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CALCIUM SPIKE IN THE LONGITUDINAL MUSCLE OF THE LOBWORM, *TYLORRYNCHUS HETEROCHAETUS*, (NEREIDAE)

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INTRODUCTION

In crustacean muscle, earthworm longitudinal muscle, leech longitudinal muscle and barnacle muscle the major role in the production of the action potential is played by divalent cations rather than sodium ions, which are believed to be most important in many other excitable cells (Fatt & Ginsborg, 1958; Fatt & Katz, 1953; Werman, McCann & Grundfest, 1961; Hagiwara, Chichibu & Naka, 1964; Hagiwara & Takahashi, 1967; Hidaka, Ito, Kuriyama & Tashiro, 1969; Ito, Kuriyama & Tashiro, 1970; Higuchi, 1967).

In the longitudinal muscle of the earthworm, Ito, Kuriyama & Tashiro (1970) concluded from the results obtained from the effects of divalent cations on the spike that Ca^{2+}, Sr^{2+} and Ba^{2+} could generate the spike in sodium-free solution, but not Mg^{2+}. Co^{2+} and Mn^{2+} blocked the generation of the spike elicited in the presence of Ca^{2+}, Sr^{2+} or Ba^{2+}. The amplitude of the spike was proportionally increased with increased external concentrations of Ca^{2+}, Sr^{2+} and Ba^{2+}. They concluded that Ca^{2+} in normal Ringer solution played an important role in determining the membrane potential in normal solution and also in the production of the action potential as a current carrier during the active state of the membrane. The present experiments were intended to investigate the properties of the muscle membrane in the Nereidae during the active state and the main factors involved in determining the membrane potential during the resting state. The experimental results were compared with the results obtained from the earthworm which were carried out under similar experimental conditions. The results led to the conclusion that the membrane potential was mainly determined by the ionic concentration gradient and permeability of K^+ across the membrane and that Na^+ had less influence on the resting membrane potential than that in the longitudinal muscle of the earthworm. The spike was mainly generated by the inward movement of Ca^{2+}. However, in the physiological solution, Na^+ (at least in part) is also important for the generation of the spike.

METHODS

The lobworm, *Tylorrynchus heterochaetus*, was pinned on the board and dissected from the dorsal side along its whole length (8–10 cm). The alimentary tract and connective tissue were carefully dissected away from the body wall and the longitudinal

muscle was removed. A 1–1·5 cm length of the excised tissue was immersed in an organ bath made of Perspex through which the solution at room temperature (20–25 °C) flowed continuously.

The normal artificial saline solution for this tissue had the following composition: NaCl 471 mM; KCl 8 mM; $CaCl_2$ 20 mM; $MgCl_2$ 12 mM; $NaHCO_3$ 10 mM; and the pH was adjusted to 7·3 using tris buffer. A single intracellular micro-electrode was used for electrical recording as well as for stimulating by means of the Wheatstone-bridge method (Kuriyama & Tomita, 1965; Hidaka, Ito, Kuriyama & Tashiro, 1969). The resistance of the micro-electrode 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 5×10^{-9} A. Tension development was measured isometrically with a transducer. The stimulating electrodes (platinum wire, 0·2 mm in diameter) were arranged along the piece of tissue as a multigrid electrode 2 mm wide, and parallel with it for 10 mm. The stimulating current therefore passed transversely across the tissue with uniform intensity.

Sodium-free tris solution was prepared with tris-(hydroxymethyl)-aminoethane ($C_4H_{11}NO_3$) titrated with a high concentration of HCl, to a pH of 7·3. To obtain the effects of various cations on the membrane activity, various amounts of $CaCl_2$, $SrCl_2$, $BaCl_2$, $MgCl_2$ and $MnCl_2$ were added to the solution containing tris Cl.

RESULTS

Mechanical properties of the longitudinal muscle

The relationship between stimulus duration and tension development in the longitudinal muscle was observed. The intensity was fixed at the maximum current level and the tissue was fixed at 10 mm. Fig. 1 shows the effects of different stimulus durations on tension development under isometric conditions. The duration of the stimulus was varied from 5 to 100 msec. The tension increased in proportion to the stimulus duration. When the stimulus duration exceeded 50 msec, the contraction was sustained for more than 30 sec. The tension, which initially increased rapidly, relaxed to a 30–40% level of the peak tension, i.e. initial rapid phasic tension development and late sustained tension development. The two components of the tension development were also observed from the longitudinal muscle of the earthworm. However, the time to reach the peak of phasic tension and to complete relaxation of the muscle was much longer in the lobworm than in the earthworm.

The slow relaxation after the tension development could be observed most clearly during the process of relaxation after tetanus. Fig. 2 shows the effects of repetitive stimulation on the tension development. The stimulus frequency was varied from 0·1 to 20 c/s. Incomplete tetanus developed when the stimulus frequency was less than 2 c/s, and complete tetanus could be obtained at a stimulus frequency of 5 c/s with 50 msec pulse duration. At a stimulus frequency of 20 c/s (50 msec pulse duration for 5 sec) complete relaxation to the resting tension took about 5 min. The duration of the sustained tonic tension developed in the Nereidae was longer than that recorded from the earthworm but it was much shorter than that recorded from the anterior byssus retractor muscle of *Mytilus edulis* (Twarog, 1967*a*). The duration of the sustained

contraction continued more than 1 hr in the case of *Mytilus edulis*. Treatment with 5-hydroxytryptamine (10^{-5} g/ml) slightly reduced the relaxation time after the tetanic stimulation. However, separation of the two components failed to occur on treatment with 5-hydroxytryptamine.

Membrane properties of the longitudinal muscle effects of various ions on the membrane potential

The relationship between the membrane potential and the external potassium concentration ($[K]_o$) was observed in the longitudinal muscle. Various concentrations of $[K]_o$ were prepared by the reduction of $[Na]_o$ in isotonic saline solution and also by

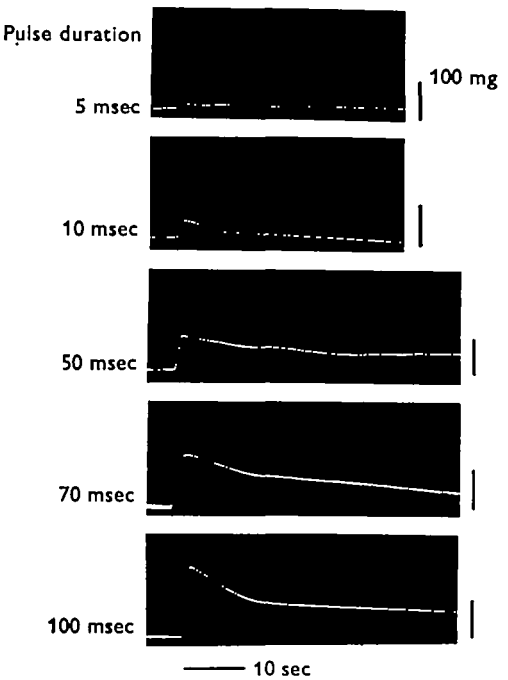

Fig. 1. Effects of different stimulus duration on tension development in longitudinal muscle under isometric conditions. Pulse durations were varied from 5 msec to 100 msec.

reduction of tris in the sodium-free solution. The mean membrane potential in the resting state was $-62{\cdot}8$ mV S.D. $= \pm 4{\cdot}3$, $n = 50$ and in the sodium-free solution it was $-71{\cdot}0$ mV S.D. $= \pm 2{\cdot}8$, $n = 50$. Increased $[K]_o$ depolarized the membrane, and reduced $[K]_o$ hyperpolarized the membrane. Fig. 3 shows the change in the membrane potential against $[K]_o$ on a logarithmic scale. In solutions containing NaCl the curve relating the membrane potential to $[K]_o$ had two different forms, one above and one below a $[K]_o$ of 18 mM. In these solutions, a linear relationship between the membrane potential and $[K]_o$ plotted on a logarithmic scale was observed when $[K]_o$ was increased up to 18 mM. The maximum decrease of the membrane potential for a tenfold change of $[K]_o$ was 39 mV measured above 18 mM $[K]_o$. In the sodium-free (tris) solution, it was 48 mV measured above 18 mM $[K]_o$. On the other hand, below 18 mM $[K]_o$, the maximum slope of the membrane potential produced by tenfold change in

$[K]_o$ was 7 mV in the isotonic potassium-saline solution and 11 mV in sodium-free (tris) solution (the mean of five different preparations). With 0·45 mM $[K]_o$, the membrane was hyperpolarized to −77 mV (±3·4, n = 50) in sodium-free solution, and with 9 mM $[K]_o$ the membrane was hyperpolarized from −61 mV (±2·9, n = 50) to

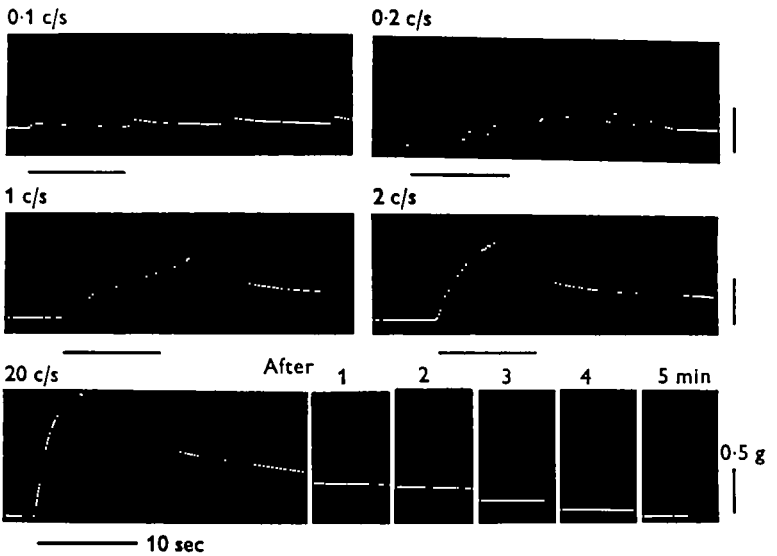

Fig. 2. Effects of repetitive stimulation on tension development in longitudinal muscle under isometric conditions. Stimulus duration (10 msec). Stimulus frequency was increased from 0·1 c/s to 20 c/s.

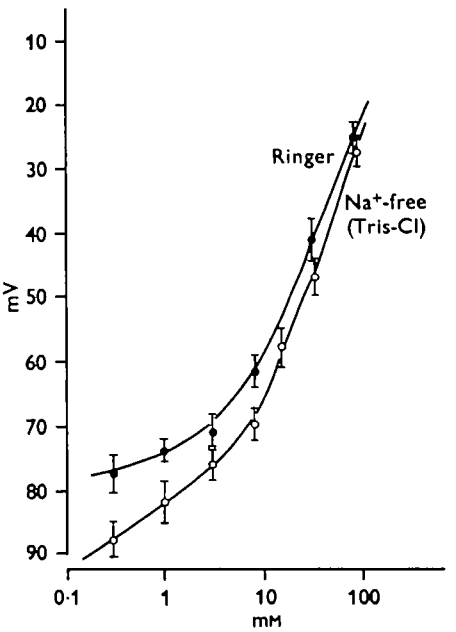

Fig. 3. Changes of the membrane potential against $[K]_o$ on a logarithmic scale. Ringer; KCl was isotonically increased by reduction of NaCl. Sodium-free; KCl was isotonically increased by replacement of tris in isotonic sodium-free (tris) solution.

-70 mV ($\pm 2{\cdot}7$, $n = 50$), indicating a very dominant influence of sodium-permeability below 9 mM $[K]_o$. However, when $[K]_o$ was increased to more than the normal concentration (9 mM), the difference resulting from the presence and absence of Na^+ became small.

Fig. 4 shows the effects of changing the concentrations of external Na^+ and Cl^- on the membrane potential. When the sodium was replaced with tris in stages, the membrane was at first hyperpolarized. However, if $[Na]_o$ was reduced below 100 mM, no more hyperpolarization was observed and the membrane potential remained nearly the same at -71 mV (-71 mV $\pm 1{\cdot}8$, $n = 50$ in 100 mM $[Na]_o$, -70 mV $\pm 2{\cdot}4$, $n = 50$ in 10 mM $[Na]_o$ and -71 mV $\pm 2{\cdot}1$, $n = 50$ in 1 mM $[Na]_o$.

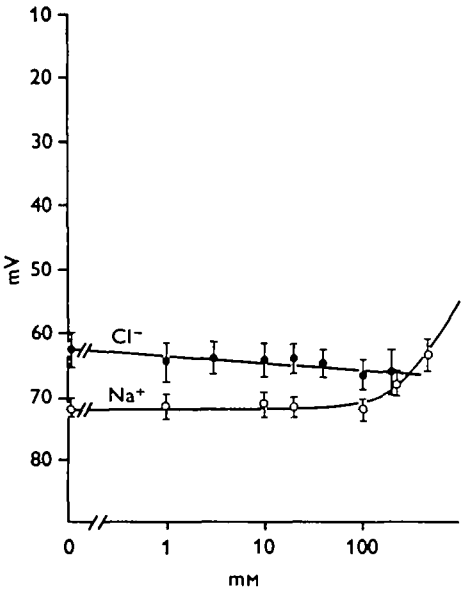

Fig. 4. Effects of $[Na]_o$ and $[Cl]_o$ on the membrane potential. Na^+; Na^+ in the solution was replaced by tris. Cl^-; Cl^- in the solution was replaced by $C_6H_5SO_3^-$.

The effects of various concentrations of Cl^- (substituted by $C_6H_5SO_3$) on the membrane potential were observed. Reduction of $[Cl]_o$ transiently depolarized the membrane, but after about 10 min the membrane potential returned to the normal level and $[Cl]_o$ did not influence the membrane potential. These observations led to the conclusion that the membrane potential was determined mainly by the concentration gradient of $[K]_o/[K]_i$ and potassium-permeability, and partly influenced by Na^+ as observed in the longitudinal muscle of the earthworm. However, the resting membrane potential was much higher in the case of the lobworm than in the earthworm, and therefore the hyperpolarization of the membrane in sodium-free solution was less than observed in earthworm.

The active membrane potential

Spontaneous spike generation and evoked spikes could be observed from the longitudinal muscle of Nereidae. Fig. 5 shows a spike elicited spontaneously (*a*) and a spike

elicited by the application of an outward current pulse (*b*). The spontaneously generated spikes showed overshoot and after hyperpolarization (undershoot potential). However, spontaneous spike generation was preceded by depolarization of the membrane, and therefore the peak of the undershoot potential did not exceed the resting membrane potential level. At a membrane potential of 62 mV ($\pm 1{\cdot}1$, $n = 50$), the mean amplitude of the overshoot potential was 18 mV ($\pm 2{\cdot}0$, $n = 50$), the maximum rate of rise of spike was 41·7 V/sec ($\pm 2{\cdot}8$, $n = 50$) and the critical membrane potential to elicit the spike was 25·4 mV ($\pm 1{\cdot}8$, $n = 50$). The spike evoked from the resting state of the membrane by an outward current pulse showed an overshoot with undershoot potential. The peak of the undershoot never exceeded the resting membrane

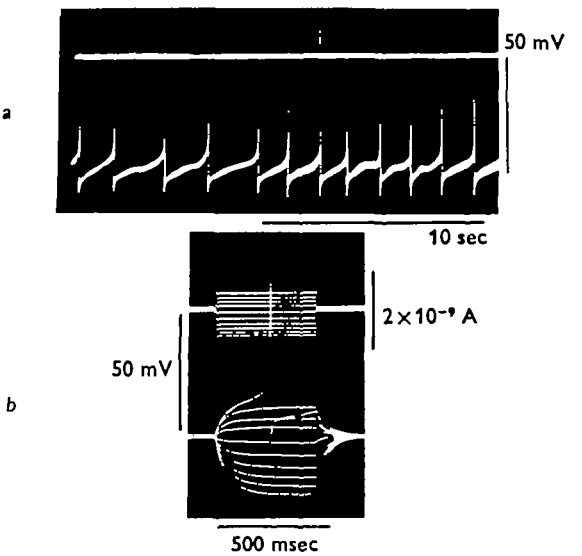

Fig. 5. Membrane activity of the longitudinal muscle. (*a*) Spontaneous spike generation. (*b*) Effects of inward and outward current pulses on the membrane. Outward current pulse elicited a spike with overshoot.

potential as also observed for spontaneous spike generation. The time constant of the membrane measured by application of weak inward current pulses was 28·4 msec (S.D. = $\pm 7{\cdot}3$, $n = 20$) at 64% of the steady state of the electrotonic potential, and the input resistance measured from the current–voltage relationship was 41·9 MΩ (S.D. = $\pm 4{\cdot}8$, $n = 20$).

The strength duration relationship for generation of the spike was studied by the intracellular polarizing method. Fig. 6 shows the intensity and duration relationship when the spikes were elicited by intracellular stimulation. The duration of the stimulus was varied from 10 to 500 msec, and the intensity used to elicit the spike ranged from 0·3–2·8 nA. The three different preparations showed wide variation in the intensity-duration relationship, and therefore the chronaxie calculated from this varied from 60 to 80 msec. This is very much longer than in skeletal muscle but similar to the chronaxie of the longitudinal muscle of the earthworm (Hidaka, Ito & Kuriyama, 1969). An example of the strength-duration relationship measured by application of outward current to elicit the spike is shown in Fig. 7.

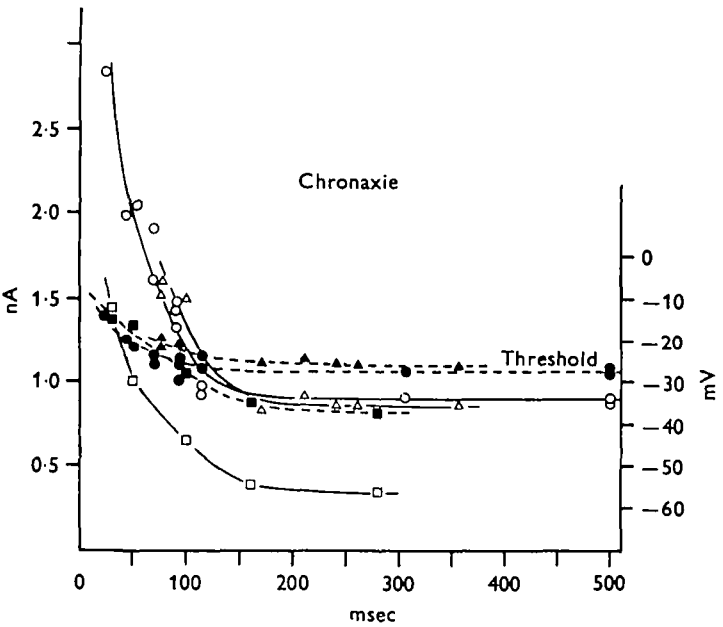

Fig. 6. Relation between the stimulus intensity and the duration of the stimulus at the threshold for eliciting the spike (Strength-duration curve). Dotted lines show the threshold potential levels for spiking. Three different preparations were used.

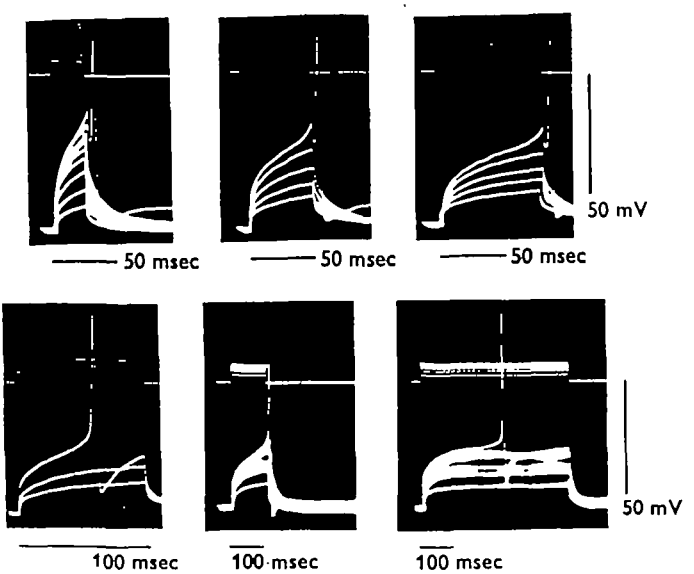

Fig. 7. Examples of the membrane activity recorded with various stimulus durations and intensities.

Spike generation in the presence of tetrodotoxin and various divalent cations

The effects of tetrodotoxin (10^{-5} g/ml) on the membrane potential, the membrane resistance and the spontaneous and evoked spike were observed. Fig. 8 shows the effect of tetrodotoxin on the spontaneously generated spike and the evoked spike.

The membrane potential, the amplitude of the spike and the maximum rate of rise of the spike were not influenced by treatment with tetrodotoxin. Furthermore, the membrane resistance measured by application of the inward and outward current pulses showed no difference before and after treatment with tetrodotoxin. These observations might suggest the possibility of sodium-independent spike generation,

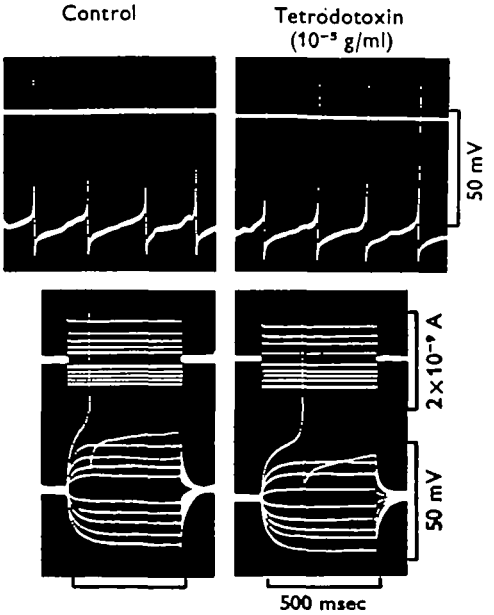

Fig. 8. Effects of tetrodotoxin (10^{-5} g/ml) on the spontaneously generated spikes and evoked spikes. Records were taken after 30 min of perfusion of the tissue with tetrodotoxin.

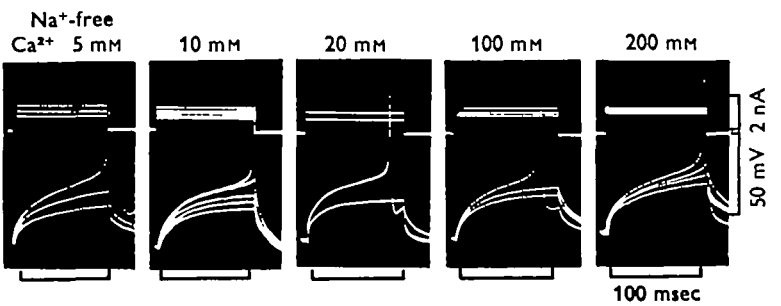

Fig. 9. Effects of various concentrations of Ca^{2+} (5–200 mM) on the membrane potential, threshold to evoke the spike and spike amplitude in sodium-free (tris) solution. The solutions were isotonic.

since in many excitable membranes, tetrodotoxin blocked the inward movement of Na^+ when the membrane was depolarized to the electrical threshold. The spike in Nereidae could be elicited in sodium-free solution.

Fig. 9 shows the effects of various concentrations of Ca^{2+} (2–200 mM) on the spike generation in sodium-free (tris) solution. The spike amplitude increased in proportion to the increased $[Ca]_o$. However, the threshold remained the same. The spike duration measured at 50% of the spike amplitude showed the shortest value in the concen-

tration of 20 mM $[Ca]_o$ (the normal concentration), but was prolonged when the concentration was varied. Fig. 10 shows the membrane potentials, threshold membrane potentials, spike amplitude and the maximum rate of rise of the spike measured at various concentrations of $[Ca]_o$ in sodium-free solution. In sodium-free solution the membrane was hyperpolarized, and no change in the membrane potential was observed in the presence of the various $[Ca]_o$. The critical membrane potential to

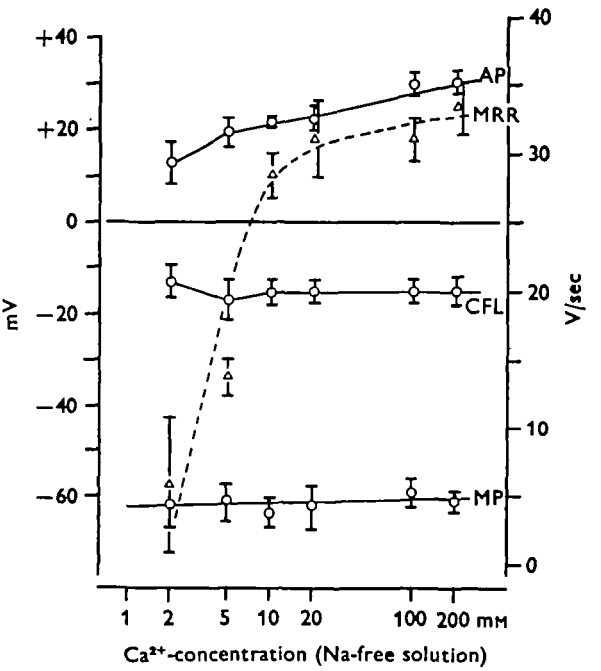

Fig. 10. Effects of various concentrations of Ca^{2+} (2–200 mM), on the membrane potential and spike generation in sodium-free (tris) solution. AP; amplitude of action potential (mV). MP; membrane potential (mV). CFL; critical firing level (mV). MRR; maximum rate of rise of the spike (V/sec).

elicit the spike remained the same in the ranges between 2 and 200 mM. However, the amplitude of the overshoot increased in proportion to the increased $[Ca]_o$. The maximum increase in amplitude of the overshoot for a tenfold change of $[Ca]_o$ was 10 mV (the mean of five different preparations). The maximum rate of rise of the spike was markedly decreased in the low concentrations of $[Ca]_o$. For example, the maximum rate of rise of the spike decreased to 6·5 V/sec ($\pm 5{\cdot}2$, $n = 10$) in 2 mM $[Ca]_o$ from 28 V/sec ($\pm 2{\cdot}8$, $n = 10$) in 10 mM $[Ca]_o$. However, when $[Ca]_o$ was increased to more than 20 mM, the maximum rate of rise of the spike remained at nearly the same value. The most marked difference observed in the results on the membrane activity in the above experimental conditions compared with those obtained from the longitudinal muscle of the earthworm was the level of the critical membrane potential for the electrical activity, i.e. in the longitudinal muscle of the lobworm the critical membrane potential remained the same with various concentrations of $[Ca]_o$, but the critical membrane potential did not remain the same in the longitudinal muscle of the earthworm (Ito *et al.* 1970).

The spike could also be elicited in the presence of Sr^{2+} and Ba^{2+} in the calcium-free and sodium-free solution. Sr^{2+} showed a similar effect of Ca^{2+} for the spike generation. The spike amplitude was increased in proportion to increased $[Sr]_o$, and the threshold to trigger the spike (-24 mV) remained the same at the various concentrations of $[Sr]_o$. However, the half duration of the spike was slightly longer at any given concentration of Sr^{2+} compared with Ca^{2+} (5 msec $\pm$ 1·1, n = 10 in 20 mM-Ca^{2+} and 18 msec $\pm$ 2·8, n = 10 in 20 mM-Sr^{2+}). Fig. 11 shows the effects of Ba^{2+} (2–20 mM) on the spike generation in sodium-free and calcium-free solution. At a concentration of 20 mM, Ba^{2+} produced the plateau phase. However, when the concentration of Ba^{2+} was below 20 mM, no plateau formation was observed. The spike amplitude in the various $[Ba]_o$ increased in proportion to $[Ba]_o$ in the ranges of 5–50 mM. The maximum increase in the overshoot against tenfold changes of $[Ba]_o$ was 18 mV.

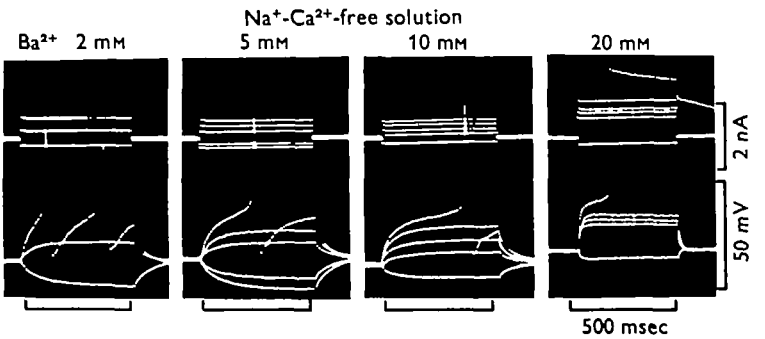

Fig. 11. Effects of various concentrations of Ba^{2+} (2–20 mM) on the membrane potential, threshold to evoke the spike and the spike amplitude in sodium-free (tris) solution.

DISCUSSION

Mechanical properties

The longitudinal muscle of Nereidae is composed of obliquely striated muscle (Ito, Kuriyama & Tashiro, unpublished observations). The striations of the contractile proteins 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 were the same as those observed from the longitudinal somatic muscle of the earthworm, i.e. obliquely striated muscle (Nishihara, 1967; Ikemoto, 1963).

The contraction developed in response to either single or repetitive stimulation, the twitch and tetanus tensions, consisted of an initial phasic tension and a sustained tension of low amplitude. These two components were also described in the earthworm (Hidaka, Kuriyama & Yamamoto, 1969) where the initial phasic tension was less sensitive to Na^+ but the sustained tension was reduced in amplitude or ceased in the absence of Na^+ (N. Tashiro, in preparation). The sustained contraction in both species was less sensitive to treatment with 5-hydroxytryptamine, unlike the 'catch' contraction of the anterior byssus retractor muscle of *Mytilus edulis* (ABRM), where Twarog (1967*a*, *b*) observed that 5-hydroxytryptamine completely blocked the generation of

the sustained tension without any influence on the phasic tension in the 'catch' muscle. An analogous mechanism for tension development in the longitudinal muscle of the earthworm and in the longitudinal muscle of the lobworm might be involved. Investigations into the mechanism of tension development are being studied by N. Tashiro. These studies might help in the understanding of the mechanism of tension development in the longitudinal muscle of Nereidae.

Membrane properties

The membrane potential of the longitudinal muscles of Nereidae was -60 mV and this value was much higher than that in earthworm. The reduction or absence of Na^+ increased the membrane potential from -60 mV to -70 mV and membrane resistance from 48 MΩ to 62 MΩ. However, the magnitude of the hyperpolarization and the increased membrane resistance compared with the normal state were smaller than those observed in the earthworm in similar ionic environments. The reduction of Cl^- (replaced by the less permeant anion $C_6H_5SO_3^-$) did not influence the membrane potential but increased the input resistance. Presumably the distribution of Cl^- across the membrane takes place in a passive manner, because in respect of membrane potential and membrane resistance the muscle fibres of Nereidae behave like the skeletal muscle fibres of the frog on replacement of Cl^- by other anions (Hutter & Padsha, 1959; Hodgkin & Horowicz, 1959; Hutter & Noble, 1961). However, in the longitudinal muscle of the earthworm the effect of Cl^- on the membrane resistance and the membrane potential may suggest that it behaved in a passive manner; but the chloride equilibrium potential (E_{Cl}) of -55 mV was much higher than the membrane potential (-35 mV). Therefore, Hidaka, Ito & Kuriyama (1969) postulated alternative explanations for the chloride-permeability of the membrane: either the reduction of $[Cl]_o$ modified the permeability of the membrane to other ions or active transport of Cl^- might be involved. In the longitudinal muscle of Nereidae, the generation of miniature inhibitory junction potentials, thought to be due to selective increase in chloride-permeability was very rare. Only when the membrane potential was hyperpolarized by inward current pulses could the miniature depolarizing potentials be recorded. These small potential changes were due to the generation of miniature excitatory junction potentials or reversed miniature inhibitory junction potentials caused by the hyperpolarization of the membrane to a value greater than the reversal potential of -60 mV, assuming that the E_{Cl} has the same value as in the resting membrane. Further studies are required to clarify the role of Cl^- in determining the membrane-potential level.

Spikes could be elicited in sodium-free solution, and tetrodotoxin had no effect on the spontaneously generated spikes or electrically evoked spikes suggesting a sodium-independent spike-generation mechanism. The spike amplitude and the maximum rate of rise of the spike were proportionally modified with $[Ca]_o$ in sodium-free solution, and Ba^{2+} and Sr^{2+} could take the place of Ca^{2+} for spike generation whether Na^+ was present or not, indicating that calcium spikes might occur in physiological solution.

The relationship between the amplitude of the overshoot potential and $[Ca]_o$ in sodium-free solution indicated that the spike amplitude was roughly linearly related to the logarithm of $[Ca]_o$. However, the maximum increase in the amplitude of the

overshoot against a tenfold change in $[Ca]_o$ was only 10 mV. If the amplitude of the overshoot depended on the $[Ca]_o$, the slope should be either 29 mV as predicted by Goldman's equation or 40 mV as predicted from the equations introduced by Kimizuka (1966). In the crustacean muscle fibre Fatt & Ginsborg (1958) reported that the relationship between the spike amplitude and $[Sr]_o$ was in reasonable agreement with the relation predicted by Goldman's equation. Hagiwara & Takahashi (1967) also reported that the overshoot potential of the barnacle muscle increased with a slope of approximately 29 mV for a tenfold increase in $[Ca]_o$ in the low range. On the other hand, Nishi, Soeda & Koketsu (1965), using frog sympathetic ganglion, reported that the spike amplitude against $[Ca]_o$ and $[Ba]_o$ could be estimated from the equations presented by Kimizuka (1966) and the maximum slope of the spike amplitude produced by a tenfold change of divalent cations was 40 mV. Presumably, in the longitudinal muscle of Nereidae Ca^{2+} was tightly bound to the membrane and stabilized the membrane by reduction in the sodium-permeability and the threshold to trigger the spike remained the same with the wide ranges of $[Ca]_o$. Hagiwara & Takahashi (1967) also reported that the amplitude of the overshoot was increased less when the $[Ca]_o$ was increased to more than 20 mM, and the slope of the potential change against $[Ca]_o$ became much smaller than that observed in the low $[Ca]_o$. The roles of Ca^{2+} on the membrane of the longitudinal muscle of the lobworm could be summarized by saying that Ca^{2+} stabilized the membrane by reduction of sodium-permeability and could carry the current during the active state of the membrane. The influx of Ca^{2+} may be controlled by Ca^{2+} which is bound to the membrane. The absence of change of the threshold to trigger the spike at various concentrations of $[Ca]_o$ suggests that calcium may be bound tightly to the membrane.

SUMMARY

1. The mechanical and electrical activities of the longitudinal somatic muscle of the nereid *Tylorrynchus heterochaetus* were studied by intra- and extracellular stimulating methods.

2. The contraction elicited by electrical stimulation under isometric conditions consisted of two components, i.e. early phasic contraction and sustained contraction. The sustained contraction lasted more than 1 min after the cessation of the tetanic stimulation.

3. The membrane potential was 62·8 mV, and spontaneous discharges with overshoot (mean 18 mV) were recorded. A similar amplitude of the spike could be recorded by the intra-cellular polarizing method.

4. The maximum slope of the membrane potential change against a tenfold change in $[K]_o$ was 39 mV in the presence of Na^+ and 48 mV in the absence of Na^+.

5. The membrane was hyperpolarized by reduction of $[Na]_o$ but not by reduction of $[Cl]_o$.

6. Tetrodotoxin (10^{-5} g/ml) blocked neither spontaneous spike generation nor spikes evoked by electrical stimulation.

7. The spike amplitude (overshoot) was proportionally increased with increased $[Ca]_o$ in the absence of Na^+. The electrical threshold and the membrane potential remained the same in the ranges of 2 and 200 mM $[Ca]_o$ in the absence of Na^+.

8. Sr^{2+} and Ba^{2+} could produce spike generation in the absence of Na^{+} and Ca^{2+}. Prolongation of the spike (plateau) was observed when 20 mM Ba^{2+} was added to the solution containing no Na^{2+} and Ca^{2+}.

9. The electrical and mechanical properties of the muscle were discussed in comparison with those observed of the longitudinal muscle of the earthworm.

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