitating arrays of thick and thin filaments. The peripheral thick filaments are surrounded by thin filaments. The central thick filaments are thicker than the peripheral thick filaments but they are not surrounded by thin filaments. These structures might be called ‘obliquely striated muscle’ to distinguish them from ordinary striated muscle and unstriated muscle (smooth muscle).

Two kinds of tubular structures are observed in the muscle cell, i.e. open tubule and packed tubule. Both types of tubule are distributed regularly; open tubules might
correspond to the so-called transverse tubules and the other might correspond to the J-rod which is thought to be analogous to the Z-line in vertebrate striated muscle cells. The open tubules are connected with the large vesicles distributed just beneath the cell membrane. However, the bridge structure distributed between the actin and the myosin in vertebrate striated muscle has not yet been observed in the somatic muscle of the earthworm. The cell membrane showed half-desmosome or desmosome structure in several places, and here the cell was tightly connected with the neighbouring cells.

Intracellular polarization by the bridge method modified the amplitude and the maximum rate of rise of the spikes generated both spontaneously and by an intracellular depolarizing current. An inward current enhanced the spike amplitude and an outward current reduced it. These results are clearly in accordance with the ionic theory (Hodgkin, 1951). An interesting feature of this muscle was plateau formation during the falling phase of the spike. The plateau phase gradually became dominant during the terminal stage of the train discharges.

Even slight depolarization of the membrane caused by train discharges or by intracellular depolarizations reduced the amplitude of the after-hyperpolarization. The reduction in amplitude of the after-hyperpolarization and the prolongation of the plateau phase were roughly inversely proportional. Conditioning hyperpolarization reduced the duration of the plateau phase and a strongly hyperpolarizing current completely eliminated it. The shape of the spikes with a plateau phase resembled that of cardiac muscle, although the mechanism of the plateau phase might be different since conditioning hyperpolarization eliminated the plateau phase in this muscle but not in cardiac muscle. Furthermore, the first component of the falling phase in the obliquely striated muscle resembles the phase of high chloride conductance in cardiac muscle (Dudel, Peper, Rüdel & Trautwein, 1967), although the chloride-deficient solution had no effect on the first component of the falling phase of the obliquely striated muscle. Plateau formation during the falling phase might be due to inactivation of the potassium-transport system due to a very low membrane potential caused by high sodium-permeability in the resting state. The variation in the critical membrane potential required to elicit a spike might be explained by inactivation of the spike-generating mechanism.

As described previously, conditioning hyperpolarization enhanced the maximum rate of rise, but it never exceeded 30 V./sec. The inward current during spike generation was less than 0.3 mA./cm². Therefore, the spike-generating system might not be as well developed as that of frog skeletal muscle (2 mA./cm²; Nastuk & Hodgkin, 1950), but might be developed as well as that of the smooth muscle cells of taenia coli (0.32 mA./cm²; Kuriyama & Tomita, 1965).

The generation mechanism of the spontaneous discharges and the mechanosensitivity of the cells are yet to be explained. However, the low membrane potential and the changeable critical membrane potential might generate spikes in the vicinity of this muscle fibre, and this might be associated with the mechanosensitivity, since the cells are connected with the neighbouring cells by desmosome or half-desmosome structure (Nishihara, 1967).

The intercellular connexions in smooth muscle have been studied in many ways. For example, by the insertion of two microelectrodes within a distance of about 100 μ synchronized spontaneous discharges were recorded from two different cells. However,
Membrane properties of the earthworm somatic muscle

even more than 10 good penetrations, neither the intracellular polarization of one cell
nor the spikes elicited by that intracellular polarization influenced the other cell in
which the second microelectrode was inserted. This is unexpected since electrical
connexion between mammalian smooth cells is supported by various observations,
e.g. from the theoretical basis of the sucrose-gap method (Burnstock & Straub, 1958),
the structural significance (Dewey & Barr, 1962) and the interpretation of the pro-
erties of the electrotonic potentials recorded under extracellular stimulation (Tomita,
1966). The present results failed to record cellular interaction within a distance of
100 μ as observed in the smooth muscle taenia coli of the guinea-pig. The propagation
of excitation between the muscle fibres is decremental and nervous elements modify
the propagation of the excitation, but the participation of a myogenic element could
not be completely ruled out. Furthermore, a stimulating electrode with a tip diameter
of less than 100 μ could not elicit propagated spikes, as observed in the circular smooth

In contrast with the muscles of the earthworm, Ascaris muscles have intercellular
electrical connexion via the syncytial structure of the arm of the somatic muscle (del
Castillo et al. 1967). Electron-microscopic observations made by Rosenbluth (1965)
and de Bell (1965) also confirmed that adjacent muscle cells were connected at the
innervation process (arms) into a functional syncytium across tight junctions (nexus).
derel Castillo et al. (1967) observed the modifications of the membrane activity recorded
from the belly region by the injections of current from the stimulating electrode in-
serted in the arm of the adjacent cell. The structural differences between the Ascaris
and earthworm muscles might appear as a different feature on the propagation of the
excitation; that is, Ascaris somatic muscle behaves as a ‘visceral muscle’ and earth-
worm somatic muscle behaves as a ‘multunit muscle’, which were classified by Bozler
(1948) from the behaviour of mammalian smooth muscles.

SUMMARY

1. The membrane properties of the longitudinal muscle fibre of the earthworm
Pheretima communissima were investigated by intra- and extracellular stimulating
methods.

2. The membrane potential was −35.4 mV., and spontaneous discharges with over-
shoot (mean +18 mV.) and after-hyperpolarization (−60 mV.) were recorded.

3. Tetrodotoxin (10⁻⁷ g./ml.) blocked nervous activity but did not influence the
spontaneous discharges or the spikes elicited in the muscle fibre by intracellular
stimulation.

4. The critical membrane potential required to elicit a spike was not constant, and
the falling phase of the spikes was markedly dependent on the level of the membrane
potential.

5. The chronaxie, measured from the intensity–duration relation to elicit a spike by
intracellular stimulation, was 55 msec.

6. When nervous activity was excluded the propagation of excitation in longitudinal
muscles was decremental.
REFERENCES


Membrane properties of the earthworm somatic muscle


