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FORCE MAINTENANCE WITH REDUCED ABILITY TO SHORTEN ACTIVELY IN BARNACLE STRIATED MUSCLE

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Summary

1. Fibres from adductor scutorum muscle of a barnacle *Tetraclita squamosa* were made to contract isometrically by electrical stimulation, and the change in the ability to shorten actively during the mechanical responses was examined by suddenly allowing the fibres to shorten under a very small load (<3 % of the force immediately before shortening) at various times after the onset of stimulation.

2. The shortening velocity (V_{sl}) was nearly constant during stimulation. After the cessation of stimulation, shortening velocity decreased steeply while isometric force decayed slowly, indicating that isometric force was maintained with reduced ability to shorten actively.

3. Similar results were obtained when the maximum rate of force redevelopment following a quick release was measured at various times during the mechanical response to electrical stimulation.

4. In these fibres, but not in fibres from frog skeletal muscle, a quick restretch following a quick release could restore the force to a level similar to that observed without a quick release. These results, together with those above, indicated a reduced cross-bridge cycling rate during the relaxation phase of mechanical responses of barnacle fibres to electrical stimulation.

5. During electrical stimulation, V_{sl} showed less dependence on $[Ca^{2+}]_o$ than was shown by isometric force.

6. These results are discussed in connection with the mechanism of force maintenance with reduced cross-bridge cycling rate.

Introduction

Some kinds of smooth muscles are known to maintain isometric force, with reduced rate of energy consumption, long after cessation of stimulation. Such an economical mechanism of force maintenance is most obvious in somatic smooth muscles of bivalve molluscs, and is called the catch mechanism. During the catch state, the muscle neither shortens actively nor redevelops isometric force following a quick release (Jewell, 1959), indicating that isometric force is passively maintained (for reviews, see Johnson, 1962; Rüegg, 1971; Twarog, 1976). An

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analogous mechanism of force maintenance has recently been found in mammalian vascular smooth muscles, and has been called the latch mechanism (Dillon *et al.* 1981; Dillon and Murphy, 1982).

The adductor scutorum muscle (ASM) of a barnacle *Tetraclita squamosa* contracts to close the operculum of the animal for many hours when the animal is exposed to air. The present experiments were therefore undertaken to investigate whether the contracted state is maintained by an economical mechanism of force maintenance in this striated muscle, as observed in other muscles. A preliminary account of this work has appeared (Iwamoto *et al.* 1988).

Materials and methods

Preparation

Specimens of a common barnacle *Tetraclita squamosa* (1.5–2 cm in height) were collected at Misaki Marine Biological Station. The adductor scutorum muscle (ASM) was isolated with small pieces of scuta attached to both ends, and carefully teased to obtain a muscle fibre bundle of about 1 mm in diameter. Observation of the fibre bundle preparation under a light microscope indicated that it consisted of muscle fibres of about 100 μm in diameter running along the entire muscle length. The sarcomere spacings of the fibres at their slack length L_0, i.e. the length at which the resting force was barely detectable, were 9–12 μm.

The preparation was mounted horizontally at L_0 (5–7 mm) in an acrylic experimental chamber (3 ml) filled with the experimental solution. One end of the preparation was glued to an extension of a force transducer, the other to a servo-control system. The standard experimental solution (artificial sea water) had the following composition (mmol l^{-1}): NaCl, 513; KCl, 10; $CaCl_2$, 10; $MgSO_4$, 50; Tris maleate, 10 (pH 8.0). When the Ca^{2+} concentration was varied, an osmotically equivalent amount of Na^+ was added or removed. The temperature of the solution was kept at 5 °C with a thermoelectric device.

Stimulation

Since the mechanical responses of the preparation to electrical stimulation were graded according to both the intensity and the duration of stimulation, transverse alternating currents (25 Hz) were applied through a pair of platinum plates covering the two opposite internal walls of the chamber to stimulate the fibres uniformly along their entire length. Reproducible maximum mechanical responses were produced when the preparation was stimulated with currents of 8 s duration (peak-to-peak intensity 14 V cm^{-1}) at intervals of 3 min (see Figs 2, 3). After the experiments, the pieces of scuta were removed from the preparation, and the fibres were blotted and weighed. The maximum isometric force per unit cross-sectional area was calculated as P_0L_0/W, where P_0 is the maximum isometric force and W is the fibre mass. The values of P_0L_0/W ranged from 2 to 5 kg cm^{-2}.

Electromagnetic servo-system and force transducer

The electromagnetic servo-system consisted of a loudspeaker voice coil and an amplifier to drive it. The system operated either in the force-control mode or in the position-control mode, the two modes being switched from one to the other by an analogue switch. The force transducer consisted of a pair of foil strain gauges attached to both sides of a phosphor-bronze plate (5 mm×10 mm×0.5 mm thick). It had a compliance of 1 μm g^{-1} and a resonant frequency of 1 kHz. The position of the moving arm of the servo-system was sensed by a displacement transducer of light source/photodiode type. The force and displacement signals were stored in a transient memory (12-bit resolution) and displayed on an x–y recorder (3025, Yokogawa Electric) or an oscilloscope (5113, Tektronix). They were analysed using a microcomputer (Fujitsu FM-16β).

Experimental procedures

The preparation was first stimulated to contract isometrically (with the servo-system in the position-control mode), and at various times after the onset of force development the preparation was suddenly allowed to shorten under a small load (<3 % of the isometric force immediately before the onset of shortening) by the servo-system operating in the force-control mode. Experiments were also made in which the preparation was subjected to a quick release (1–2 % of L_0) at various times after the onset of isometric force development, so that the force was just reduced to zero and then started to redevelop (see Fig. 2). In some experiments, the preparation was released quickly by up to 7 % of L_0, so that the fibres were slackened and had to shorten freely before developing isometric force again (slack test, Edman, 1979).

Experiments with frog muscle fibres

To compare the mechanical responses of barnacle ASM fibres with those of frog skeletal muscle fibres, experiments were also performed with single fibres from the anterior tibialis muscle of a frog *Rana japonica*. The fibres were made to twitch isometrically at L_0 (4–5 mm) with 1 ms supramaximal current pulses. The Ringer's solution had the following composition (mmol l^{-1}): NaCl, 115; KCl, 2.5; $CaCl_2$, 1.8; Tris maleate, 10 (pH 7.2).

Results

Change in the ability to shorten actively during the mechanical response to electrical stimulation

In response to 8 s of electrical stimulation, the ASM fibre bundle preparation showed isometric force development which reached a maximum 2–3 s after the cessation of stimulation. Examples of length and force records when the preparation was allowed to shorten under a very small load (<3 % of force immediately before shortening) at three different times after the onset of force

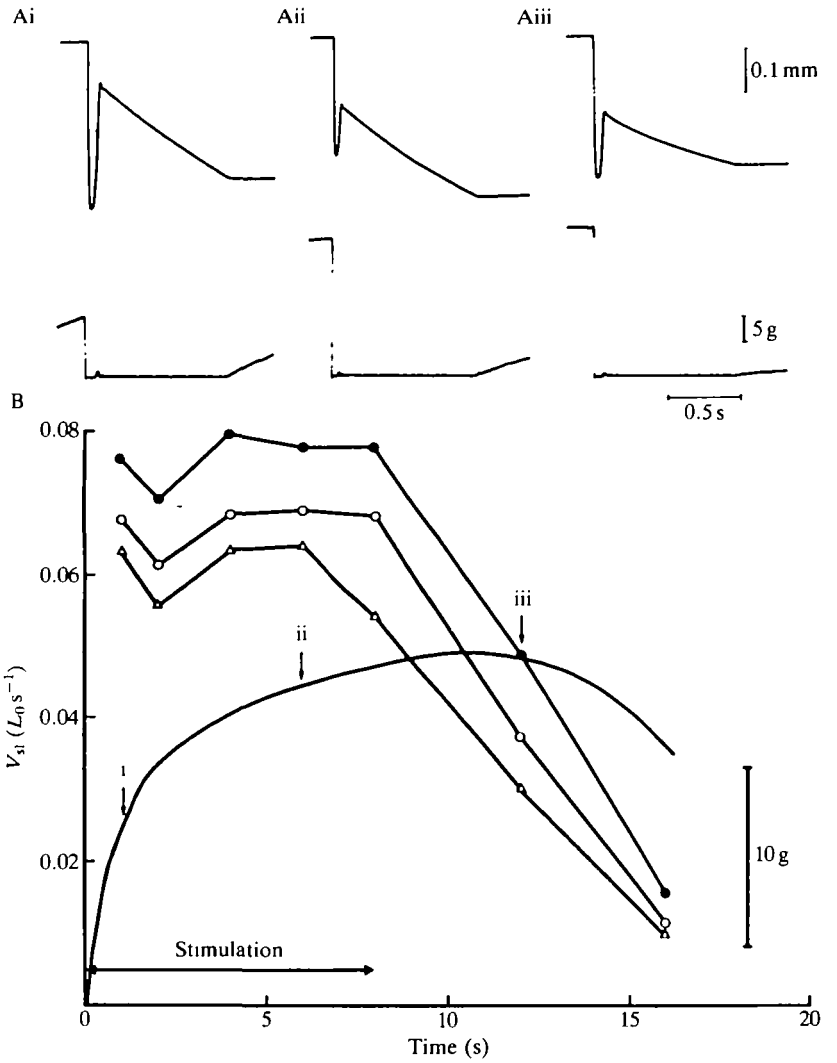

Fig. 1. Change in the shortening velocity (V_{sl}) during the mechanical response of barnacle ASM fibres to 8 s of alternating current stimulation. (A) Examples of length and force records when the fibres were suddenly allowed to shorten under a very small load 1 s (i), 6 s (ii) and 12 s (iii) after the onset of stimulation. (B) Relationship between V_{sl} and time after the onset of stimulation, together with the time course of the mechanical response. Values of V_{sl} were measured 200 (●), 400 (○) and 600 ms (△) after the onset of shortening. Arrows indicate times at which records i, ii and iii were obtained. L_0, slack length.

development are shown in Fig. 1A. Shortening velocity, V_{sl}, obtained by differentiating the length record, is plotted against time after the onset of the mechanical response in Fig. 1B. V_{sl} was nearly constant during stimulation, but decreased steeply after its cessation, though isometric force still continued to rise.

Thus, V_{sl} measured around the peak isometric force was only about 60 % of that measured in the early rising phase of isometric force, and decreased to about 20 % of the maximal value when measured 8 s after the cessation of stimulation, while the force stayed at about 70 % of the maximal value. These results clearly indicate that the ability to shorten actively decays much faster than isometric force after the cessation of stimulation.

The measured V_{sl} had a value close to that of the unloaded shortening velocity V_0, so experiments were made in which V_0 was measured by the slack test method during the mechanical response to electrical stimulation. Fig. 2 illustrates a typical result. Quick releases of various amplitudes were applied to the preparation 2 s (Fig. 2Ai) and 10 s after the onset of stimulation (Fig. 2Aii), and the time from the onset of quick release to the onset of force redevelopment was measured for each release and plotted against the amplitude of release (Fig. 2B). The values of V_0 can be estimated from the slopes of the curves in Fig. 2B. The slopes of the curves were not constant but decreased gradually with time. Nevertheless, the above result shows that, in agreement with the changes in V_{sl} shown in Fig. 1, V_0 is much smaller after stimulation than during stimulation, though the isometric force immediately before release is almost the same for the two cases.

Changes in the rate of force redevelopment during the mechanical response to electrical stimulation

The ability to shorten actively can also be estimated from the maximum rate of redevelopment of isometric force following a quick release which reduces the force to zero, since the contractile components start to shorten internally, pulling the slackened series elastic component during the redevelopment of isometric force. Fig. 3A shows a typical example of experiments in which such quick releases (about 2 % of L_0) were applied at various times during the mechanical response to electrical stimulation. The maximum rate of force redevelopment, obtained by differentiating the force record using the microcomputer, is plotted against time after the onset of stimulation in Fig. 3B. In agreement with the changes in V_{sl} shown in Fig. 1B, the maximum rate of force redevelopment was nearly constant during stimulation and started to decrease steeply after the cessation of stimulation while isometric force was still rising.

Force changes in response to a quick restretch following a quick release

To characterize further the relaxation phase of the mechanical response, in which the ability to shorten actively decays much faster than isometric force, the ASM fibres were first released quickly to reduce the force to zero during the relaxation phase and then quickly restretched to their initial length 2–5 s after the preceding quick release. A quick restretch could bring the reduced force back to the level observed when no release was given (Fig. 4A). When similar length changes were applied to frog muscle fibres during the relaxation phase of an isometric twitch, a quick restretch did not bring the reduced force back to the level

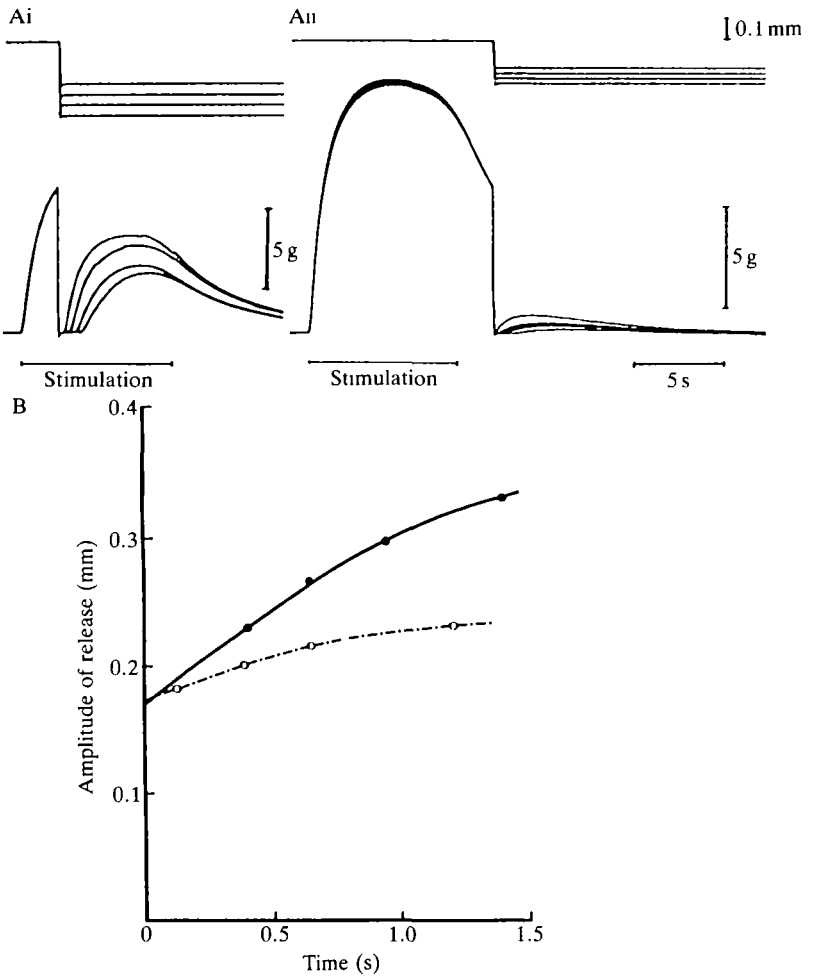

Fig. 2. Unloaded shortening velocity (V_0) measured by the slack test method during and after electrical stimulation. (A) Superimposed length and force records when quick releases of four different amplitudes were applied to the fibres 2 s (i) and 10 s (ii) after the onset of stimulation. (B) Relationship between the amplitude of quick release and the time from the onset of release to the onset of force redevelopment 2 s (●) and 10 s (○) after the onset of stimulation. Values of V_0 are estimated from the slopes of the curves.

to be expected without release. Instead, the level was as observed for a release without a restretch (Fig. 4B).

Effect of changes in external Ca^{2+} concentration on the shortening velocity and isometric force during stimulation

The value of V_{sl} was nearly constant during electrical stimulation, and decreased steeply after the cessation of stimulation (Fig. 1). To obtain information about the dependence of V_{sl} on the intracellular Ca^{2+} concentration during stimulation, the

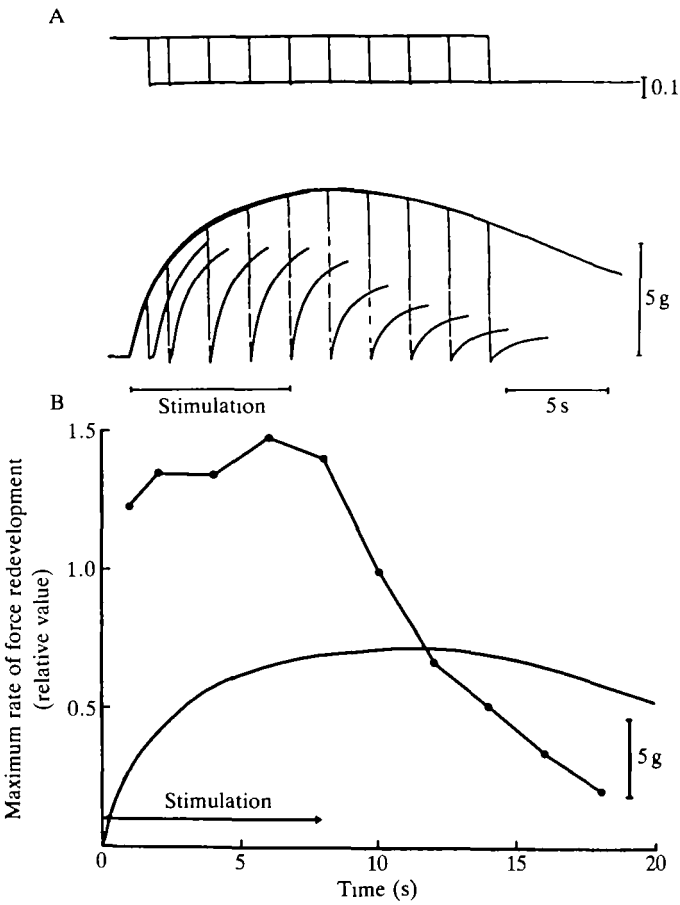

Fig. 3. Changes in the rate of force redevelopment during the mechanical response to 8 s of electrical stimulation. (A) Superimposed length and force records when quick releases (about 2% of L_0) were applied at various times during the mechanical response to 8 s of electrical stimulation to reduce the force just to zero. After each release, the time course of force redevelopment for the first 2 s is shown. (B) Relationship between the maximum rate of force redevelopment and the time after the onset of stimulation, together with the time course of the mechanical response. The rates are expressed relative to the maximum rate of isometric force development in response to stimulation.

preparation was stimulated at various external Ca^{2+} concentrations ($[Ca^{2+}]_o$). Since evidence has been presented that the mechanical response of barnacle muscle to electrical stimulation is associated with the inward movement of external Ca^{2+} (Keynes *et al.* 1973; Atwater *et al.* 1974), it was expected that the intracellular Ca^{2+} concentration during stimulation would be altered by changing $[Ca^{2+}]_o$.

Although both V_{sl} and isometric force increased reversibly when $[Ca^{2+}]_o$ was increased from 2 to 100 mmol l^{-1}, V_{sl} showed less dependence on $[Ca^{2+}]_o$ than was shown by isometric force (Fig. 5A).

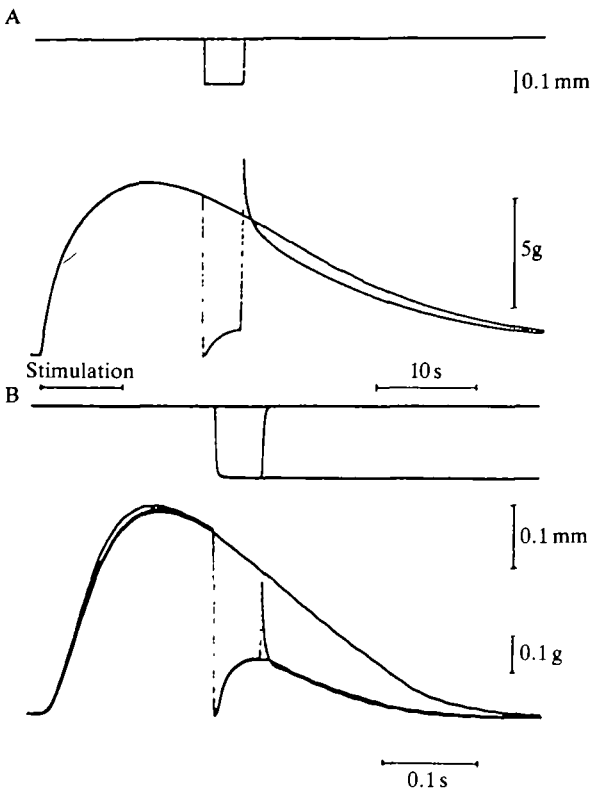

Fig. 4. Comparison between barnacle and frog muscle fibres of the force changes in response to a quick restretch preceded by a quick release. (A) Superimposed length and force records, showing the mechanical response of barnacle ASM fibres to 8 s of electrical stimulation without length change and with a quick release followed by a quick restretch. After restretch, the reduced force rises to the force level without length changes (except for a transient viscoelastic rise in force). (B) Superimposed length and force records showing an isometric twitch without length changes, with a quick release, and with a quick release followed by a restretch after 25 ms. Quick releases were applied during the late relaxation phase (500 ms after the onset of the current pulse). Note that a quick restretch produces a transient viscoelastic rise in force, but the subsequent force trace is the same as that without quick restretch.

The relationship between V_{sl} and isometric force as calcium concentration was changed was calculated from the data shown in Fig. 5A, and was found to be quite different from that observed during relaxation in another bundle in the standard experimental solution (Fig. 5B). The slope of the curve for V_{sl} at varied $[Ca^{2+}]_o$ was modest, but the curve for V_{sl} during the relaxation phase was almost vertical. This result indicates that V_{sl} is controlled by different factors in the two cases.

Discussion

In barnacle striated muscle, the relaxation phase following electrical stimulation

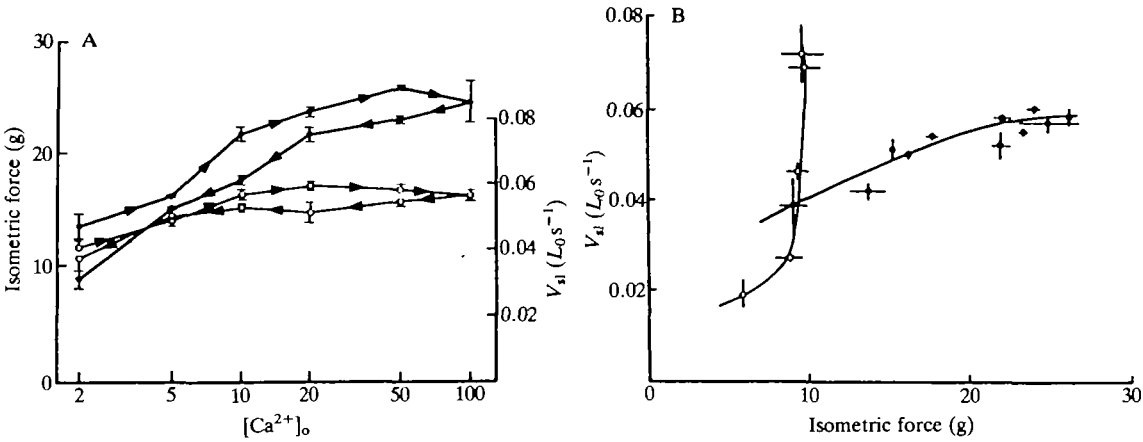

Fig. 5. Effect of changing $[Ca^{2+}]_o$ on V_{sl} and isometric force. (A) Dependence on $[Ca^{2+}]_o$ of V_{sl} and isometric force during electrical stimulation. V_{sl} (○) and isometric force (●) were measured 6 s after the onset of 8 s of electrical stimulation. $[Ca^{2+}]_o$, measured in mmol l^{-1}, is given on a logarithmic scale. Arrowheads on the solid lines indicate whether data points were obtained with increasing or decreasing $[Ca^{2+}]_o$. (B) Relationship between V_{sl} and isometric force. ●, V_{sl} and isometric force measured 6 s after the onset of 8 s of electrical stimulation at various $[Ca^{2+}]_o$ (replotted from Fig. 5A). ○, V_{sl} and isometric force measured in the late phase of the mechanical response to 8 s of stimulation (taken 6, 8, 10, 12 and 16 s after the onset of stimulation) in the standard experimental solution, in a separate ASM fibre bundle. Each data point with vertical bar represents mean of three different experiments obtained from the same preparation with S.D.

exhibited an isometric force which decayed much more slowly than the ability to shorten actively as examined by measuring V_{sl} (Fig. 1), V_0 (Fig. 2) and the rate of force redevelopment following a quick release (Fig. 3). This maintenance of a high isometric force with a markedly reduced ability to shorten actively is characteristic of the catch state observed in other muscles (see Introduction).

It is generally believed that muscle contraction results from an alternating cycle of attachment and detachment between myosin heads (cross-bridges) and actin filaments. In the contraction model of Huxley (1957), muscle shortening and isometric force generation can be described in terms of the rate constants for attachment (f) and detachment (g) of the cross-bridges. The value of P_0 is determined both by the number of force-generating cross-bridges within a half sarcomere and by the quantity $f/(f+g)$, while the value of V_0 reflects the attachment–detachment cycling rate of individual cross-bridges but is independent of the number of the cross-bridges involved. On this basis, the steep decay of V_{sl} during the relaxation phase, close to V_0, may be taken to result from the reduced cycling rate of each cross-bridge. This implies that an economical force maintenance mechanism exists not only in smooth muscle but also in striated muscle; if the reduced cycling rate were due to a nearly proportional decrease in f and g to keep

$f/(f+g)$ almost unchanged, the ability to shorten actively would be reduced while the level of isometric force would not change appreciably.

Another indication of the reduced cross-bridge cycling rate during the relaxation phase was given by a comparison between barnacle and frog muscle of the force changes in response to a quick restretch following a quick release (Fig. 4). In frog muscle fibres, the value of V_0 is known to be constant during the entire course of an isometric twitch, indicating a constant cross-bridge cycling rate (Haugen, 1987), and there is accumulating evidence that, during unloaded or lightly loaded shortening, the number of attached cross-bridges decreases as a result of increased g (Julian and Sollins, 1975; Brenner, 1983). The observation that the force trace following a quick restretch applied during the relaxation phase of a twitch was the same as that without a restretch (Fig. 4B) can be accounted for by assuming that a quick release also decreased the number of attached cross-bridges and that their number was not increased by a subsequent restretch. In barnacle muscle, in contrast, a quick release applied during the relaxation phase of the mechanical response may decrease the number of attached cross-bridges only slightly when their cycling rate has been reduced. Thus, a subsequent restretch may bring the reduced force level back to the force level without a quick release by pulling slackened, but still attached, cross-bridges.

In mammalian vascular smooth muscle, dephosphorylation of myosin light chains is associated with a decrease in the ability to shorten actively, which might provide a mechanism for a decrease in cycling rate of cross-bridges (Dillon *et al.* 1981; Dillon and Murphy, 1982). This implies that, during a decrease in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in the relaxation phase, dephosphorylation of the myosin light chain would be very sensitive to the change in $[Ca^{2+}]_i$. As shown in Fig. 5, however, V_{sl} in barnacle muscle was less sensitive to the change in $[Ca^{2+}]_o$ than was the level of isometric force during stimulation, indicating the involvement of other unknown mechanisms for regulation of cross-bridge cycling rate.

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