EFFECT OF MECHANICAL VIBRATION ON ACTIVE TENSION IN THE LONGITUDINAL RETRACTOR MUSCLE OF A SEA CUCUMBER STICHOPUS JAPONICUS

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Summary

1. The effect of mechanical vibration on active tension in an echinoderm somatic smooth muscle was studied using the longitudinal retractor muscle (LRM) of a sea cucumber Stichopus japonicus.

2. The steady contracture tension in LRM fibres maximally activated with 10^{-3} mol l^{-1} acetylcholine (ACh) was reduced by vibrations (peak-to-peak amplitude, 0.5–2.5 % of \( l_0 \), where \( l_0 \) is the slack length of the muscle; frequency, 5–100 Hz). The extent of reduction of active contracture tension increased with increasing amplitude of vibration, but it did not change appreciably with increasing frequency of vibration.

3. The steady contracture tension in LRM fibres submaximally activated with 10^{-5} mol l^{-1} ACh was more markedly reduced by vibrations than was that in maximally activated fibres.

4. The vibration-induced reduction of active contracture tension disappeared when temperature was lowered from 20–23 to 0 °C.

5. The development of contracture tension in LRM fibres activated with ACh was not affected by mechanical vibration.

6. These results are discussed in connection with the vibration-induced decrease in the rate of breakage of the actin–myosin linkages responsible for isometric force generation.

Introduction

It is known that, when an isometrically tetanized vertebrate skeletal muscle is subjected to mechanical vibration, the mean tension of the corresponding tension oscillation is below the level of isometric tension before the application of vibration, although the mean muscle length is the same as the isometric muscle length (Buchthal and Kaiser, 1951; Matthews, 1966, Joyce et al. 1969). This reduction of the mean tension during vibration may be explained in terms of the Huxley contraction model (Huxley, 1957), in which tension in a muscle is generated by the alternate formation and breakage of cross-linkages between actin and myosin; the vibration-induced sliding of myofilaments increases the rate of breakage of actin–myosin linkages and decreases the total number of

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actin–myosin linkages responsible for isometric force generation. Vibration-induced reduction of mean active tension has also been observed in mammalian cardiac muscle (Vukas et al. 1978), mammalian vascular smooth muscle (Ljung and Sivertsson, 1972, 1975; Ljung and Hallgren, 1975; Klemt et al. 1981) and molluscan somatic smooth muscle (Ljung and Hallgren, 1975; Kobayashi et al. 1985), and these results have also been explained in terms of the direct action of vibration on actin–myosin linkages.

The present experiments were undertaken to study the effect of vibration on the steady contracture tension produced by acetylcholine (ACh) in an echinoderm somatic smooth muscle, the longitudinal retractor muscle (LRM) of a sea cucumber Stichopus japonicus. This investigation examined whether the vibration-induced reduction of the mean tension below the level of isometric tension was affected (1) by submaximal activation of the LRM fibres, in which the number of actin–myosin links is reduced, and (2) by lowering the temperature, which reduces the cycling rate of actin–myosin linkages.

Materials and methods

Sea cucumbers, Stichopus japonicus, with a relaxed body length of 20–25 cm were collected at the Misaki Marine Biological Station and kept in aerated sea water at 16–18 °C for 10 days prior to experiments. They were identified by the Biological Station staff members. The longitudinal retractor muscle (LRM) was carefully isolated from the inner surface of the body wall. All experiments were performed with a small strip of the LRM (0.5–1 mm in diameter). A pair of stainless-steel wire connectors (0.2 mm in diameter and 3 mm in length; Sugi, 1972) was tied to both ends of the preparation with braided silk thread. The preparation was mounted horizontally at its slack length (l₀, 6.5–8 mm) in an experimental chamber (2 ml) filled with the experimental solution (artificial sea water), which had the following composition (mmol l⁻¹): NaCl, 497; KCl, 10; CaCl₂, 20; MgCl₂, 52 (pH adjusted to 7.2 with NaHCO₃). One end of the preparation was connected to a tension transducer (AE-801, Aksjeselskapet; compliance 0.1 mm N⁻¹; resonant frequency 5 kHz), while the other end was connected to a servo-motor (G-100PD, General Scanning) which contained a displacement transducer (differential transformer) sensing the position of the motor arm. The compliance of the motor arm at the point of attachment of the preparation was 2.7 mm N⁻¹.

The preparation was first made to contract isometrically by applying acetylcholine (ACh, 10⁻⁵ or 10⁻³ mol l⁻¹), which is known to be effective in producing sustained contracture tension in the LRM (Sugi et al. 1982) and, when the steady contracture tension had developed, sinusoidal mechanical vibrations (peak-to-peak amplitude 0.5–2.5 % of l₀; frequency 5–100 Hz) were applied to the preparation with the servomotor, driven by a power amplifier to which sinusoidal voltages were sent from a waveform generator (model 164, Wavetek). Vibrations were applied either repeatedly at intervals of 20–40 s during a single ACh-induced contracture (see Fig. 1A,B), or only once in each of a series of ACh-induced contractures, with similar results. In some experiments, vibrations were also applied during the whole course of contracture tension development. Experimental data were obtained only from preparations that showed reproducible ACh-induced contracture tension development with steady levels of peak
isometric tension. The preparation was relaxed by washing out ACh and was kept in the relaxed state for 5–10 min before it was reactivated with ACh. The solutions in the chamber were renewed using a water vacuum suction tube. The length and tension changes of the preparation were recorded with a thermal pen recorder (WR3101, Graphtec) or with a digital oscilloscope (type 310, Nicolet).

Most experiments were carried out at room temperature (20–23 °C). In some experiments, the temperature of the experimental solution was lowered to 0 °C with a thermoelectric device (RMT6, Lauda).

Results

General features of the vibration-induced reduction of active tension in the LRM fibres

Fig. 1 shows typical records when sinusoidal mechanical vibrations at a constant frequency and of varying amplitude (Fig. 1A) or at a constant amplitude and of varying frequency (Fig. 1B) were applied on the steady contracture tension in the LRM fibres, which were activated with a supramaximal concentration of ACh ($10^{-3}$ mol l$^{-1}$). On application of vibration, the mean tension of the corresponding tension oscillation decreased to a steady level below the level of isometric force before the application of vibration; after removal of vibration, the tension again increased to a level as high as, or slightly below, the initial isometric force. Fig. 2 shows the dependence of the extent of reduction of active tension below the isometric level on the amplitude of vibration at a constant frequency (Fig. 2A) and on the frequency of vibrations at a constant amplitude (Fig. 2B). The extent of reduction of active contracture tension increased to 25–50 % of the initial steady isometric force when the amplitude of vibration was increased from 0.5 to 2.5 % of $l_0$ (Fig. 2A), but it did not change when the frequency of vibration was increased from 5 to 100 Hz (Fig. 2B). Similar results were obtained from seven other preparations. The vibration-induced reduction of active tension was not noticeable with vibrations below 0.5 % of $l_0$.

To determine whether the state of the contractile system remains unchanged during the period of steady contracture tension generation, small step changes in fibre length (release followed by restretch) were applied repeatedly during a single contracture. As shown in Fig. 1C, the rate of redevelopment of tension following each step decrease in fibre length (2 % of $l_0$) did not change appreciably, irrespective of the time after application of ACh, indicating that the contractile system is in the same active state during the course of steady isometric tension generation.

Enhancement of the vibration-induced reduction of active tension in submaximally activated LRM fibres

In the Huxley contraction model (Huxley, 1957), the magnitude of active isometric tension is determined by the number of actin–myosin linkages involved in force generation. To examine the effect of a decrease in the number of actin–myosin linkages on the vibration-induced reduction of active tension, the LRM fibres were made to contract not only with a supramaximal concentration of ACh ($10^{-3}$ mol l$^{-1}$), but also with a submaximal concentration of ACh ($10^{-5}$ mol l$^{-1}$), producing about 50 % of the
maximum ACh-induced contracture tension (Fig. 3, Sugi et al. 1982). In both cases, vibrations were applied during the period of steady contracture tension generation.

A typical result is shown in Fig. 4. For a given amplitude of vibration (0.5–2.5 % of $l_0$), the extent of the reduction of active tension was much larger when the preparation was submaximally activated (70–80%) than when it was maximally activated (25–50%). This enhancement of the vibration-induced reduction of active tension was also observed in three other preparations.

Effect of low temperature on the vibration-induced reduction of active tension

To study how the vibration-induced reduction of active tension was affected when the cycling rate of actin–myosin interactions in LRM fibres was reduced, experiments in which the temperature of the experimental solution was lowered to 0°C were also carried out. The magnitude of peak contracture tension with $10^{-3}\text{ mol} l^{-1} \text{ ACh}$ at 0°C was about 50% higher than that at 20–23°C. As shown in Fig. 5A, the mean tension during the vibration-induced tension oscillation showed little or no decrease with time and remained at almost the same level as that of the isometric tension before the application of vibration,

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Fig. 1. Effect of sinusoidal vibration on the steady contracture tension in the maximally ACh-activated LRM fibres at 21°C. (A) Reduction of active contracture tension with vibrations at a constant frequency (20 Hz) and of five different peak-to-peak amplitudes (0.5, 1, 1.5, 2 and 2.5 % of $l_0$). (B) Reduction of active contracture tension with vibrations at a constant peak-to-peak amplitude (1.5 % of $l_0$) and of five different frequencies (5, 10, 20, 50 and 100 Hz). (C) Tension changes in response to small step length changes (2 % of $l_0$) applied during the period of steady contracture tension. Records A, B and C were obtained from different preparations. In this and subsequent figures, the upper and lower traces show fibre length and tension changes, respectively.
irrespective of the amplitude and frequency of vibration. The application of small step changes in fibre length (2% of \( l_0 \)) indicated that the rate of tension redevelopment remained the same during the course of ACh-induced contractures at 0°C (Fig. 5B), although the rate of tension redevelopment was reduced in proportion to the initial rate of

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**Fig. 2.** Dependence of the extent of reduction of active tension on the amplitude and the frequency of vibration. The extent of reduction of active tension is expressed by the steady mean tension \( (P) \) relative to the steady isometric tension before application of vibration \( (P_0) \), as illustrated in the inset. (A) Relationship between the extent of reduction of active tension \( (P/P_0) \) and the amplitude of vibration (at a frequency of 20 Hz). (B) Relationship between the extent of reduction of active tension \( (P/P_0) \) and the frequency of vibration (at an amplitude of 1.5% of \( l_0 \)). Data points are obtained from the records shown in Fig. 1.

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**Fig. 3.** Vibration-induced reduction of active contracture tension in LRM fibres maximally activated with \( 10^{-3} \text{ mol l}^{-1} \) ACh (A) and submaximally activated with \( 10^{-5} \text{ mol l}^{-1} \) ACh (B). In both A and B, the LRM fibres were subjected to vibrations at 20 Hz and at five different amplitudes (0.5, 1, 1.5, 2 and 2.5% of \( l_0 \)) at a temperature of 20°C. Records A and B were obtained from the same preparation.
development of contracture tension at 0°C compared with the corresponding values at 20–23°C (compare Fig. 1C). The marked decrease in the extent of the vibration-induced reduction of active tension at low temperature was also observed in four other preparations. The half rise-time of tension redevelopment following the step decrease in fibre length (2% of $l_0$) at 0°C (about 2.5 s) was more than six times longer than that at 20–23°C (less than 0.4 s). Similar results were obtained from three different preparations.
Vibration-induced tension oscillation

Fig. 6A shows the time course of the vibration-induced contracture tension oscillation in maximally activated LRM fibres at room temperature (23 °C). The tension changes were sinusoidal in shape, irrespective of the amplitude and frequency of vibrations used. The tension–length loop was narrow and symmetrical with clockwise rotation (Fig. 6B). These features of the tension changes were the same in submaximally activated fibres. When, however, vibration was applied to the LRM fibres at 0 °C (Fig. 6C), the corresponding tension–length loop became a straight line (Fig. 6D), indicating that there was no phase difference between the length and tension changes.

Effect of vibration on the development of ACh-induced contracture tension

As shown in Fig. 7, the time course of development of ACh-induced contracture tension was not affected by continuous application of vibration (1–2.5 % of \( l_0 \)) starting before the application of ACh and lasting throughout the ACh-induced contracture. This indicates that vibration has no inhibitory action on the ACh-induced activation of the contractile system in the LRM fibres, in contrast with the situation in rat portal vein smooth muscle, in which vibration has an inhibitory action on tension development induced by high K+ concentration or noradrenaline (Klemt et al. 1981).

Discussion

Similarities and differences between the present results and the results obtained from other muscles

The present experiments have shown that a vibration-induced reduction of active tension occurs in the LRM fibres of a sea cucumber Stichopus japonicus, as in other types
of muscle (cat skeletal muscle, Joyce et al. 1969; rabbit papillary muscle, Vukas et al. 1978; rat portal vein, Ljung and Sivertsson, 1972, 1975; Ljung and Hallgren, 1975; Klemt et al. 1981; rabbit thoracic aorta, Ljung and Sivertsson, 1975), indicating that this phenomenon is common to different kinds of smooth muscle and to skeletal muscle. In rat portal vein smooth muscle, the vibration-induced reduction of active tension is independent of the type of stimulation and does not affect the spontaneous electrical activity of muscle fibres (Ljung and Sivertsson, 1972, 1975; Sjöqvist and Ljung, 1980); the time course of tension recovery after removal of vibration is exponential and is not affected by the type of stimulation (Ljung and Sivertsson, 1975; Klemt et al. 1981). In sea cucumber LRM fibres, we observed that vibrations also effectively reduced K⁺-induced contracture tension, although the K⁺-induced contracture tension declined with time after reaching its peak (Sugi et al. 1982) and was therefore unsuitable for systematically studying the effect of vibration. After removal of vibration, the tension returned to the initial level with an exponential time course (Figs 1 and 3). The similarities in these features of the vibration-induced reduction of active tension in several kinds of smooth muscle may be taken as evidence that the vibration-induced reduction of active tension in smooth muscles results from the direct action of vibration on the contractile system, as has been proposed for skeletal muscle (Joyce et al. 1969).

In the present study, the effect of vibration on active tension could be observed clearly with vibrations of 1–2.5 % of \( l_0 \) (Figs 1–4) at 20–23 °C, which is comparable with the report of Joyce et al. (1969), who used vibrations of around 1 % of \( l_0 \). Larger amplitudes of vibration are required to reduce active tension in mammalian cardiac and vascular tissues (Ljung and Sivertsson, 1975; Vukas et al. 1978; Klemt et al. 1981). This variation of vibration amplitude may be because, in these tissues, only part of the applied length change is transmitted to the contractile system. Sea cucumber LRM is composed of smooth muscle fibres running parallel to each other, and no connective tissues are visible in the space between the fibres (Suzuki and Sugi, 1982), reflecting its function in locomotion. It is therefore not surprising to find that the amplitude of vibrations required to reduce active tension in LRM fibres is comparable to that for mammalian skeletal muscle (Joyce et al. 1969).
The reduction of active tension was not affected by the frequency of vibration (Figs 1B and 2B). This observation differs from the more pronounced reduction of active tension in rat portal vein and rabbit thoracic aorta as the frequency of vibration is increased from 5 to 100 Hz (Ljung and Sivertsson, 1975). At present, we have no explanation for this discrepancy. One possibility is that, in their preparations, some passive components, connected in series with the contractile component, might become more compliant with increasing frequency of vibration, thus producing a transient reduction of active tension, as Ljung and Sivertsson (1975) did not use vibrations long enough for the reduced active tension to reach a steady value.

Mechanisms underlying the vibration-induced reduction of active tension

Rack and Westbury (1969) produced smooth and steady isometric tension in cat soleus muscle by subdividing the ventral root and supplying stimulating pulses to different motor units in rotation. They demonstrated that the steady tension in such a submaximally activated muscle is reduced much more markedly with vibration than is the steady tension in a maximally tetanized muscle. As ACh-induced contracture tension is graded according to the concentration of ACh applied (Sugi et al. 1982), we were able to compare the effects of vibration on LRM fibres maximally activated with ACh and those submaximally activated with ACh. In agreement with Rack and Westbury (1969), submaximal ACh-induced contracture tension was reduced much more markedly by vibration than was maximal ACh-induced contracture tension (Figs 3 and 4). As the magnitude of active isometric force is believed to be determined by the number of actin–myosin linkages (Huxley, 1957), these results indicate that, in both skeletal and smooth muscles, vibration reduces the number of actin–myosin linkages responsible for isometric tension generation more markedly in submaximally activated fibres than in maximally activated ones.

If a molluscan somatic smooth muscle (the anterior byssus retractor muscle of *Mytilus edulis*) is subjected to vibrations during the catch state, in which the tension is maintained passively, probably by the actin–myosin linkages breaking at an extremely slow rate (Rüegg, 1971), the catch tension is also reduced with vibration but shows no subsequent recovery (Ljung and Hallgren, 1975; Kobayashi et al. 1985), reflecting the absence of active cycling of actin–myosin linkages. In the present study, we examined the effect of vibration on steady ACh-induced contracture tension in LRM fibres at 0 °C, in which the rate of active actin–myosin cycling was reduced to less than one-sixth of the rate at 20–23 °C, as judged from the rate of tension redevelopment after a step decrease in fibre length (Fig. 5). The extent of reduction of the actin–myosin cycling rate is comparable with the reduction of shortening velocity of frog skeletal muscle fibres under a given load (Buchthal and Kaiser, 1951). Rather unexpectedly, we observed a marked decrease in the extent of the vibration-induced reduction of active tension in LRM fibres at 0 °C (Fig. 5A). In the Huxley contraction model (Huxley, 1957), the magnitude of isometric tension for a given muscle length is proportional to \( f/(f+g) \), where \( f \) and \( g \) are rate constants for the formation and breakage, respectively, of actin–myosin linkages. If \( f \) and \( g \) are assumed to be equal at 20–23 °C \([f/(f+g)=0.5]\), the observation that the ACh-induced contracture tension increased by about 50% \([f/(f+g)=0.75]\) as temperature was reduced...
from 20–23 to 0 °C indicates that $g$ decreases more markedly than $f$, so that $g$ becomes $f/3$ at 0 °C. On this basis, the effect of low temperature on the vibration-induced reduction of active tension may indicate that the value of $g$ is no longer appreciably affected by vibration after its marked reduction at 0 °C. Much more experimental work is needed to identify the mechanisms underlying the vibration-induced reduction of active tension in muscle.

References


