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MECHANICAL PROPERTIES OF THE LONGITUDINAL AND CIRCULAR MUSCLE IN THE EARTHWORM

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INTRODUCTION

The somatic muscle of the earthworm consists of longitudinal and circular muscle layers. Ultrastructural studies have shown that these muscle fibres are obliquely striated, but their fine structural organization is fundamentally similar to that of cross-striated muscle fibres (Hanson, 1957; Kawaguti & Ikemoto, 1959; Ikemoto, 1963; Nishihara, 1967; Heumann & Zebe, 1967).

The mechanical properties of the longitudinal muscle of the earthworm have previously been investigated (Hidaka, Kuriyama & Yamamoto, 1969). The present experiments were intended to investigate the mechanical properties of the longitudinal muscle further and to compare this muscle with the circular muscle.

The results obtained in the present experiments suggest the possibility that calcium-dependent spikes preceded the onset of the mechanical response of the longitudinal muscle, while in the circular muscle sodium-dependent spikes trigger the mechanical response.

METHODS

The earthworm, *Pheretima communissima*, was dissected from the dorsal side along its length (5–8 cm) and the alimentary tract was carefully removed from the body wall. Two different strips were obtained from the body wall, one being cut along the longitudinal muscle fibres, and the other along the circular muscle fibres at right angles to the longitudinal muscle fibres. Both strips had a similar size of about 10 mm long and 1 mm wide.

The preparation was suspended in the chamber which contained 1 ml of the earthworm Ringer solution. Normal earthworm Ringer solution had the following composition (mM): Na, 140; K, 2·7; Ca, 1·8; Mg, 1·0; Cl, 148·3. The solution was adjusted to pH 7·3 with tris-buffer. In sodium-free solution, NaCl was substituted with an equivalent amount of LiCl, tris (hydroxymethyl)-aminomethane chloride or glucose. Drugs used were atropine sulphate (Tanabe) at a concentration of $3{\cdot}5 \times 10^{-5}$ M $\doteqdot 10^{-5}$ g/ml, *d*-tubocurarine chloride (Sigma), $1{\cdot}4 \times 10^{-5}$ M $\doteqdot 10^{-5}$ g/ml, and tetrodotoxin (Sankyo), 3×10^{-6} M $\doteqdot 10^{-6}$ g/ml.

Stimulating currents were applied longitudinally to the preparation with platinum-black foil electrodes. The tension was measured isometrically with a mechano-electric transducer, as previously described by Hidaka *et al.* (1969). All experiments were carried out at room temperature (20–23 °C) during winter time (November to March).

RESULTS

Tension development in the longitudinal and circular muscles

It was very difficult to separate either the longitudinal muscle or the circular muscle from the cuticle and the other muscle layer. It was therefore assumed that the tension recorded from a strip (10 mm length) cut along the longitudinal muscle fibres was produced mainly by the longitudinal muscle, although it contained circular muscle

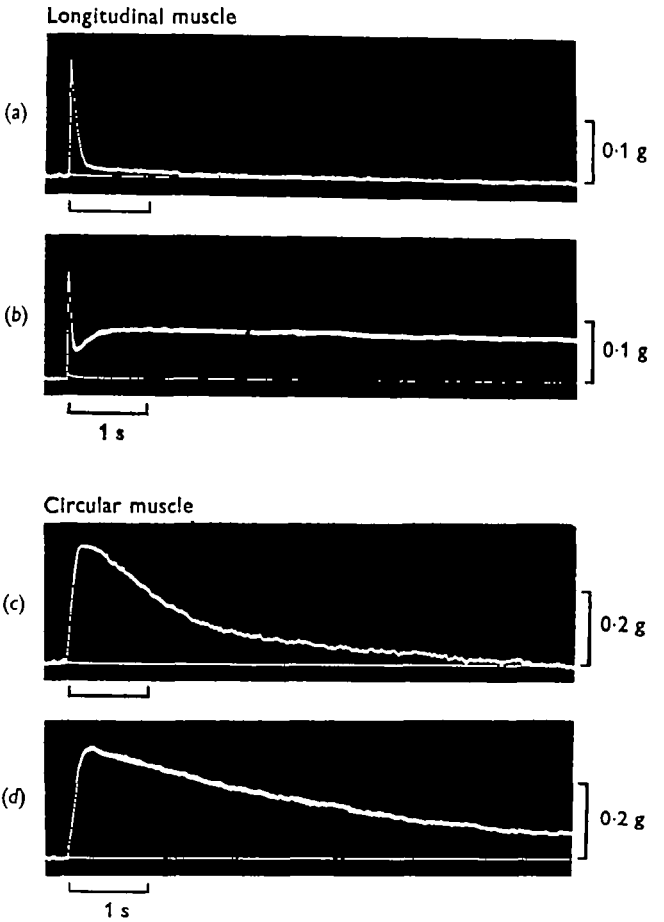

Fig. 1. Various types of tension development of the longitudinal (*a*, *b*) and circular muscles (*c*, *d*). Longitudinal muscle: (*a*) the phasic contraction produced by electrical stimulation (10 ms) of low intensity (1·8 V/cm), and (*b*) the phasic and tonic contraction at increased intensity (3·0 V/cm). Circular muscle: (*c*) and (*d*) tension responses of two different preparations to current pulses of same intensity (3·0 V/cm) and duration (10 ms).

fibres running at right angles across it for a short length (1 mm). Similarly, the tension from a strip dissected along the circular muscle fibres was assumed to represent predominantly the response of the circular muscle. The description of the magnitude of the tension development was, therefore, only qualitative.

The upper two records (*a*, *b*) in Fig. 1 show the typical features of tension develop-

ent in the longitudinal muscle. A low intensity of stimulus (1·8 V/cm) for 10 ms elicited only a phasic contraction (*a*), and a current pulse of higher intensity (3·0 V/cm) of the same duration elicited a phasic contraction followed by a very prolonged tonic contraction, lasting for about 1·5 min (*b*). The time to peak tension of the phasic contraction was 70–80 ms and that of the tonic contraction varied between 0·8 and 1·5 s. In most preparations the duration of the phasic contraction was 100–200 ms, and the falling phase of the phasic contraction was superseded by the second tension development, i.e. the tonic contraction. Occasionally, a rather slow phasic contraction was observed, the duration of which was 500 ms or more. In such a preparation the magnitude of the tonic contraction was relatively small.

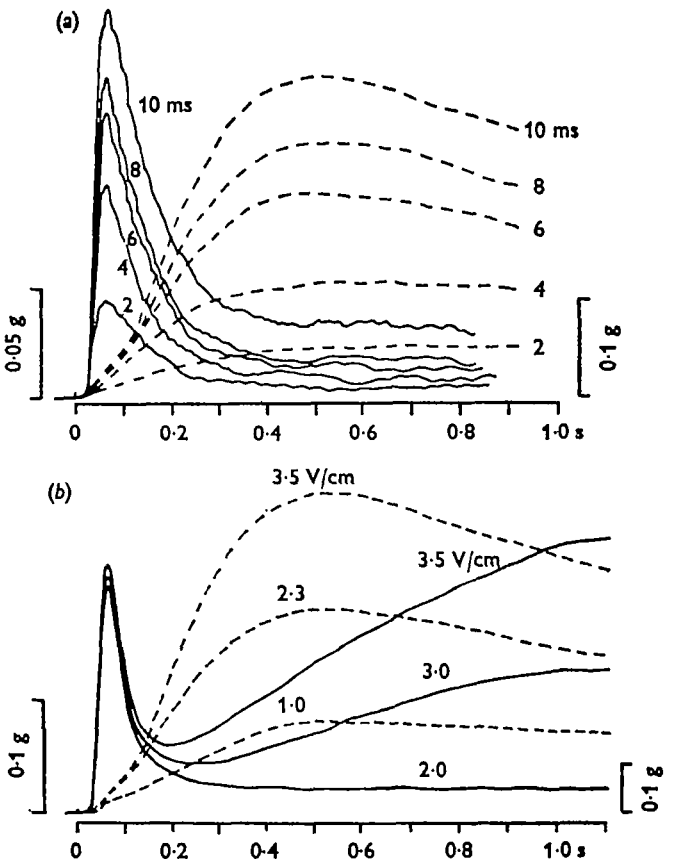

Fig. 2. Superimposed records of tension development in the longitudinal (continuous lines) and circular muscles (broken lines). (*a*) Responses to current pulses of various durations (2–10 ms) at constant intensity (2·2 V/cm). (*b*) Responses to current pulses of various intensities (1·0–3·5 V/cm) at constant duration (10 ms). Left bar: tension calibration for longitudinal muscle and right bar for circular muscle.

Tension development recorded from two different preparations of the circular muscle is shown in the lower two records (*c*, *d*) of Fig. 1. The rate of rise of tension response in the circular muscle was slower than that of the phasic tension in the longitudinal muscle. The time to peak tension was 300–500 ms. The circular muscle

slowly relaxed and did not usually show clear phasic and tonic components. In some preparations, however, the relaxation appeared to consist of two phases as seen in the record (*c*).

Superimposed tracings in Fig. 2*a* illustrate the tension developments in the longitudinal and circular muscles elicited by current pulses of various durations (2–10 ms) at a constant intensity (2·2 V/cm). The degree of tension development increased roughly in proportion to the stimulus duration both in the longitudinal and circular muscles. Fig. 2*b* shows superimposed tracings of tensions produced by electrical stimulation with various intensities (1–3·5 V/cm) at a constant duration (10 ms). The phasic contraction was already maximum at an intensity at which the tonic contraction was still small.

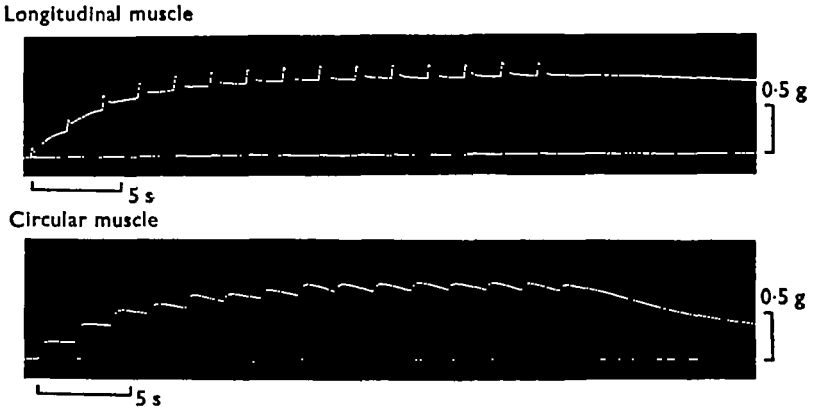

Fig. 3. Tension development of longitudinal and circular muscle elicited by successive stimuli (0·5 c/s in frequency). For details see text.

Fig. 3 shows the tension responses produced by successive stimuli (0·5 c/s in frequency). In the longitudinal muscles each tonic contraction summed up to a certain level and then remained constant, whereas a separate phasic contraction was elicited by each stimulus (upper record). The tension of the circular muscle also summed as in the longitudinal muscle, but began to relax soon after cessation of stimulation (lower record).

Effects of removal of external sodium ions

Fig. 4 shows tension development of the longitudinal muscle after 15 min exposure to sodium-free solution (NaCl was substituted with LiCl, tris-Cl or glucose), elicited by two stimuli applied at an interval of 5 s. According to the previous report (Hidaka, *et al.* 1969), both the phasic and the tonic tensions were enhanced. In the present experiments, the phasic contraction was potentiated, except in lithium solution. However, the tonic contraction was reduced in sodium-free solutions.

Fig. 5 shows that the tonic contraction disappeared completely 1 h after immersion in sodium-free (tris) solution, while the phasic contraction was enhanced. It is of interest to study how the magnitude of the contraction is related to external sodium concentrations. In Fig. 6, the magnitudes of the phasic and tonic contractions of a longitudinal muscle are plotted against sodium concentrations on a logarithmic scale. The responses were elicited by electrical stimulation applied every 5 min between

and 45 min after immersion in each test solution. After control responses had been recorded in normal solution (140 mM-Na), sodium concentrations were reduced in steps by replacement with tris-Cl.

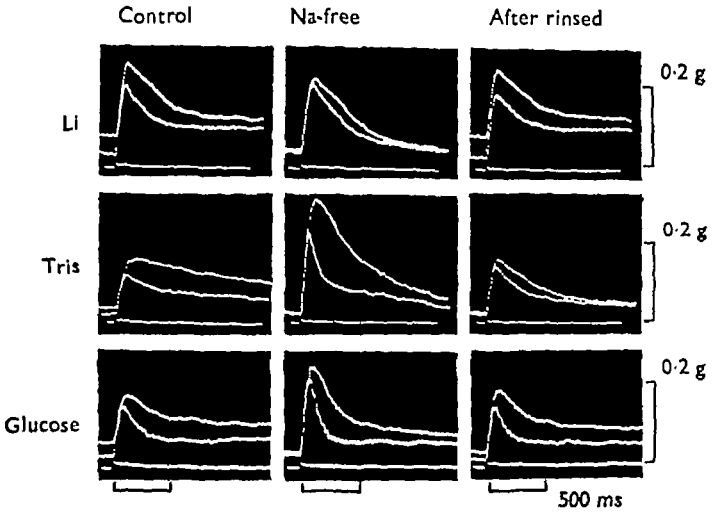

Fig. 4. Effects of LiCl, tris-Cl and glucose substituted for NaCl on tension development of longitudinal muscle. Paired pulses (10 ms and 2·0 V/cm) applied at interval of 5 s. Note tonic contraction reduced in sodium-free solution.

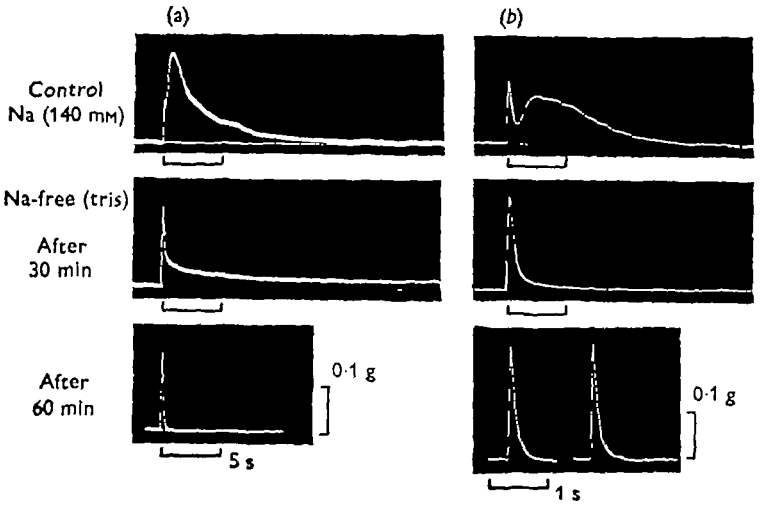

Fig. 5. Tension development of two different preparations (*a*, *b*) of longitudinal muscle in sodium-free (tris) solution. Note potentiation of phasic contraction and abolition of tonic contraction.

There were fluctuations in the magnitude of the phasic and tonic contractions in the normal solution. However, in low-sodium solutions the phasic contraction (empty circles) had a tendency to increase and the tonic contraction (filled circles) to decrease on successive stimuli. Potentiation of the phasic contraction was roughly inversely proportional to the logarithm of external sodium concentration. Reduction of the tonic

contraction was less dependent on the external sodium concentrations at more than 9 mM, but in sodium-free solution no tonic contraction was observed. This suggests that a small amount of external sodium is necessary to maintain the tonic contraction The effect of removal of sodium ions was reversible.

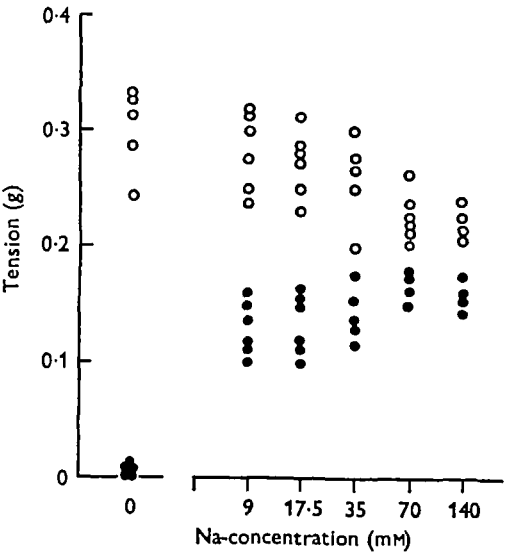

Fig. 6. Effects of varying external sodium concentration on tension development of the longitudinal muscle. Each point indicates the magnitude of phasic (○) and tonic (●) contraction elicited by electrical stimulation every 5 min during 20–45 min after immersion in test solution.

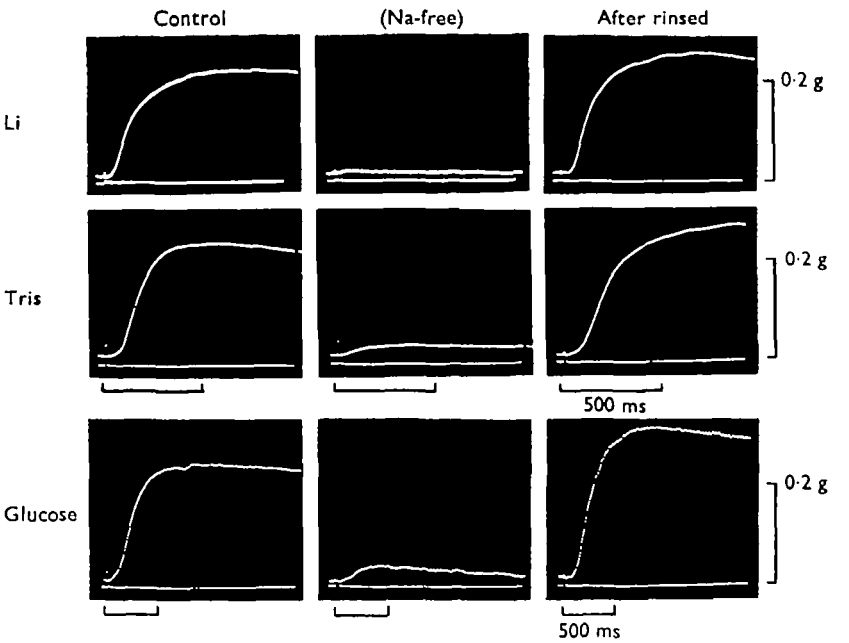

Fig. 7. Effects of LiCl, tris-Cl and glucose substituted for NaCl on tension development in circular muscle. Responses in sodium-free solution were obtained 20–30 min after immersion.

Tension development of the circular muscle was nearly abolished 10 min after immersion in sodium-free (Li, tris and glucose) solutions but a small contraction usually remained, as shown in Fig. 7. Replacement of sodium with lithium was the most effective in suppressing the contraction. These effects were also reversible.

Tetrodotoxin (TTX, 3×10^{-6} M) had no effect on either the phasic or the tonic contractions in the longitudinal muscle, whereas the tension development of the circular muscle was reduced markedly by TTX (Fig. 8), as observed in frog muscle fibres which generate the sodium spike (Narahashi *et al.* 1960). The effects of TTX were very similar to removal of Na ions.

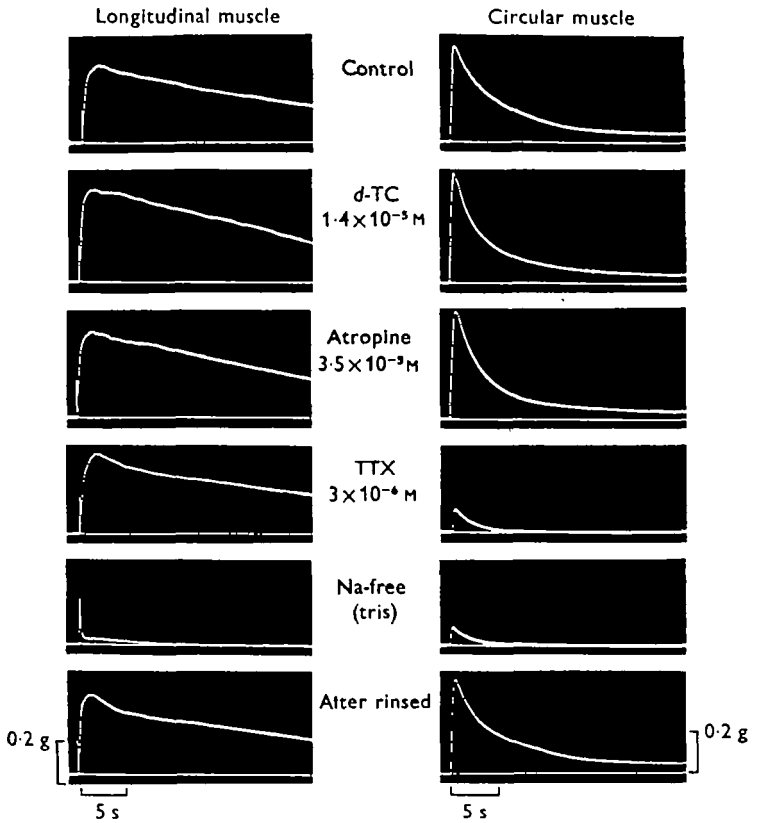

Fig. 8. Effects of *d*-tubocurarine (*d*-TC), atropine, tetrodotoxin (TTX) and sodium-free solution (substituted with tris) on the tension development in longitudinal and circular strips. Each tension development was measured 30 min after immersion in each test solution. The solutions were replaced with the new test solution successively in the order shown.

Substitution of the external Cl ions with *d*-glutamate did not modify the contraction and relaxation in the longitudinal or in the circular muscle. This confirms a previous observation (Hidaka *et al.* 1969).

In muscles immersed in sodium-free solution the resting tension gradually increased after about 15 min, and reached a maximum after about 25 min. Repetitive stimulation speeded up the increase in resting tension. During the next half hour in sodium-free solution, the muscle spontaneously relaxed, except in the lithium solution, in which no

relaxation was observed. Similarly no relaxation occurred in muscles treated with ouabain (unpublished observation). The increase in resting tension was not observed in solutions containing more than 9 mM sodium.

Effects of d-*tubocurarine and atropine*

In order to examine the possibility that nervous elements in the muscles are involved in the contraction, cholinergic blocking agents were used. Fig. 8 also shows the effects of *d*-tubocurarine and of atropine on tension development of the longitudinal and circular muscles. Neither atropine ($3{\cdot}5 \times 10^{-5}$ M) nor *d*-tubocurarine ($1{\cdot}4 \times 10^{-5}$ M) had an effect on the phasic and tonic contractions of the longitudinal muscle, or on the contraction of the circular muscle.

DISCUSSION

Contractions observed in the present experiments are probably not mediated by nervous elements but caused by direct stimulation of the muscle membrane. Hidaka *et al.* (1969*b*) demonstrated that *d*-tubocurarine (10^{-6} g/ml) completely blocked the neuromuscular transmission. However, contractions in the longitudinal and the circular muscle are not affected by *d*-tubocurarine. Furthermore, TTX (10^{-6} g/ml) had no effect on contractions in the longitudinal muscle, in spite of its blocking action on peripheral nerves (Hidaka, Ito & Kuriyama, 1969). Although the neuromuscular transmitter at the circular muscle is not known, the suppressive action of TTX and of removal of external sodium ions on contraction is likely to be exerted directly on the muscle membrane. Current pulses of more than 1 ms in duration are usually necessary to elicit the contraction, suggesting direct stimulation of the muscle.

The thickness of the circular muscle layer is only $\frac{1}{6} - \frac{1}{8}$ of the thickness of the longitudinal muscle layer (H. Nishihara, personal communication), and the preparation of the circular strip contains longitudinal muscle fibres. Therefore, there is the possibility that the tension obtained from the circular strip results not only from the circular muscle but also partly from the longitudinal muscle, which could produce tension at an angle to the fibre axis due to shearing of the sets of myofilaments which form the obliquely striated system. This is very unlikely, however, because there is no close relationship between the time courses of contraction of the longitudinal and circular strips. Furthermore, their responses are affected differently by various solutions, or by drugs. The assumption that tension from the circular strip represents the response of circular muscle and that tension from the longitudinal strip is caused by the longitudinal muscle seems to be valid.

It is well established that the spike in the longitudinal muscle of the earthworm is due to inward fluxes of calcium ions through the membrane, because the spike can be elicited in sodium-free solution or in the presence of TTX, and is strongly dependent on the external calcium concentration (Hidaka, Ito & Kuriyama, 1969; Hidaka *et al.* 1969*a*, *b*; Ito, Kuriyama & Tashiro, 1969*a*, *b*, 1970). The findings that the phasic contraction of the longitudinal muscle is observed in sodium-free solution and is not affected by TTX, suggest that the phasic contraction is triggered by a spike which is caused by calcium influx. The graded response in contraction may be a result of an increase in the number of active fibres. The tonic contraction in the longitudinal

muscle has quite different properties from the phasic contraction and will be described in the following paper (Tashiro & Yamamoto, 1971).

The internal fine structure of the circular muscle fibre is very similar to that of the longitudinal muscle fibres (H. Nishihara, personal communication). However, their mechanical responses are different in many respects. The contraction in the circular muscle is nearly abolished in sodium-free solution and also in the presence of TTX. Therefore, it may be speculated that sodium-dependent spikes precede the onset of the mechanical response in this muscle.

SUMMARY

1. The mechanical properties of the longitudinal and circular muscles of the earthworm, *Pheretima communissima*, were studied in various solutions.

2. In the longitudinal muscle, field stimulation elicited two distinct waves of tension development, i.e. phasic and tonic contractions. But in the circular muscle, these components were not distinguishable.

3. The phasic contraction in the longitudinal muscle increased in sodium-free (tris) solution while the tonic contraction was abolished. Neither the phasic nor the tonic contraction, however, was influenced by tetrodotoxin (3×10^{-6} M), *d*-tubocurarine ($1{\cdot}4 \times 10^{-5}$ M), or atropine ($3{\cdot}5 \times 10^{-5}$ M).

4. The contraction in the circular muscle was suppressed in sodium-free solution and also by tetrodotoxin (3×10^{-6} M), but was not affected by *d*-tubocurarine ($1{\cdot}4 \times 10^{-5}$ M) or by atropine ($3{\cdot}5 \times 10^{-5}$ M).

5. It is speculated that the phasic contraction of the longitudinal muscle is triggered by a calcium spike, and the contraction in the circular muscle is preceded by a sodium spike in muscle fibres.

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