THE PHASIC AND TONIC CONTRACTION IN THE LONGITUDINAL MUSCLE OF THE EARTHWORM

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INTRODUCTION

In the preceding paper (Tashiro, 1971) it was reported that the mechanical response of the longitudinal muscle of the earthworm to electrical stimulation consisted of two distinct phases: the phasic and tonic contractions. The phasic contraction was enhanced in sodium-free solution, while the tonic contraction was abolished.

The tonic contraction is characterized by a prolonged maintenance of tension, which is similar to the 'catch' contraction in the anterior byssal retractor muscle (ABRM) of *Mytilus edulis*. The mechanical properties of the ABRM have been studied extensively by Jewell (1959), Johnson & Twarog (1960), Lowy & Hanson (1962), Lowy, Millman & Hanson (1964), Rüegg (1964), Nauss & Davies (1966) and Twarog (1967b). There are two hypotheses for the 'catch' mechanism. One is the 'parallel hypothesis' and proposes that the tension maintenance during the tonic contraction results from interaction between paramyosin molecules without involvement of the contractile actomyosin system (Johnson, 1962; Rüegg, 1964); the other, the 'linkage hypothesis', proposed that the actomyosin cross linkages break at a very slow rate during the tonic contraction (Lowy et al. 1964).

From the ultrastructural point of view, Ikemoto (1963) has suggested that the contraction of the muscle fibres of the earthworm body wall takes place in two stages. The first stage corresponds to the contraction of cross-striated muscles, and the second stage, bending of thick filaments, seems to be carried out very slowly because a considerable time would be required for the bending and for the drift between the neighbouring sets of myofilaments.

The main purpose of the present experiments was to find out whether Ikemoto's hypothesis can explain the mechanical properties of the longitudinal muscle, or whether the 'catch' mechanism is a better explanation for the tonic contraction in the earthworm muscle.

METHODS

The preparation, the experimental procedure, and the solutions used in the present experiments were the same as described in the preceding paper (Tashiro, 1971). Electrical field stimulation was applied longitudinally to elicit the contraction. Its duration was between 5 and 20 ms, and its intensity was 1–4 V/cm expressed as the potential gradient along the tissue.

$MnCl_2$, $CoCl_2$ and $NiCl_2$ were simply added to the normal solution. The concentra-

tion of the salts was usually below 5 mM so that the change in tonicity can be neglecte▇
When divalent cations of more than 5 mM were used, the corresponding concentration
of NaCl in the solution was reduced. Drugs used were 5-hydroxytryptamine (5-HT),
acetylcholine (ACh) and γ-aminobutyric acid (GABA).

The redevelopment of tension after a quick release at various phases of the contrac-
tion was studied by means of Ritchie's method (Ritchie, 1954).

The experiments were carried out during winter time (November to March) and at
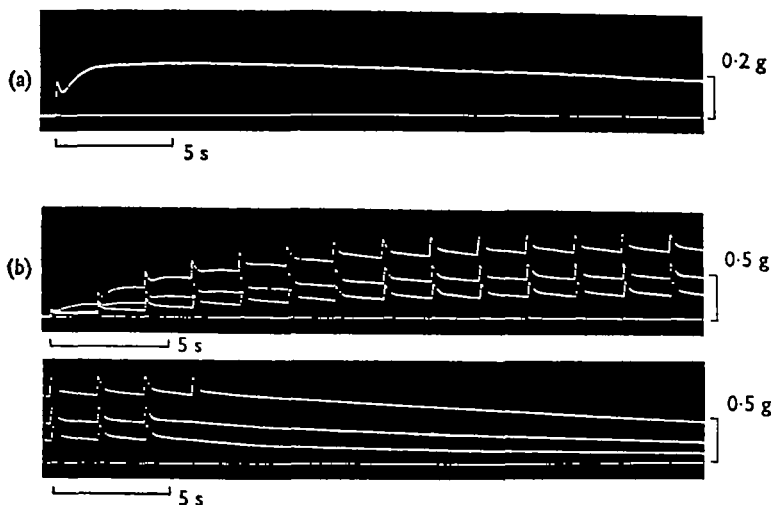room temperature (20–23 °C).

Fig. 1. Tension development in the longitudinal muscle of the earthworm. (a) The phasic and
tonic contraction elicited by a current pulse (10 ms, 3·2 V/cm). (b) Superimposed records of
tension responses produced by repetitive stimulation with current pulse (10 ms) of three dif-
ferent intensities (2·0, 2·5, 3·2 V/cm) at frequency of 0·5 c/s. The bottom records were
continuous with the upper ones. (a, b) Different preparations.

RESULTS

Characteristics of tension development produced by electrical stimulation

Longitudinally applied current pulses elicited two waves of contraction, the phasic
and tonic contractions, in the longitudinal (obliquely striated) muscle of the earthworm.
Fig. 1a illustrates characteristic tension development of the longitudinal muscle
elicited by a current pulse of 10 ms in duration and 3·2 V/cm in intensity. Fig. 1b
shows superimposed records of tension responses produced by repetitive stimulation
with current pulses of constant duration (10 ms) and three different intensities (2·0,
2·5, 3·2 V/cm) at constant frequency (0·5 c/s). The tension of the tonic contraction
elicited by repetitive stimulation increased gradually and finally reached a plateau, on
which the phasic contraction appeared in response to each stimulus. As the intensity
of current pulses was increased, the summed tonic tension increased several times
while the phasic tension did not increase so markedly. After the cessation of stimula-
tion the tonic contraction decayed very slowly with a half-time of relaxation of 25–55 s.

Fig. 2 illustrates the relationship between the peak tension of the phasic and tonic

ntractions and the intensity of current pulse at various current durations (5–20 ms). The phasic contraction increased with increasing stimulus intensity up to a certain magnitude. The tonic contraction increased gradually at first then steeply beyond a critical intensity. This finding indicates that longer duration or higher intensity of current pulse is needed to elicit the tonic contraction compared with the phasic contraction.
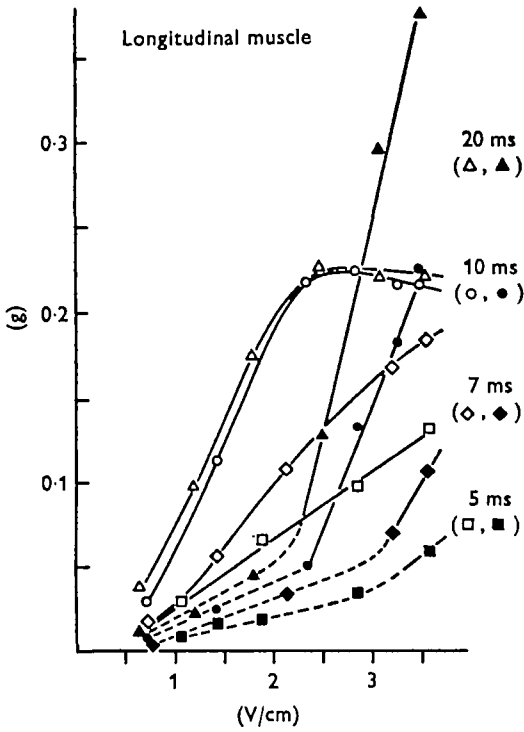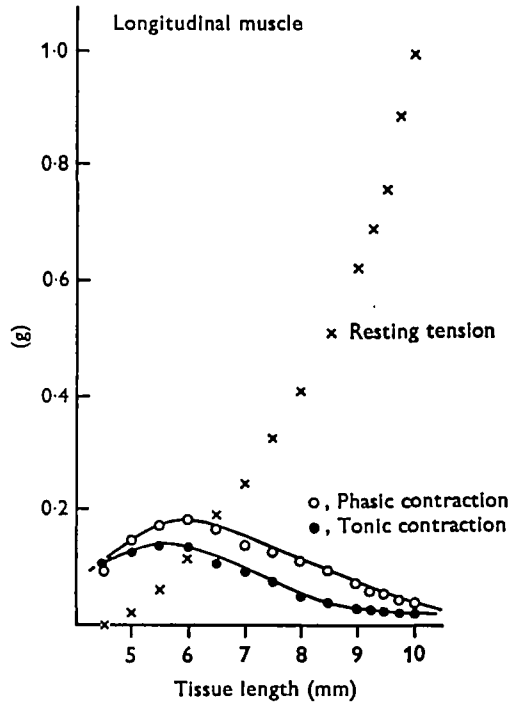

Fig. 2   Fig. 3

Fig. 2. Relationship between peak tension of phasic (open) and tonic (filled) contraction and intensity of current at constant duration (5–20 ms). Results were obtained from the same preparation.

Fig. 3. Length–tension curve. Phasic (○) and tonic (●) contractions elicited by current pulses (10 ms, 3·5 V/cm), were measured at various tissue lengths. Resting tensions (×) were measured after the relaxation had been completed.

Length–tension curve

Twarog (1967*a*) observed in the ABRM of *Mytilus* that the 'catch' tension increased to a maximum with increasing muscle length. Fig. 3 shows the relationship between the tissue length and tension developed in the earthworm muscle. The phasic and tonic contractions were elicited by current pulses (10 ms in duration and 3·5 V/cm in intensity) and were measured at various tissue lengths after the stress relaxation had finished. Both the phasic and the tonic contraction were affected similarly by length changes, as observed in *Mytilus* muscle. The length of the preparation could be extended by stretch to more than twice the resting length at zero tension.

'Active state' of the phasic and tonic contractions

The method of Ritchie (1954) was employed to follow the decay of the active state. The redevelopment of tension after a quick release is illustrated in Fig. 4. The preparation was approximately 15 mm in length and 3 mm in width. The decay of the active state obtained during the phasic contraction resembled that in other muscles such as the frog sartorius and semitendinosus (Ritchie, 1954; Mashima, 1967), and the papillary muscle of the cat (Abbott & Mommaerts, 1959). When the release was carried out just after the peak of the phasic tension, the redevelopment of tension appeared with a long latency as seen in the curve 3–3′ in the bottom tracings in Fig. 4. During the early phase of the tonic contraction the tension lost by a quick release was redeveloped, indicating that this phase was in the 'active state'. However, the active state decreased after 30 s to a very small level, although the tonic contraction lasted for more than 1 min.
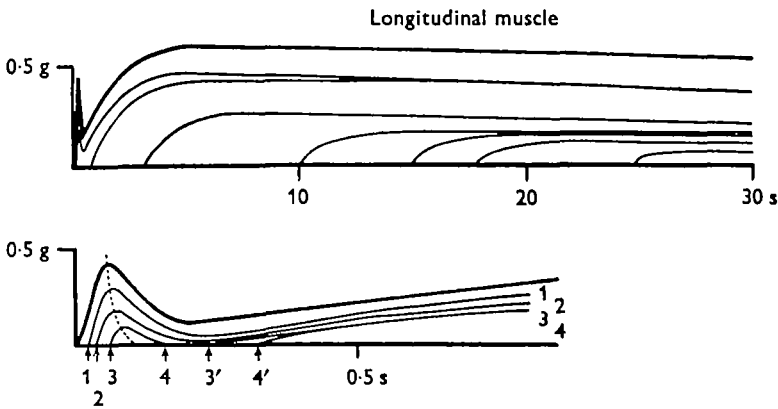

Fig. 4. Superimposed records of tension redevelopment after quick releases made during phasic and tonic contractions. Thick uppermost lines of upper and lower traces show the normal contraction. Upper traces show redevelopment of tension during tonic contractions on slow time base, and lower traces redevelopment of tension during phasic contraction and earlier phase of tonic contraction on fast time base.

Effects of temperature and 5-hydroxytryptamine (5-HT)

Fig. 5 shows the effect of changes in temperature and of 5-HT (2.5×10^{-6} M) on the phasic and tonic contractions. Current pulses (3.5 V/cm, 10 ms) elicited the phasic and tonic contractions at room temperature (20 °C). The tonic contraction was dependent on temperature, as observed in the 'catch' tension of the ABRM (Johnson, 1966; Twarog, 1967b). The tonic contraction disappeared at 30 °C, while it increased with lowering temperature from 20 to 10 or 5 °C. The rates of rise and fall of the phasic contraction were proportional to temperature, but the magnitude of the phasic contraction was the largest at 10–15 °C. Although the excitability of the membrane may be affected by temperature, it is possible that the stiffness of the series elastic element is temperature dependent, affecting the velocity of shortening as observed in other tissues (Jewell & Welkie, 1958; Abbot & Mommaerts, 1959; Yeatman, Parmley & Sonnenblick, 1969).

At a temperature of 20 °C, 5-HT (2.5×10^{-6} M) abolished the tonic contraction

cited by a 5 ms current pulse, and reduced the magnitude of the phasic tension (Fig. 5). However, by increasing the duration of the current pulse to more than 20 ms the tonic contraction was restored to some extent, and the magnitude of the phasic contraction was also regained.

Effects of acetylcholine (ACh) and γ-aminobutyric acid (GABA)

It has already been shown that in the earthworm ACh is released from the excitatory nerve terminal (Hidaka *et al.* 1969*b*; Ito, Kuriyama & Tashiro, 1969*a*, *b*). When the preparation was immersed in a solution containing $5 \cdot 5 \times 10^{-4}$ M ACh, a sustained (tonic) contracture was produced after a transient (phasic) contraction. A phasic
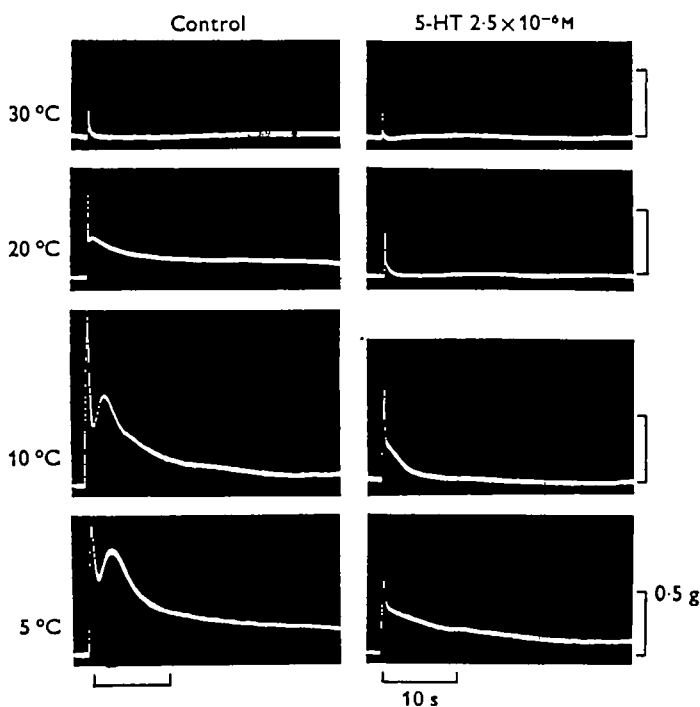

Fig. 5. Effects of 5-HT on the tension at various temperatures. Intensity and duration of current pulses were constant ($3 \cdot 0$ V/cm, 5 ms). See text for details.

contraction elicited by electrical stimulation ($3 \cdot 5$ V, 10 ms) appeared upon the sustained contracture, but no tonic contraction was produced even by a strong stimulating current pulse ($5 \cdot 0$ V/cm, 20 ms). Fig. 6 shows the typical features of the effects of various concentrations of ACh on the tension development elicited by electrical stimulation.

Fig. 7 shows the effects of various concentrations of ACh on tension development of the longitudinal muscle elicited by electrical stimulation ($3 \cdot 5$ V/cm, 10 ms). The points are the values recorded every 5 min between 20 and 40 min after the preparation was immersed in the test solution. The absolute tension of the phasic contraction was roughly constant and independent of ACh concentrations (2×10^{-5} to 5×10^{-4} M),

although the magnitude of the phasic contraction measured from the resting tension decreased at a high concentration of ACh (more than 1.1×10^{-4} M) because of an increase in the resting tension. The tonic contraction was reduced by ACh as shown in Fig. 7 (filled circles). These phenomena were reversible.

Hidaka et al. (1969 b) and Ito et al. (1969 b) demonstrated that GABA is an inhibitory chemical transmitter and selectively increases the chloride conductance in the earthworm somatic muscle, as observed in other tissues (Hoyle, 1955; Hoyle & Wiersma, 1958; Cerf et al. 1959; Kravitz, Kuffler & Potter, 1963; Usherwood & Grundfest, 1965; Takeuchi & Takeuchi, 1965, 1966). GABA (10^{-6} to 5×10^{-4} M) had no effect on the phasic or tonic contractions of somatic longitudinal muscle of the earthworm.
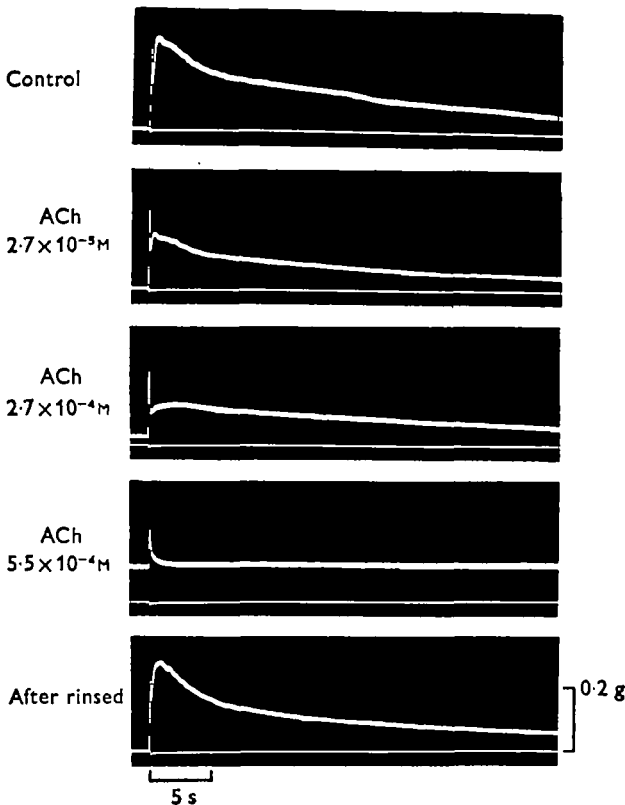

Fig. 6. Phasic and tonic contractions elicited by current pulses (3·5 V/cm, 10 ms) in solutions containing various concentrations of acetylcholine (ACh).

Effects of the divalent cations of nickel, cobalt and manganese

As reported in a previous paper (Ito, Kuriyama & Tashiro, 1970) the spike in the earthworm somatic muscle is suppressed by divalent transition metal ions, cobalt and manganese, in sodium-free solution. Fig. 8 shows comparison of suppressing effects of these divalent cations on the tension development. These phenomena were reversible. The magnitude of the effect produced at the same concentration (2 mM) was in the order of manganese > cobalt > nickel. At a low concentration (0·5–1·0 mM) of nickel, the tonic contraction was usually slightly potentiated.

In Fig. 9, the relationships between tension and external concentration of divalent ions are plotted both for the tonic and for the phasic contraction. The phasic contraction was suppressed in proportion to the logarithm of concentration of added divalent cations, while the tonic contraction was reduced steeply after the phasic contraction was nearly abolished.
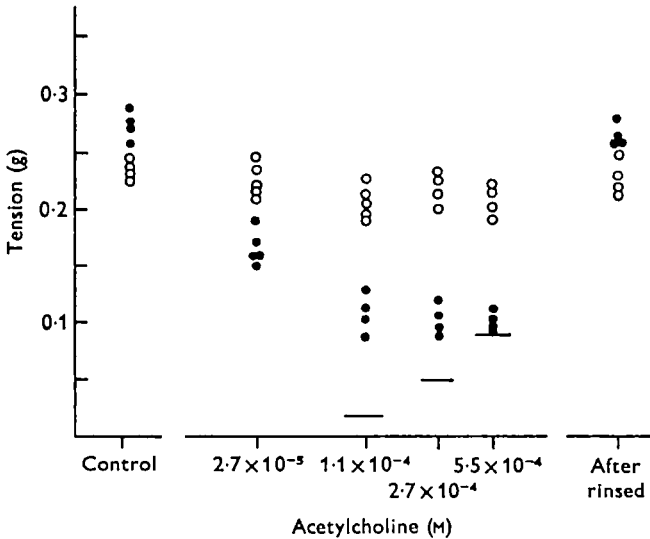

Fig. 7. Effects of various concentrations of ACh on tension developed. Each point indicates peak tension of phasic (O) and tonic (●) contractions. These were elicited by electrical stimulation every 5 min between 20 and 40 min after the preparation was immersed in test solution. Horizontal bars show magnitude of contracture produced by ACh.
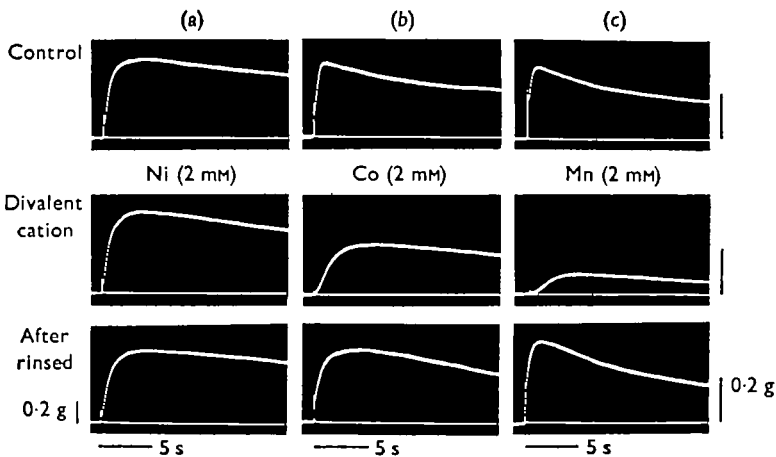

Fig. 8. Effects of 2 mM concentration of transition-metal ions (Mn^{2+}, Co^{2+}, Ni^{2+}) on the tension. Records were taken 30 min after transition-metal ions had been added.
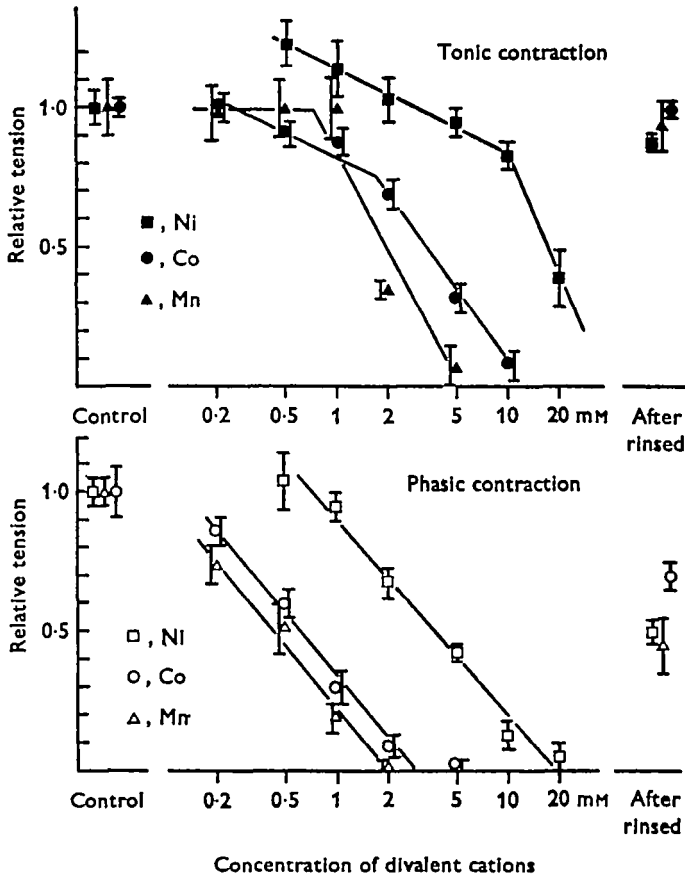
Fig. 9. Effects of transition-metal ions (Mn²⁺, Co²⁺, Ni²⁺) on tonic contraction (top) and phasic contraction (bottom). Vertical bars show the standard deviation. Values taken between 15 and 45 min after muscle was immersed in each test solution.

DISCUSSION

Electronmicroscopic studies of somatic (obliquely striated) muscles of the earthworm and *Ascaris* have shown that the contractile apparatus of these muscles consists of thick and thin filaments, but the alignment is staggered, so that sarcomeres run obliquely with respect to the fibre axis (Hanson, 1957; Kawaguti & Ikemoto, 1959; Ikemoto, 1963; Heumann & Zebe, 1967; Nishihara, 1967; Rosenbluth, 1967). It has been considered that in the earthworm there are probably two mechanisms of length change during contraction: the thin filaments slide between the thick filaments, as occurs in cross-striated muscles, and in addition bending of the thick filaments occurs as a result of shortening of the thin filaments. The contraction of these muscle fibres, therefore, may take place in two stages: the first stage corresponds to the contraction of the cross-striated muscle, and the second stage, the bending of the thick filaments, is carried out very slowly.

As demonstrated in the preceding paper (Tashiro, 1971) phasic and tonic contractions are observed in response to electrical stimulation. The tonic contraction starts

during the falling phase of the phasic contraction and relaxes gradually over a few minutes.

The properties of these two contractions differ in other respects besides the differences in time course. (i) Stronger and longer stimulation is necessary to elicit the tonic than the phasic contraction. (ii) The tonic contraction is easily fused after summation by repetitive stimulation, but the phasic contraction appears on the tonic contraction at each stimulus. (iii) In sodium-free solution the phasic contraction is enhanced whereas the tonic contraction is abolished (Tashiro, 1971). (iv) At 30 °C the tonic contraction is abolished, but the phasic contraction still appears. (v) 5-HT and ACh suppress the tonic more than the phasic contraction. These observations suggest that the underlying mechanisms for these contractions are different. However, the fact that the transition-metal ions abolish only the phasic contraction without greatly affecting the tonic contraction does not support Ikemoto's hypothesis (Ikemoto, 1963). If the tonic contraction were due to the shearing effect of obliquely arranged myofilaments, the phasic contraction would be a prerequisite for the tonic contraction.

The tonic contraction is qualitatively similar to the 'catch' contraction in the ABRM of *Mytilus*. The tonic tension development in the earthworm muscle lasts much longer than the 'active state'. In the ABRM the 'catch' tension persists after the 'active state' has ceased (Jewell, 1959; Johnson & Twarog, 1960). Furthermore, the tonic contraction in the earthworm and the 'catch' contraction in the ABRM are both suppressed by 5-HT and by raising the temperature to 30 °C.

It is now generally believed that an action potential precedes and initiates the mechanical response, and that calcium ions play an essential part in muscle contraction (cf. Sandow, 1965). It has already been demonstrated in the earthworm somatic muscle that the spike is caused mainly by an inward movement of calcium ions (Hidaka *et al.* 1969*a, b*; Ito *et al.* 1970). The spike in the longitudinal muscle can be observed in sodium-free solution and in the presence of tetrodotoxin, but it is suppressed by transition-metal ions, such as manganese and cobalt. In the presence of ACh (5.5×10^{-4} M) and of 5-HT (2.5×10^{-6} M), and at a temperature of 30 °C, the spike can be elicited (unpublished observation). Thus, under all conditions in which the phasic contraction is produced, the spike also occurs. It may therefore be concluded that the phasic contraction is triggered by the spike in the longitudinal somatic muscle, supporting the previous assumption (Tashiro, 1971).

It is postulated by many investigators that there are two different calcium-binding sites in skeletal and smooth muscle fibres: a superficial site (binds calcium loosely) and a sequestered site (binds calcium tightly) (Durbin & Jenkinson, 1961; Frank, 1963; Edman & Schild, 1962; Daniel, 1965; Hinke, 1965; van Breemen & Daniel, 1966; Hurwitz, von Hagen & Joiner, 1967). Excess potassium ions release the superficial calcium, and ACh releases the sequestered calcium.

In the earthworm muscle it is also possible that there are two sites of calcium storage, and that the phasic contraction is produced by calcium ions carried from the outside and probably also released from the superficial site and the tonic contractions by calcium ions from the sequestered site. It is difficult to locate the sites in the structures, but the membrane may be the superficial site and the sarcoplasmic reticulum the sequestered site. The spike carries calcium inward and may release calcium transiently from the membrane causing the phasic contraction, and also from the reticulum with a

slow time course, causing the tonic contraction. The release of calcium from, or uptake into, the reticulum is affected by various conditions, such as sodium-free saline, high temperature or 5-HT, modifying the tonic contraction.

SUMMARY

1. In extension of preceding studies on the mechanical properties of the longitudinal muscle fibres of the earthworm, the phasic and tonic contractions were analysed under various conditions.

2. The phasic contractions appeared on the tonic contraction which was maintained at a steady level by repetitive stimulation, and the tonic contraction decayed very slowly after cessation of stimulation.

3. The length–tension curve indicated that the phasic and tonic contractions were both affected similarly by changing the muscle length.

4. The mechanical 'active state' was investigated during the phasic and tonic contractions by means of the quick-release method. During the phasic contraction the ability to redevelop the tension was observed. During the early phase of the tonic contraction the muscle was in the 'active state', but the tonic contraction continued even after the tension redevelopment had nearly ceased.

5. The tonic contraction was absent when the temperature was raised to about 30 °C. 5-Hydroxytryptamine (2.5×10^{-6} M) decreased the tonic more than the phasic contraction. The tonic contraction disappeared completely in the presence of acetylcholine (5.5×10^{-4} M), while the phasic contraction was reduced but not abolished. γ-aminobutyric acid (10^{-6} to 5×10^{-4} M) had no effect on the contractions.

6. Transition-metal ions suppressed mainly the phasic contraction in the order of $Mn^{2+} > Co^{2+} > Ni^{2+}$. The decrease in the phasic contraction was proportional to the logarithm of concentration of added divalent cations. The tonic contraction was also reduced but at higher concentration than the phasic contraction.

7. It is postulated that, by depolarization produced by electrical stimulation or by acetylcholine, calcium ions are released from the plasma membrane and also from the sarcoplasmic reticulum. The phasic contraction might be generated mainly by calcium ions released from the membrane, and the tonic contraction might be caused mainly by calcium ions released from the sarcoplasmic reticulum.

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