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MECHANICAL ACTIVATION IN SLOW AND TWITCH SKELETAL MUSCLE FIBRES OF THE FROG

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SUMMARY

1. Slow and twitch muscle fibres of the frog were studied with a two-micro-electrode point voltage-clamp method. Slow fibres were identified in pyriformis and cruralis muscles by their appearance in the light microscope, electrical characteristics, and rate of sarcomere shortening or of tension development.

2. The relation between the amplitude and duration of threshold depolarizing pulses was determined in sartorius twitch and pyriformis slow fibres. Strength–duration relations for contractile activation are very similar in the two fibre types.

3. The effect of a brief subthreshold pulse on the threshold voltage level decays with a half-time of 1–2 msec at 9 °C in both slow and twitch fibres. This fast decay, thought to reflect voltage-dependent deactivation of Ca^{2+} release following repolarization, is followed by a slower decay of greatly different rates in the two fibre types. The slower components of decay might reflect the rate of background Ca^{2+} removal by the sarcoplasmic reticulum.

4. Reducing external Ca^{2+} levels to about 0·1 μM with 2·5 mM-EGTA has no effect on the shapes of strength–duration curves for both slow and twitch fibres, suggesting that activator Ca^{2+} in both fibre types originates entirely from intracellular stores.

5. 'Tonic' contractions were studied using voltage-clamped short cruralis slow fibres at 20 °C. Reducing external Ca^{2+} to about 0·1 μM had no effect on the steepness of the steady-state tension–voltage relation or on the ability of slow fibres to maintain maximal tension during long (200 sec) depolarizations to membrane potentials of up to +50 mV.

6. Functional similarities in activation kinetics of slow and twitch fibres are discussed in relation to the sensing of tubular membrane potential by the sarcoplasmic reticulum, to Ca^{2+} release from it, and to possible mechanisms involved in these processes. Processes leading to the rapid turning on and off of Ca^{2+} release in response to changes in tubular membrane potential are probably similar in slow and twitch fibres. However, the apparent lack of voltage- and time-dependent inactivation of Ca^{2+} release in slow fibre points to a major difference in the two types of muscle.

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INTRODUCTION

Slow (tonic) skeletal muscle fibres of the frog differ in properties from twitch fibres in several respects (see reviews by Peachey, 1968; Hess, 1970; Costantin, 1975*a*, *b*; Lännergren, 1975). These slow fibres are polyneuronally innervated every few millimetres along their length (Kuffler & Vaughan Williams, 1953*a*; Nasledov, 1965; Nasledov & Thesleff, 1974). They cannot generate action potentials, and their resting membrane resistance is extremely high, resulting in very large membrane time constant, space constant, and input resistance (Adrian & Peachey, 1965; Stefani & Steinbach, 1969). Slow synaptic potentials can thus sum both temporally and spatially to activate and regulate contraction over the entire length of muscle fibre.

A maximally activated slow fibre shortens or develops tension about ten times more slowly than a twitch fibre does and similarly relaxes much more slowly following a contraction (Aidley, 1965; Lännergren, 1975). Prolonged depolarization, however effected, results in maintained tension, and relaxation occurs only when the membrane is repolarized (Kuffler & Vaughan Williams, 1953*b*; Nasledov, 1969).

Several factors can be expected to influence this slow rate of contractile activation. Slow membrane potential change might be a possible cause. Alternatively, voltage-dependent delivery of Ca^{2+} to the myofibrils might well be the rate limiting factor for tension development. While a very low rate of Ca^{2+} release in slow fibres has been suggested (e.g. Zhukov, 1967; Miledi, Parker & Schalow, 1977*a*), supporting evidence is equivocal.

Franzini-Armstrong (1973) showed that the fine structures of triad contacts between the sarcoplasmic reticulum and transverse tubules are nearly identical in frog slow and twitch fibres and suggested that the regulation of Ca^{2+} release from the sarcoplasmic reticulum following transverse tubule depolarization may be similar in these two very different fibre types. It is thus possible that a given membrane potential change could result in functionally equivalent amounts of Ca^{2+} delivered to slow and twitch myofibrils at similar rates, with the time course of the ensuing contractions being effectively regulated by the properties of the activated contractile filaments. Of particular importance would be the rates of actomyosin cross-bridge turnover (Barany, 1967; Floyd & Smith, 1971; Costantin, Podolsky & Tice, 1967) and of Ca^{2+} uptake by the sarcoplasmic reticulum.

Threshold contractions in response to step depolarizations can be studied identically in slow and twitch fibres with a method involving a visually determined end-point. Although such experiments only provide an indirect measure of Ca^{2+} release, they ought to give indications of similarities or differences in voltage- and time-dependent processes regulating slow and twitch fibre mechanical activation. Similarly, measurement of tension in short slow fibres under voltage-clamp conditions can provide additional information on the nature of the tonic contraction. Particularly of interest is the relative importance of Ca^{2+} release from internal stores as opposed to Ca^{2+} influx across the surface membrane in maintaining myoplasmic Ca^{2+} at a level high enough to saturate the contractile apparatus.

This paper is the first of a series in which the properties of slow and twitch fibres are compared. The present paper deals with some characteristics of mechanical

activation in both fibre types. Results of our experiments on membrane electrical properties and voltage-dependent charge movements will be presented in the second and third paper, respectively (Gilly & Hui, 1980*a*, *b*). Some of the results reported here have appeared in preliminary form (Gilly & Hui, 1977*a*, *b*; Chandler, Gilly & Hui, 1978).

METHODS

Frogs and muscles

All experiments (except in Fig. 2) were performed on English frogs (*Rana temporaria*) maintained either in the dark at 4 °C or exposed to running tap water at 15–20 °C. Those kept at room temperature were fed regularly. Twitch fibre experiments were carried out at the pelvic ends of sartorius muscles. Contractile thresholds of slow fibres were determined at the femoral ends of pyriformis muscles (Stefani & Steinbach, 1969). Tension measurements employed bundles of short fibres isolated from the cruralis muscle (Gilly, 1975).

Micro-electrodes and impalement procedure

Voltage-sensing electrodes were filled with 3 M-KCL, and current-passing electrodes with 2 M-K citrate. Twitch fibre electrodes had resistance 4–8 MΩ and tip potential < 2 mV. Good impalements of slow fibres required much higher resistance electrodes (see also Stefani & Steinbach, 1969), and 70–100 MΩ electrodes bevelled to 30–40 MΩ were most suitable. Tip potentials after bevelling were usually less than 5 mV. Capacitance compensation of the electrometer was usually adjusted to give an effective electrode time constant of about 100–150 μsec. Current-passing electrodes for all slow and some twitch fibre experiments were shielded to within 1 mm of their tips with conductive silver paint (Acheson Colloids Electrodag 416) and insulated with fast curing epoxy resin or nail polish.

In a twitch fibre experiment the voltage-recording electrode was inserted first, and if the resting potential was more negative than −80 mV, the current electrode was inserted while passing small inward current pulses. Such a procedure was impossible with slow fibres, as insertion of even a high resistance electrode led to considerable depolarization due to a large electrode shunt conductance relative to the fibre's input conductance. Usually this depolarization exceeded contraction threshold, and a damaging contracture occurred around the electrode, leaving the fibre unsuitable for study. In order to avoid this problem advantage was taken of the long membrane time constant (seconds). The citrate electrode was connected to the input of a voltage-follower circuit and inserted first. Upon entering the fibre, the electrode was immediately switched to the output of a d.c. current source of about −5 nA. The voltage electrode was then inserted and membrane potentials generally more negative than −100 mV were recorded.

Electronics

The voltage-clamp circuit was similar to that described by Almers (1971). Command pulses to the voltage-clamp circuit were rounded with time constants of 40 μsec for twitch and 100–150 μsec for slow fibres.

Membrane potential, bath potential, and electrode current were displayed on a Tektronix 565 oscilloscope and photographed for analysis. Total electrode-holding current was also monitored on a Brush recorder as a check on fibre condition.

Contractile threshold determination

Thresholds were studied in the manner described by Costantin (1974). Depth of focus observed with a water immersion objective (40×, Zeiss) was < 10 μm in Ringer solution. It was thus possible to focus on the upper or lower surface of the fibre, minimizing interference due to sub-threshold contraction artifacts around the micro-electrodes (see also Adrian, Costantin & Peachey, 1969). In some cases, optical sections could be obtained through top and bottom surfaces so that strength–duration curves could be obtained on both. There was rarely a difference of more than a few mV between the two surfaces, even with the shortest pulses used, and mean threshold values are given in the Results section.

All fibres were clamped at a holding potential of −90 mV. At the lowest temperature of these experiments (9 °C) twitch fibre threshold contractions for all pulse durations were quite brisk and readily determined to within 1 mV. On the other hand, slow fibres responded to 2 and 3 msec pulses with relatively little visible movement, therefore threshold determinations were not so clear-cut, but never required more than a range of 5 mV to go from no detectable motion to an unequivocal contraction. Threshold was taken as the mid-point of such a span in pulse amplitudes.

TABLE 1. Composition of solutions (mM)

Solution	NaCl	KCl	Tetra-ethyl-ammon-ium Cl	$CaCl_2$	$MgCl_2$	$MnCl_2$	EGTA
A (normal Ringer)	115	2·5	—	1·8	—	—	—
B	96	2·5	—	1·8	10	—	—
C	98·7	2·5	—	—	10	—	—
D	96	2·5	—	—	10·8	—	2·5
E	96	2·5	—	1·8	—	10	—
F	—	2·5	115	1·8	—	—	—
G	—	2·5	115	—	10·8	—	2·5

All solutions were buffered at pH 7·0 with 1·1 mM-Na_2HPO_4 and 0·4 mM-NaH_2PO_4, except solution E which was buffered with 1 mM-Tris.

Force and tension measurements

Bundles of distal cruralis fibres 0·1–0·5 mm thick were used. The distal end of the muscle was pinned out, while the proximal tendon was attached to the force transducer via a few links of gold chain. Individual fibres, stretched to sarcomere lengths of 2·5–3·0 μm, were impaled near their mid points with two micro-electrodes and voltage clamped as described above. Force exerted by the clamped fibre, damped by the bundle's resting compliance, was thus recorded in response to depolarizing membrane potential steps. Fibre diameter was measured in both the horizontal (d_1) and vertical (d_2) directions, and the area $\frac{1}{4}(\pi d_1 d_2)$ was used to calculate tension (force/area).

The force transducer consisted of two matched Pixie semiconductor elements mounted in such a way that the ratio of their resistances changed linearly according to the applied force. Resonant frequency of the arrangement was 250 Hz and the output was filtered with a 2 msec time constant.

Solutions

All solutions are given in Table 1. Tetrodotoxin (10 μg/ml) was added to all solutions for twitch fibre experiments (except in Fig. 1).

RESULTS

Slow fibre identification

Slow fibres can be distinguished from twitch fibres under the microscope. Pl. 1 shows portions of two living pyriformis muscle fibres. The fibre on the right is slow and the other is twitch. Slow fibres could be readily recognized by their zig-zag Z-lines. Twitch fibres in this portion of the muscle were usually of small diameter (~ 60 μm) with well aligned sarcomeres, prominent longitudinal striations, and many small black dots which are presumably lipid droplets (Engel & Irwin, 1967). Twitch fibres in pyriformis and cruralis muscles outside the region containing slow fibres, as well as in sartorius muscles, generally lacked the lipid droplets and were of larger

diameter ($\sim 100\ \mu$m). Smith & Ovalle (1973) have described a similar zonation of fibre types in *Xenopus* muscles.

Voltage-clamped slow fibres can also be identified electrically. Pyriformis and cruralis twitch fibres had input resistances of about 1 MΩ; values for slow fibres were generally around 10 MΩ. Even more characteristic was the shape of the current transient required to produce a small step of membrane potential. Currents in slow fibres always showed long tails, sometimes requiring several seconds to reach steady state. Such slow transients are a consequence of the high membrane resistance which allows a relatively long length of cable to experience a slow voltage transient following a step in membrane potential at one end of the fibre.

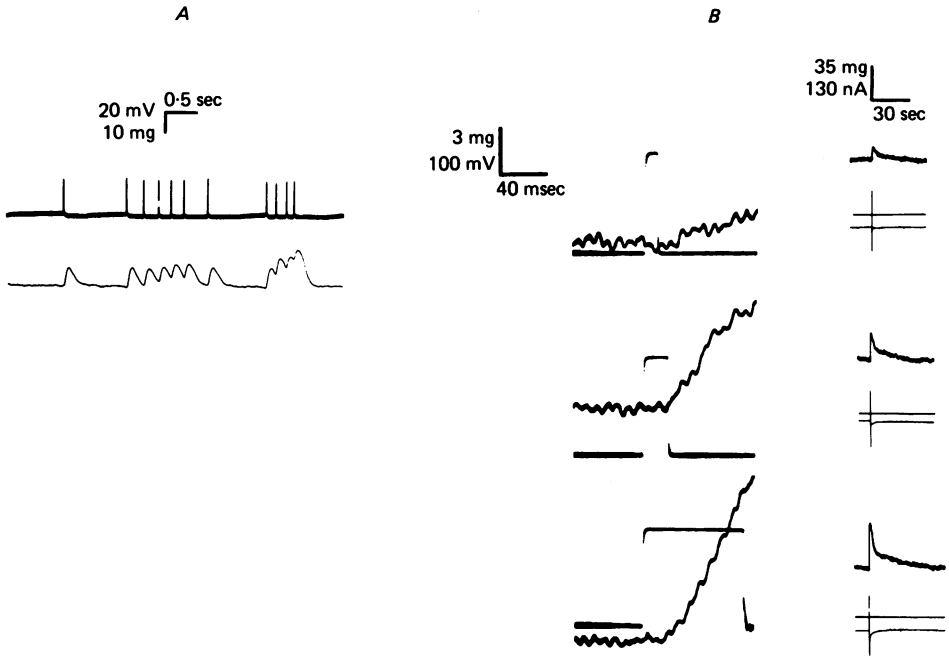

Fig. 1. Time course of contractile responses following fast voltage changes. Membrane potential transients and contractile responses are shown from twitch (*A*) and slow (*B*) fibres from the cruralis muscle at 20 °C. *A*, action potentials (upper traces) and twitches in the absence of tetrodotoxin. Nornal Ringer, solution A. Resting potential = −75 mV during trace; full height of action potentials is not shown. Fibre D151-76. *B*, membrane potential steps and onset of contraction shown on left. Complete contractions and total current transients shown on right, Straight line on current trace is zero current base line. Note difference in time scales from that of *A*. Solution F with 10 mM-$CaCl_2$ added and phosphate buffer replaced by 1 mM-PIPES. Holding potential = −100 mV. Maximum steady-state force with long depolarizations = 53 mg. Fibre length = 6 mm; sarcomere length = 2·75 μm. Fibre 0181-77.

When a fibre was allowed to contract, the contraction time course provided a third criterion for identification of fibre type. To demonstrate this point, tension exerted by twitch and slow cruralis fibres at 20 °C are compared in Fig. 1. These twitch fibres were too long (5–7·5 mm) to allow measuring uniform longitudinal tension with the point voltage-clamp method used. Hence, action potentials, which

served to depolarize the entire fibre length quickly and relatively synchronously, were used to elicit contraction as shown in Fig. 1*A*. Fusion of tension was not reached with the final train of action potentials.

Fig. 1*B* shows contractile responses of a slow fibre to strong depolarizing pulses of 10–80 msec duration. The time course of contraction is markedly different from that in the twitch fibre. Relaxation proceeds very slowly in the slow fibre, and brief depolarizations delivered several hundred milliseconds apart would lead to complete fusion.

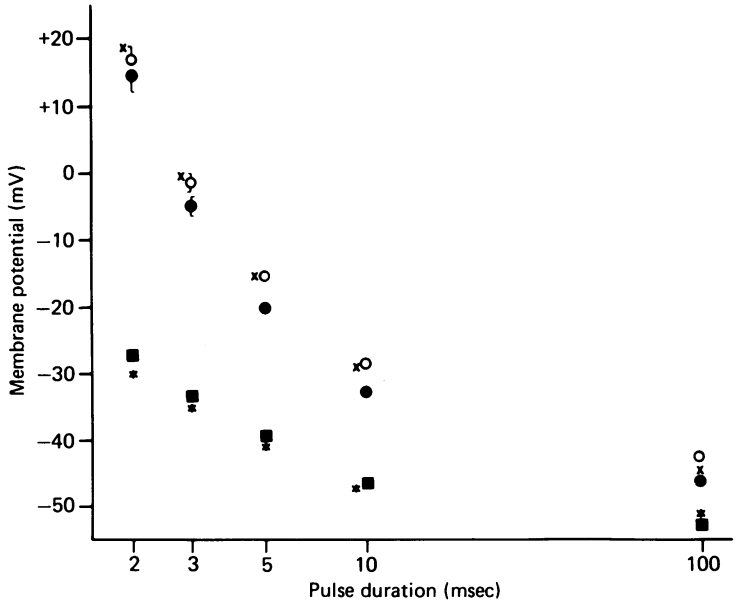

Fig. 2. Twitch fibre strength–duration curves at 9° and 20 °C. Comparison of *R. temporaria* and *R. pipiens*. All frogs were maintained at room temperature. Means ± S.E. of means are plotted; error bars are omitted where smaller than the symbol. ●: *R. temporaria*, eighteen fibres at 9 °C; ○: *R. pipiens*, four fibres at 9 °C; ■: *R. temporaria*, six fibres at 20 °C; ⁎: data of Costantin (1974) on *R. pipiens*.

Strength–duration relation for twitch fibres

Adrian, Chandler & Hodgkin (1969) first studied the relation between mechanical threshold and the necessary amplitude for voltage pulses of various durations in sartorious fibres of *Rana temporaria*. Costantin (1974) later extended their findings using isolated single fibres of *Rana pipiens* and improved optics, enabling determination of short pulse thresholds with activation of only a superficial annulus of myofibrils. This latter method avoids problems associated with tubular spread of depolarization and permits studying threshold contractile responses in a region of the muscle fibre where the membrane potential is best known. Results of both studies qualitatively agree for fibres at 4 °C. As pulse duration decreases below about 10 msec, the amplitude required to elicit a threshold mechanical response increases markedly, resulting in a smooth strength–duration relation.

We also studied strength–duration relations in sartorius fibres of *R. temporaria* and *R. pipiens*. Results are shown in Fig. 2 (filled and open symbols) and compared with the results of Costantin (1974) on *R. pipiens* (crosses and asterisks). At 9° and 20 °C, curves for each species are much the same.

Strength–duration relation for slow fibres

Strength–duration curves are shown in Fig. 3 for slow fibres (open symbols) and twitch fibres (filled symbols) from warm-adapted frogs. At 9 °C, the curves superimpose for short pulses but diverge at 100 msec to values of $-51 \pm 0{\cdot}8$ mV (S.E. of mean) in slow and $-46 \pm 0{\cdot}8$ mV in twitch fibres. Continuous curves are drawn according to the model of Adrian *et al.* (1969) and fit both fibre types equally well (see also legend to Fig. 3). Mean values of thresholds in slow and twitch fibres for each duration studied at 20 °C are indistinguishable ($P > 0{\cdot}05$) by Student's *t*-test.

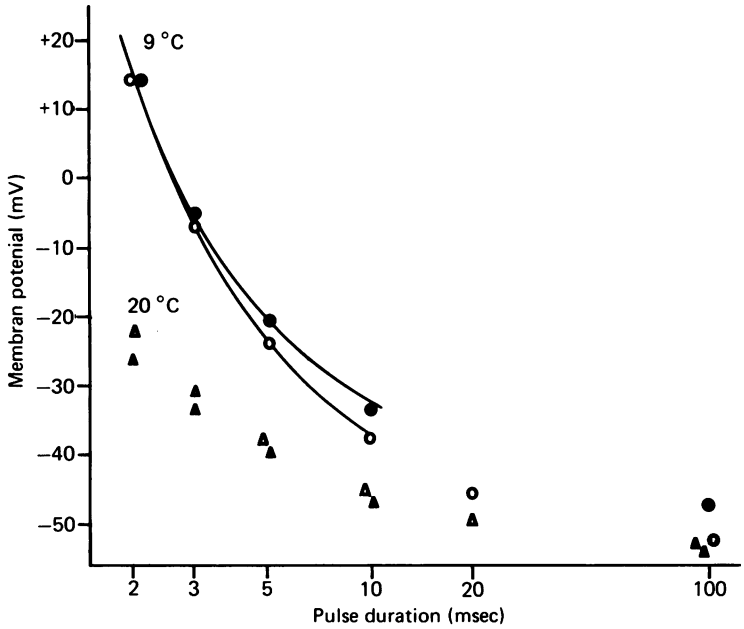

Fig. 3. Comparison of strength–duration curves for twitch and slow muscle fibres. All fibres were from warm-adapted frogs. Twitch data are re-plotted from Fig. 2 (● at 9 °C and ▲ at 20 °C). Means are plotted from fourteen slow fibres at 9 °C (○) and twenty-one slow fibres at 20 °C (△); error bars are omitted for clarity. S.E. of means $\leqslant \pm 1$ mV for pulses 10 msec and longer, $\leqslant \pm 2{\cdot}8$ mV for 5 msec and shorter at 9 °C. All S.E. of means at 20 °C $\leqslant \pm 1$ mV. At 20 °C, no corresponding values in the two fibre types are significantly different ($P < 0{\cdot}05$) by *t*-test. At 9 °C, only 10 and 100 msec values are different ($P < 0{\cdot}05$). Continuous curves are drawn according to the model of Adrian, Chandler & Hodgkin (1969): $(V_T + C)t_c = B_T$, where V_T is threshold membrane potential (mV), t_c is pulse duration (msec), and C and B_T are constants fitted to the data as described by Costantin (1974). $B_T = 127 \pm 4{\cdot}7$ mV. msec (S.E. of mean) and $C = 49{\cdot}1 \pm 0{\cdot}9$ mV in slow fibres; $B_T = 116 \pm 5{\cdot}6$ mV. msec and $C = 43{\cdot}5 \pm 1{\cdot}0$ mV in twitch fibres.

Long pulse thresholds were also measured in both types of fibres. In slow fibres the mean difference between 100 and 800 msec thresholds was $3{\cdot}9 \pm 0{\cdot}2$ mV (eleven fibres) at 9 °C and $3{\cdot}0 \pm 0{\cdot}4$ mV (eight fibres) at 20 °C. Twitch fibres showed somewhat smaller differences between these two durations: three fibres at 9 °C gave a $0{\cdot}9 \pm 0{\cdot}3$ mV difference, while eight fibres at 20 °C gave $2{\cdot}0 \pm 0{\cdot}3$ mV. Hence, a slow fibre at 9 °C takes more time to reach a rheobase which is 8 mV more negative than

that in a twitch fibre. A few observations with 2000 msec pulses showed no further decrease in threshold in either fibre type.

It must be recognized that the shape of a strength–duration curve is governed by several processes. Long pulse thresholds represent a somewhat complicated situation in which C^{2+} release or influx (or both) should just balance re-uptake by the sarcoplasmic reticulum. On the other hand, short pulse thresholds at low temperature should be more relevant to the study of the rise in myoplasmic Ca^{2+}, as Ca^{2+} uptake ought to be negligible in this case (Podolsky & Costantin, 1964). Our results show that short pulse thresholds are indistinguishable in slow and twitch fibres, suggesting that the early steps of Ca^{2+} release from the sarcoplasmic reticulum may be somewhat similar in both fibre types. Furthermore, the finding that rheobase is more negative in slow than in twitch fibres would be consistent with similar rates of Ca^{2+} release but a slower rate of uptake in slow fibres.

As noted above, the twitch fibres of Fig. 3 and all slow fibres in this report are from warm-adapted frogs. Different results were obtained from cold-adapted (4 °C) frogs. Twitch fibres from cold-adapted frogs had a 2 msec threshold at 9 °C about 10–15 mV below the corresponding value in warm-adapted frogs (see also Kovacs & Schneider, 1978). This effect decreased with increasing pulse duration; 100 msec values were identical. Slow fibres from cold-adapted frogs have more drastic changes in contractile as well as membrane electrical properties and behave somewhat like denervated slow fibres (Miledi, Stefani & Steinbach, 1971). Detailed studies of these changes in cold adaption were not carried out.

Two-pulse experiments: decay of mechanical excitability

Another series of experiments was aimed at determining the time course of decay in mechanical excitability by studying the temporal summation of two subthreshold depolarizations (Adrian *et al.* 1969; Costantin, 1974). One can estimate the level of excitability remaining at any time Δt after a near-threshold first pulse of amplitude V_1, by comparing amplitudes of a second test pulse eliciting a threshold contraction, $V_2(\Delta t)$, and of a single threshold pulse of the same duration, V_T (see inset to Fig. 4). Thus, the fraction of mechanical excitability at a time Δt following a subthreshold pulse can be defined as:

$$\frac{V_T - V_2(\Delta t)}{V_T - V_2(0)},$$

where $V_2(0)$ is test pulse amplitude at zero pulse separation.

In practice, summation of brief depolarizations of identical duration (3 msec in twitch and 5 msec in slow fibres) was studied. V_1 was set at 90–95% of threshold (V_T), maximizing the voltage range covered by V_2. Several tests at different pulse separations were bracketed by checks on V_T, and mens of these two V_T values were used for analysing results from each block. V_T usually increased by a few mV during the time course of an experiment. Variations in $V_2(0)$ were similarly checked for and dealt with.

Results from twitch and slow fibres are compared in Fig. 4. Both twitch (Adrian *et al.* 1969; Costantin, 1974), and slow fibres show a very rapid intitial decay of mechanical excitability (Fig. 4*A*). Thus, the effect of a subthreshold pulse disappears in slow as well as in twitch fibres with a half-time of less than 2 msec at 9 °C. A second slower component of excitability decay is also obvious in both fibre types but, unlike the fast component, this component decays at different rates. The slow component has a time constant of 5–6 msec in the twitch fibre in Fig. 4*A* and of greater than 300 msec in slow fibres (Fig. 4*B*).

Costantin (1974) has argued that the fast decay of excitability in a twitch fibre may represent the rapid voltage-dependent deactivation of Ca^{2+} release upon repolarization, whereas the slow decay probably progresses at a rate similar to that for removal of background Ca^{2+} by the sarcoplasmic reticulum and intracellular buffering sites (Taylor, Rüdel & Blinks, 1978; Miledi, Parker & Schalow, 1977*b*; see also Endo, 1977). Following this argument, our results suggest similar kinetics of deactivation of Ca^{2+} release in both fibre types, but a much slower rate of Ca^{2+} re-uptake from the myofilament space in slow fibres. The latter idea is consistent with the lower density of sarcoplasmic reticulum in slow fibres (Page, 1965; Bailey & Peachey, 1975*a*, *b*; and in preparation).

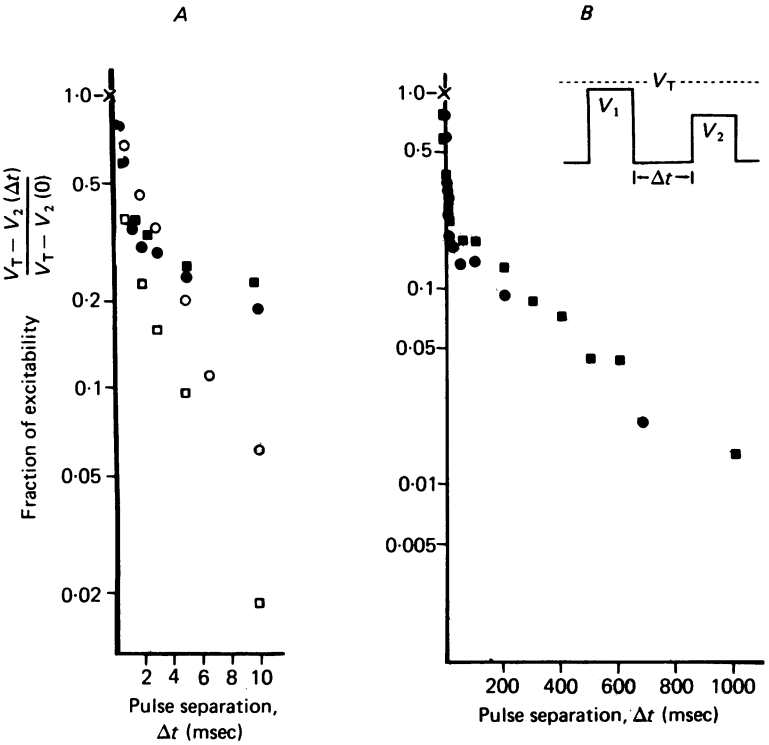

Fig. 4. Decay of mechanical excitability induced by a brief subthreshold pulse in slow and twitch fibres. Fraction of excitability is determined as described in text and depicted in inset. Pulse duration is 5 msec for slow and 3 msec for twitch. All fibres were from warm-adapted frogs. *A*, fast excitability decay in two twitch (○, □) and two slow (●, ■) fibres. Cross represents value shared by all fibres at time zero. ○: fibre 7191–76, 9·1 °C; □: fibre 7201-76, 9·0 °C; ●: fibre 5281-76, 8·7 °C; ■: fibre 5271-76, 9·5 °C. *B*, slow excitability decay in the two slow fibres of *A*. Note difference in time scales.

Effect of divalent cations on strength–duration relations

Both removal of extracellular Ca^{2+} (Stefani & Chiarandini, 1973; Oota, Kosaka & Nagai, 1976; Barrett & Barrett, 1978) and addition of Mn^{2+} ions (Oota, Takauji & Nagai, 1972; Chiarandini & Stefani, 1973; Sakai, Kurihara & Yoshioka, 1974) have been reported to have inhibitory effects on mechanical activation in frog twitch fibres. Ca^{2+} removal in slow fibres has also been reported to abolish contractility (Lüttgau, 1963). On the other hand, several studies have shown that external Ca^{2+}

ions do not appear to be critical for normal activation in both frog twitch (Armstrong, Bezanilla & Horowicz, 1972; Sandow, Pagala & Sphicas, 1975) and slow fibres (Nasledov, Zachar & Zacharova, 1966).

To test the involvement of external Ca^{2+} on mechanical activation, we studied the effect of variation of external divalent cation concentrations on the shape of strength–duration curves in both twitch and slow fibres. 10 mM-Mg^{2+} was added to solutions of greatly lowered Ca^{2+} levels (solutions C and D) to minimize fibre deterioration. In these solutions, twitch fibres maintained normal resting potentials and input resistances for at least several hours (see also Lüttgau, 1963). Slow fibres were also studied in these same Ca^{2+}-free, high Mg^{2+} solutions.

Single slow and twitch fibres were studied both in normal Ringer solution and in one of altered divalent cation composition. After obtaining a strength–duration curve in one solution with a twitch fibre, the electrodes were withdrawn. The solution was drained from the chamber (< 10% remained), and new solution was added. After changing the solution three times at 5 min intervals, the electrodes were re-inserted. If the resting potential upon reimpalement was − 80 mV or better, another strength–duration curve was obtained.

Unfortunately, this procedure was not practicable in slow fibre experiments as removal of the electrodes usually resulted in a powerful contracture. Electrodes were therefore kept in place with the fibre clamped, and the bathing solution was removed by suction while the test solution was introduced from a syringe. Usually a volume 5–6 times that of the bath was flushed through the chamber over about 1 min. This was repeated two or three times at 5–10 min intervals, and the extent of solution change was as thorough as that for twitch fibres.

Results on twitch fibres will be described first. Since all Ca^{2+}-free solutions contained added Mg^{2+}, it was necessary to obtain a control set of strength–duration curves on a fibre in normal Ringer solution and solution B (1·8 mM-Ca^{2+} and 10 mM-Mg^{2+}). The effect of 10 mM-Mg^{2+} on a twitch fibre is shown in Fig. 5*A*. Values obtained in normal Ringer solution are given by filled symbols, those in high Mg^{2+} by open ones. Thresholds at all pulse durations are shifted $4{\cdot}9 \pm 0{\cdot}4$ mV (S.E. of mean) in the depolarizing direction. Another fibre studied showed a similar shift of $6{\cdot}2 \pm 0{\cdot}5$ mV. These shifts are close to those reported for long pulse thresholds (Costantin, 1968; Dörrscheidt-Käfer, 1976). Since thresholds at all durations are shifted equally, the most straightforward explanation is that of a surface charge effect due to the high Mg^{2+} as discussed by the above authors. Such a decrease in fixed negative surface charge density should simply be equivalent to a change in holding potential of − 5 mV.

Fig. 5*B* shows the result of Ca^{2+} removal (solution D) on another twitch fibre. With 10·8 mM-$MgCl_2$, 2·5 mM-EGTA, and an assumed Ca^{2+} contamination of 50 μM, the free Ca^{2+} level should be about 10^{-8} M, assuming the apparent Ca-EGTA binding constant to be $1{\cdot}5 \times 10^6$ M^{-1} at 10 mM-Mg^{2+} (Portzehl, Caldwell & Rüegg, 1964; Godt, 1974). Again, a uniform shift of $5{\cdot}0 \pm 0{\cdot}8$ mV was found. Thus, with 10 mM-Mg^{2+} present, the contractile threshold at any pulse duration does not depend on whether extracellular Ca^{2+} is 1·8 mM or 0·01 μM, showing that external Ca^{2+} ions play no crucial role in regulating contractile activation.

Fig. 5*C* shows the effect of 10 mM-Mn^{2+} on strength–duration curves of a twitch fibre. While the shift due to Mn^{2+} is definitely larger than that caused by Mg^{2+}, it is qualitatively similar. Three fibres studied gave a mean shift of $11{\cdot}3 \pm 1{\cdot}2$ mV. Thus, any inhibitory action of Mn^{2+} on twitch tension might be simply due to a duration-

independent, surface charge-type shift in thresholds of 10–15 mV, which could be significant for an action potential.

Similar experiments were carried out on slow fibres. Fig. 6 shows strength–duration curves at 20 °C in both normal and Ca^{2+}-free Ringer solutions. In the Ca^{2+}-free experiment of Fig. 6*A*, EGTA was not present, while in that of Fig. 6*B*, 2·5 mM-

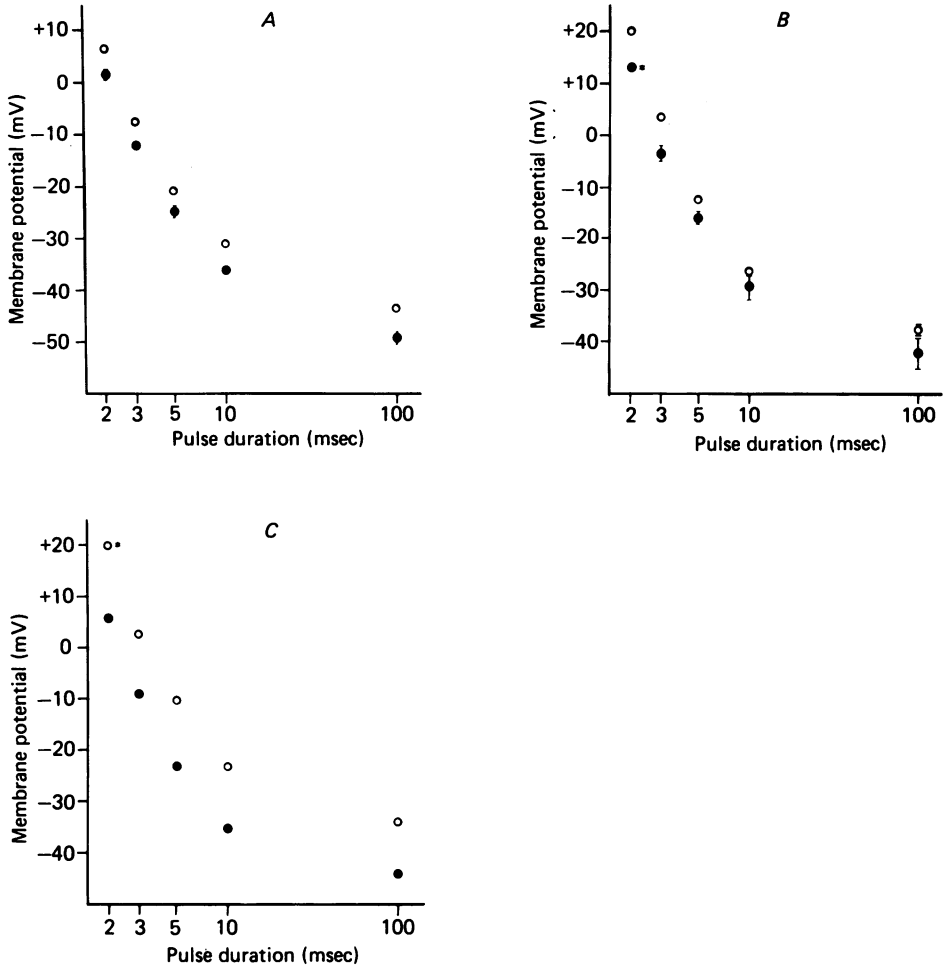

Fig. 5. Strength–duration curves from twitch fibres in normal Ringer solution and in a solution of altered divalent cation concentration. ● : values in normal Ringer solution; ○: values in test solution. Means are plotted with error bars indicating ± 1 S.E. of means within one run. Where absent, error bars are smaller than the symbol, except for those points denoted by * for which only one determination was made. *A*, effect of 10 mM-Mg^{2+} (solution B). Fibre 9094-75, tested first in normal Ringer solution. Initial resting potential: − 85 mV; following first run and change to solution B: − 89 mV; 8·5 °C. *B*, effect of Ca^{2+} removal with 2·5 mM-EGTA and 10·8 mM-Mg^{2+} (solution D). Fibre 0015-75, tested first in solution D. Initial resting potential: − 82 mV; following first run and change to normal Ringer: − 86 mV; following second run: − 77 mV; 9 °C. *C*, effect of 10 mM-Mn^{2+} (solution E). Fibre 0094-75, tested first in Solution E. Initial resting potential: − 86 mV; following first run: − 85 mV; following change to normal Ringer solution: − 85 mV; following second run: − 83 mV; 8·9 °C.

EGTA was included. Results were first obtained in normal Ringer (filled circles) and then in the Ca^{2+}-free solution (open circles). Filled squares are results obtained after changing back to normal Ringer solution. As in twitch fibres, the only action of Ca^{2+} removal in the presence of 10 mM-Mg^{2+} was a uniform depolarizing shift of $5{\cdot}0 \pm 0{\cdot}3$ mV in Fig. 6*A* and $3{\cdot}6 \pm 0{\cdot}4$ mV in Fig. 6*B*. Two other fibres studied, one at 20 °C (solution C) and one at 9 °C (solution D), gave comparable shifts of $4{\cdot}9 \pm 0{\cdot}6$ mV and $5{\cdot}6 \pm 0{\cdot}4$ mV, respectively.

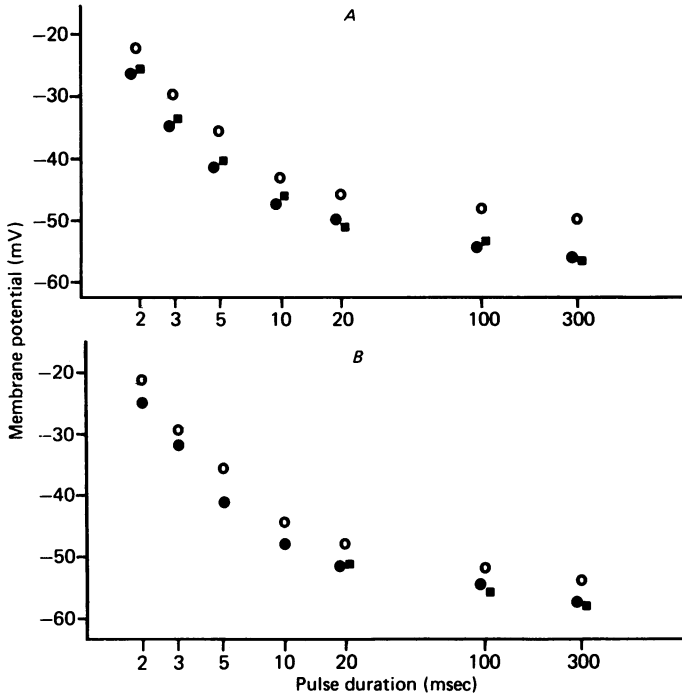

Fig. 6. Strength–duration curves for slow fibres in normal Ringer and in a Ca^{2+}-free solution. ●: initial values in normal Ringer solution; ○: values in Ca^{2+}-free solution; ■: values in normal Ringer solution following the Ca^{2+}-free run. Error bars are omitted for clarity. *A*, effect of 0 Ca^{2+} and 10 mM-Mg^{2+} (solution C). Means of two to three trials are plotted; all S.E. of means $\leqslant \pm 1$ mV, but only one determination was made for 2 msec in normal Ringer solution. Fibre 4071-76. Initial input resistance (R_{in}) and holding current (I_H) in normal Ringer solution: 18 MΩ, −1 nA; following first run: 9·5 MΩ, −17·5 nA. Values could not be measured after solution change due to development of leak through microscope objective to ground; 19 °C. *B*, effect of Ca^{2+} removal with 2·5 mM-EGTA and 10·8 mM-Mg^{2+} (solution D). Means of two to five trials are plotted; all S.E. of means $\leqslant \pm 1$ mV except 20 msec point in normal Ringer solution (± 2 mV). Only one determination made for 2 msec in solution D. Fibre 3314-76. Initial R_{in} and I_H in normal Ringer solution: 11 MΩ, −3·8 nA; following first run: 9 MΩ, −5·2 nA; following change to solution D: 3 MΩ, −32·5 nA; 20 °C.

The lack of effect of external Ca^{2+} on the shape of strength–duration curves implies that external Ca^{2+} is not required in either fibre type for activating contraction. Results presented so far are consistent with a common sequence of processes in mechanical activation shared by slow and twitch fibres.

Tonic contraction in slow fibres: effect of external Ca^{2+}

Since frog slow fibres are cabable of maintaining tension in response to membrane depolarization for extremely long times, whereas twitch fibres spontaneously relax under similar conditions, it is possible that slow fibres depend on a secondary mode of activation to maintain the tonic contraction. Ca^{2+} influx for a slow fibre depolarized by 32 mM-KCl (to about -30 mV, Stefani & Steinbach, 1969) is probably < 1 p-mole/cm^2.sec (Shanes, 1961). Stefani & Uchitel (1976) give a similar value based on indirect electrical measurements, and a net flux of this magnitude would raise the total internal Ca^{2+} level of a 50 μm diameter slow fibre by 0·8 μM/sec. If ~ 100 μM total Ca^{2+} were required for maximal activation as in a twitch fibre (Costantin *et al.* 1967; Costantin, 1975*b*), this modest Ca^{2+} influx might result in a prolonged contraction even if release from internal stores was inactivated.

To evaluate the importance of Ca^{2+} entry, the time course of a long contraction of a maximally activated slow fibre can be compared in normal Ringer and in a Ca^{2+}-free solution. Results concerning the necessity of external Ca^{2+} in maintaining K^+ contractures are conflicting (compare Lüttgau, 1963; Nasledov *et al.* 1966; Lännergren, 1967). Discrepancies may have been due to differences in solution compositions (none contained EGTA or high Mg^{2+}), length of exposure times to divalent-cation-free solutions (up to tens of minutes), and a possible species difference between *Xenopus* and *Rana* concerning the degree of spontaneous relaxation during prolonged depolarization.

The voltage-clamp method described above was applied to the middle of short cruralis slow fibres and the tension recorded (see also Heistracher & Hunt, 1969). Ca^{2+}-free solutions again contained high Mg^{2+}. NaCl was replaced by tetraethylammonium (TEA^+) chloride (solutions F and G) to block delayed K^+ conductances (Gilly & Hui, 1980*a*). Under these conditions steady-state voltage decrement at the fibre end was small, probably 5–10 %,

Fig. 7*A* shows records of membrane potential and tension from a slow fibre in Ca^{2+}-free Ringer (solution G) and the open circles in Fig. 7*B* shows the corresponding steady-state tension–voltage relation (Fig. 7*B*). Filled circles in Fig. 7*B* are from another fibre in 1·8 mM-Ca^{2+}. These curves are in good agreement with those obtained from K^+ contractures in isolated slow fibres (Nasledov *et al.* 1966; Nasledov, 1969). Saturation of tension for the largest depolarizations suggests that the entire fibre length was maximally activated. The curve in the Ca^{2+}-free solution was shifted 5 mV in the depolarizing direction, which may be attributed to the surface charge effect of 10·8 mM-Mg^{2+}. The important point of Fig. 7*B* is that removal of extracellular Ca^{2+} does not affect the shape of the steady-state tension–voltage curve.

Fig. 8*A* shows longer tonic contractions from a slow fibre in 1·8 mM-Ca^{2+}. Relaxation during the long (200 sec) contractions was variable in the same fibre. For example, the fibre in Fig. 8*A* showed little droop in tension with repeated testing, mainly because the initial peak of tension declined. Other fibres behaved in the opposite manner, i.e. the intial peak was relatively constant and the final level progressively decreased. Tension at 200 sec was always greater than 60 % of the initial peak, however, and the rate of spontaneous relaxation was not obviously affected by membrane potential.

Removal of extracellular Ca^{2+} from the bathing medium with EGTA (solution G) did not change the nature of these tonic contractions (Fig. 8*B*). Maximal force was well maintained for 200 sec in the absence of external Ca^{2+}. Similar results were observed on three other fibres.

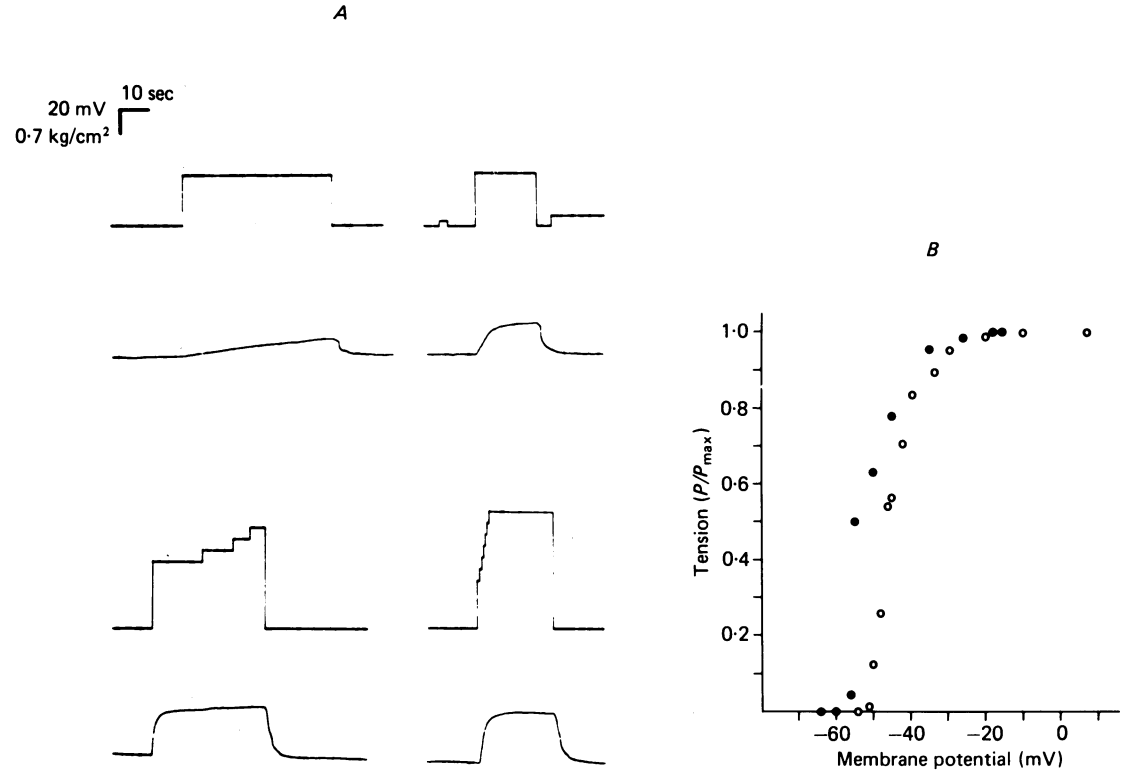

Fig. 7. Membrane potential–tension relation in voltage-clamped short slow fibres. *A*, membrane potential changes (upper traces) and corresponding contractile responses of a slow fibre bathed in solution G. Staircase change of membrane potential for the largest pulse was routinely used to minimize the likelihood of the electrodes being pulled out of the fibre during the period of rapid sarcomere shortening immediately following a large change in membrane potential. Fibre 6302-76. Fibre diameter = 55·7 μm; P_{max} = 1·3 kg/cm²; sarcomere length = 2·75 μm; 18 °C. *B*, values of normalized tension at pulse end are plotted against membrane potential. ○: data from fibre of *A*, solution G (Ca^{2+}-free). ●: data from another fibre in solution F (1·8 mM-Ca^{2+}). Fibre 6152-76. Fibre diameter not measured; maximum force at −15 mV = 120 mg; sarcomere length = 2·1 μm; 17·7 °C.

Ideally, comparison of contractions in 1·8 and 0 mM-Ca^{2+} should be made on the same fibre. Fig. 9*A* shows a long contraction of a maximally activated fibre in 1·8 mM-Ca^{2+} Ringer solution. This fibre showed no droop in tension, and additional depolarization did not alter the level of tension. Following the change to the Ca^{2+}-free EGTA-Ringer (solution G), the contraction in Fig. 9*B* was obtained. Peak tension was unchanged and well maintained for about the first 75 sec, at which time the current passing electrode failed.

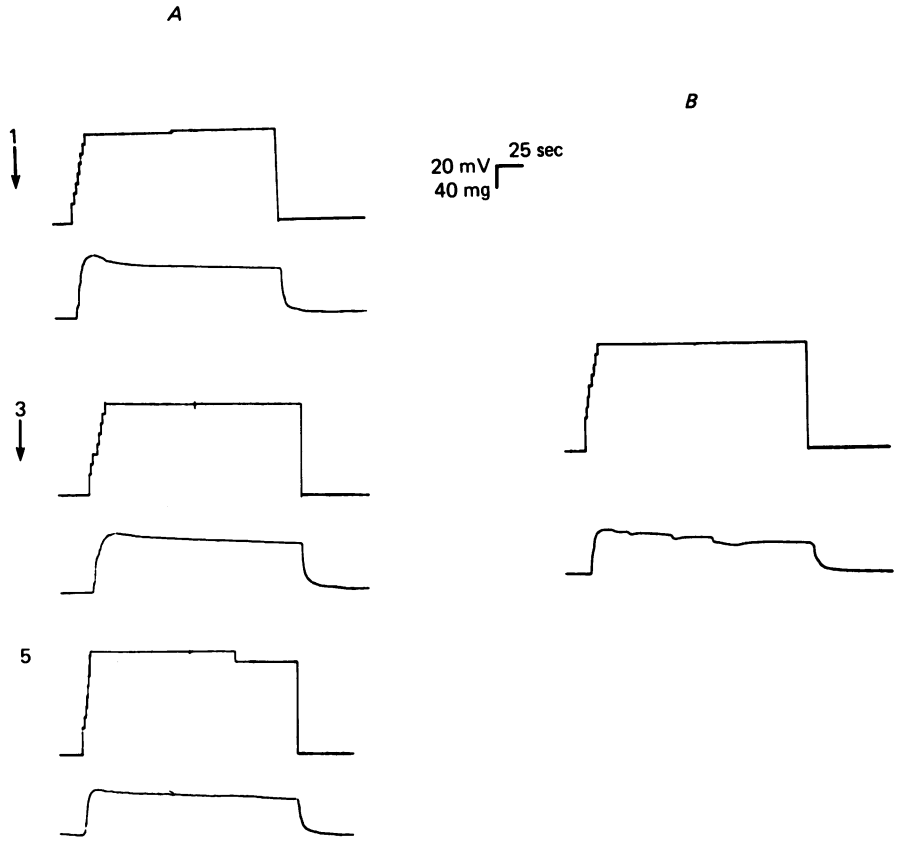

Fig. 8. Force developed by slow fibres during prolonged depolarizations. Upper traces show membrane potentials and lower traces show tensions. Pulse duration is 200 sec. *A*, first, third, and fifth records obtained from a fibre in solution F (1·8 mM-Ca^{2+}). Several minutes were allowed for recovery between tests. Fibre 6241-76. Diameter not measured; 20·3 °C. *B*, one of two similar records from another fibre in solution G (Ca^{2+}-free). Small notches in tension records are probably due to damage at impalement sites. Fibre 6242-76. Diameter not measured; 20·4 °C.

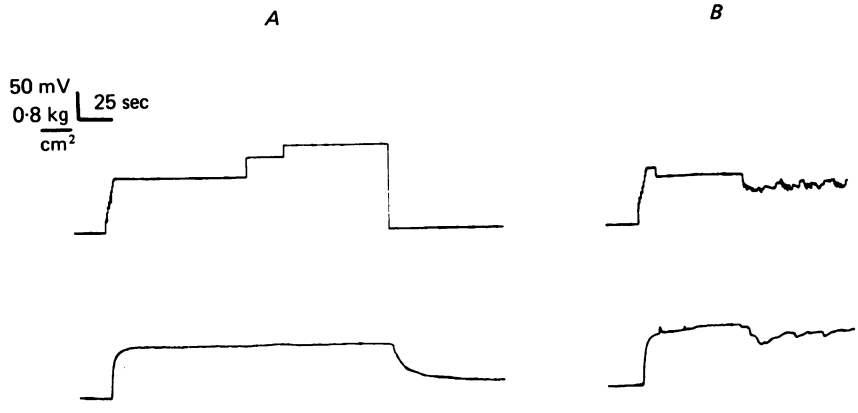

Fig. 9. Tension of a slow fibre studied in 1·8 mM-Ca^{2+} and Ca^{2+}-free Ringer solutions. *A*, in 1·8 mM-Ca^{2+} Ringer (solution F); *B*, in Ca^{2+}-free Ringer (solution G). Upper traces show membrane potential and lower traces show tension. Pulse duration is 200 sec. Note similar nature of contractions in both solutions. Current-passing electrode failed during response in *B*. Fibre 7144-76. Fibre diameter = 75·7 μm; P_{max} = 1·4 kg/cm²; sarcomere length = 3·0 μm; 19·5 °C.

From these results, it seems that external Ca^{2+} ions play no essential part in the slow fibre's ability to maintain maximal tension during prolonged depolarizations. Furthermore, in these experiments the Ca^{2+} release process in slow fibres does not show any sign of voltage-dependent inactivation on a time scale of several hundred seconds (at 20 °C) at membrane potentials of up to +50 mV.

DISCUSSION

While the 'steady-state' voltage dependence of tension measured with K^+ contractures in frog twitch (Hodgkin & Horowicz, 1960) and slow (Nasledov, 1969) muscle fibres is known to be similar, information on the kinetics of mechanical activation in slow fibres had been lacking. Frog slow fibres were believed to have poorly developed internal membrane (sarcoplasmic reticulum and tubule) systems, and a prevailing line of thought has been that the increase in myoplasmic Ca^{2+} upon depolarization might be mainly due to an influx of extracellular Ca^{2+} and be slow enough to limit the rate of tension development. Results presented in this report suggest that neither of these ideas is correct. It seems more likely that the elevation in Ca^{2+} concentration is a result of rapid release from the sarcoplasmic reticulum just as in twitch fibres. Recently, C. H. Bailey & L. D. Peachey (in preparation) found far more tubule : sarcoplasmic reticulum junctional contacts in frog slow fibres than were previously thought to exist, making a rapid release of Ca^{2+} more tenable.

The similarity of strength–duration curves in slow and twitch fibres suggests that information concerning changes in tubular membrane potential is probably transferred to the sarcoplasmic reticulum by similar mechanisms in the two kinds of fibres. A possible location for this transfer is the triadic feet (Franzini-Armstrong, 1973, 1975). Moreover, the glycerol shock procedure, thought to disrupt the triad, is effective in interfering with contractile activation in slow as well as in twitch fibres (Nasledov, Mandelstam & Radzjukewich, 1972). This aspect of excitation–contraction coupling will be covered in the third paper of this series (Gilly & Hui, 1980*b*).

Traces in Fig. 1*B* show that slow fibres develop tension with a latency similar to the value (5–10 msec) obtained by Heistracher & Hunt (1969) from voltage-clamped snake twitch fibres. This finding is of interest and is consistent with the hypothesis that the gating of Ca^{2+} release channels (or whatever mechanism) leading to the rise in myoplasmic Ca^{2+}, shares similar voltage-dependent kinetics in slow and twitch fibres. The slow rate of tension development in slow fibres may be partly due to a slow release of Ca^{2+}, but, more likely, may result from slow steps following release.

The rate of rise in myoplasmic Ca^{2+} concentration has been measured by Miledi *et al.* (1977*a*, *b*) who used arsenazo III as a Ca^{2+} indicator. They found a much slower time course of Ca^{2+} release in slow than in twitch fibres. Part of the difference in kinetics may be due to the fact that the twitch fibres were studied under voltage clamp and the slow fibres were studied using steps of current. Since the membrane time constant of a slow fibre is on the order of 1 sec, it is difficult to interpret current-clamp results on a millisecond time scale. Besides, their absorbance signals in slow fibres were filtered electrically with a 10 msec time constant whereas in twitch fibres only a 0·5 msec time constant was used. Nonetheless, the rate of rise in myoplasmic Ca^{2+} concentrations may very well be slower in slow than in twitch fibres.

Following activation of the thin filaments, the time course of the contraction–relaxation cycle in a muscle fibre is influenced by such factors as the kinetics of cross-bridge turnover, Ca^{2+} dissociation from troponin, Ca^{2+} interaction with intracellular Ca^{2+} buffers such as parvalbumin, and eventual Ca^{2+} uptake by the sarcoplasmic reticulum. Any one or a combination of these events may be much slower in slow fibres, enabling them to contract slowly, maintain tension during prolonged depolarization and retain subthreshold excitability for a relatively long period, as reflected by direct tension measurements and the double-pulse experiments.

A main difference in the regulation of Ca^{2+} release in slow and twitch fibres (Caputo & DeBolaños, 1979) concerns the apparent absence of voltage- and time-dependent inactivation of Ca^{2+} release in slow fibres. This is interesting as it provides a basis for our observations of maintained tonic contractions in the absence of Ca^{2+} influx.

Miledi *et al.* (1977*a*) have also studied maintained depolarizations in slow fibres with the arsenazo III method and concluded that external Ca^{2} probably did play a role in supporting the tonic contraction. In the presence of extracellular Ca^{2+} the observed absorbance change was well maintained during long (100 sec) depolarizations to + 20 mV, in agreement with the lack of relaxation described in this report. With external $Ca^{2+} < 10^{-7}$ M, however, they found the absorbance change slowly returned to some level near the resting base line during such a depolarization (see their Fig. 3*b*). Since they did not record tension, the level to which ionized Ca^{2+} decreased in relation to Ca^{2+} binding by the thin filaments is unclear.

It may be that Ca^{2+} release in a slow fibre does inactivate very slowly but incompletely, and the residual steady-state release can saturate the contractile apparatus. Our data cannot rule out such a possibility. Similarly, we cannot rule out the possibility of separate fast and slow voltage-dependent Ca^{2+} release systems located in the sarcoplasmic reticulum membranes.

Tonic contractions in slow muscle fibres pose a variety of interesting questions, one of which involves long term balance of Ca^{2+} movements into and out of the sarcoplasmic reticulum. During prolonged depolarization with maintained maximal tension, Ca^{2+} might be continuously recycled via competing sarcoplasmic reticulum processes of release and uptake. This need not be energetically wasteful. If the mechanism of Ca^{2+} release in a slow fibre involves a sarcoplasmic reticulum membrane permeability increase to Ca^{2+} ions, the rate of release might be self-limiting and fall to a very low value during a long depolarization, as the driving force for Ca^{2+} movement out of the sarcoplasmic reticulum could be greatly reduced at high myoplasmic Ca^{2+} levels.

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REFERENCES

ADRIAN, R. H., CHANDLER, W. K. & HODGKIN, A. L. (1969). The kinetics of mechanical activation in frog muscle. *J. Physiol.* **204**, 207–230.

ADRIAN, R. H., CONSTANTIN, L. L. & PEACHEY, L. D. (1969). Radial spread of contraction in frog muscle fibres. *J. Physiol* **204**, 231–257.

ADRIAN, R. H. & PEACHEY, L. D. (1965). The membrane capacity of frog twitch and slow muscle fibres. *J. Physiol.* **181**, 324–336.

AIDLEY, D. J. (1965). Transient changes in isotonic shortening velocity of frog rectus abdominis muscles in potassium contracture. *Proc. R. Soc.* B **163**, 215–223.

ALMERS, W. (1971). The potassium permeability of frog muscle membrane. Ph.D. thesis, University of Rochester, Rochester, N.Y.

ARMSTRONG, C. M., BEZANILLA, F. & HOROWICZ, P. (1972). Twitches in the presence of ethylene glycol bis (β-aminoethylether)-*N*,*N*′-tetraacetic acid. *Biochim. biophys. Acta* **267**, 605–608.

BAILEY, C. H. & PEACHEY, L. D. (1975*a*). High voltage stereoscopy of selectively stained SR and T-system in frog slow fibers. *J. cell Biol.* **67**, 15a.

BAILEY, C. H. & PEACHEY, L. D. (1975*b*). The sarcoplasmic reticulum of frog slow and twitch muscle fibers as revealed by stereoscopic high voltage electron microscopy. *33rd Ann. Proc. Electron Microscopy Soc. Amer.*, ed. BAILEY, G. W., pp. 552–553.

BARANY, M. (1967). ATPase activity of myosin correlated with speed of muscle shortening. *J. gen Physiol.* **50**, 197–216.

BARRETT, J. N. & BARRETT, E. F. (1978). Excitation–contraction coupling in skeletal muscle: blockade by high extracellular concentrations of calcium buffers. *Science, N.Y.* **200**, 1270–1272.

CAPUTO, C. & DEBOLANOS, P. F. (1979). Membrane potential, contractile activation and relaxation rates in voltage clamped short muscle fibres of the frog. *J. Physiol.* **289**, 175–189.

CHANDLER, W. K., GILLY, W. F. & HUI, C. S. (1978). Electrical properties of amphibian slow muscle fibers. In *Biophysical Aspects of Cardiac Muscle*, ed. MORAD, M. pp. 31–44. New York: Academic Press.

CHIARANDINI, D. J. & STEFANI, E. (1973). Effects of manganese on the electrical and mechanical properties of frog skeletal muscle fibres. *J. Physiol.* **232**, 129–147.

COSTANTIN, L. L. (1968). The effect of calcium on contraction and conductance thresholds in frog skeletal muscle. *J. Physiol* **195**, 119–132.

COSTANTIN, L. L. (1974). Contractile activation in frog skeletal muscle. *J. gen. Physiol.* **63**, 657–674.

COSTANTIN, L. L. (1975*a*). Contractile activation in skeletal muscle. *Prog. Biophys. molec. Biol.* **29**, 197–224.

COSTANTIN, L. L. (1975*b*). Activation in striated muscle. In *Handbook of Physiology. The Nervous System, I.* ed. KANDEL, E. pp. 215–259. Washington, D.C.: Williams & Wilkins.

COSTANTIN, L. L., PODOLSKY, R. J. & TICE, L. W. (1967). Calcium activation of frog slow muscle fibres. *J. Physiol.* **188**, 261–271.

DÖRRSCHEIDT-KÄFER, M. (1976). The action of Ca^{2+}, Mg^{2+} and H^+ on the contraction threshold of frog skeletal muscle. Evidence for surface charges controlling electro-mechanical coupling. *Pflügers Arch.* **362**, 33–41.

ENDO, M. (1977). Calcium release from the sarcoplasmic reticulum. *Physiol. Rev.* **57**, 71–108.

ENGEL, W. K. & IRWIN, R. L. (1967). A histochemical-physiological correlation of frog skeletal muscle fibers. *Am. J. Physiol.* **213**, 511–518.

FLOYD, K. & SMITH, I. C. H. (1971). The mechanical and thermal properties of frog slow muscle fibers. *J. Physiol* **213**, 617–631.

FRANZINI-ARMSTRONG, C. (1973). Studies of the triad. IV. Structure of the junction in frog slow fibers. *J. cell Biol.* **56**, 120–128.

FRANZINI-ARMSTRONG, C. (1975). Membrane particles and transmission at the triad. *Fedn Proc.* **34**, 1382–1389.

GILLY, W. F. (1975). Slow fibres in the frog cruralis muscle. *Tissue & Cell* **7**, 203–210.

GILLY, W. F. & HUI, C. S. (1977*a*). Contractile activation in slow and twitch muscle fibers of the frog. *Nature, Lond.* **266**, 186–188.

GILLY, W. F. & HUI, C. S. (1977*b*). External calcium ion and mechanical activation in frog slow fibers. *Biophys. J.* **17**, 6a.

GILLY, W. F. & HUI, C. S. (1980*a*). Membrane electrical properties of frog slow muscle fibres. *J. Physiol.* **301**, 157–173.

GILLY, W. F. & HUI, C. S. (1980*b*). Voltage-dependent charge movement in frog slow muscle fibres. *J. Physiol.* **301**, 175–190.

GODT, R. E. (1974). Calcium-activated tension of skinned muscle fibers of the frog. Dependence on magnesium adenosine triphosphate concentration. *J. gen. Physiol.* **63**, 722–739.

HEISTRACHER, P. & HUNT, C. C. (1969). The relation of membrane changes to contraction in twitch muscle fibres. *J. Physiol.* **201**, 589—611.

HESS, A. (1970). Vertebrate slow muscle fibers. *Physiol. Rev.* **50**, 40–62.

HODGKIN, A. L. & HOROWICZ, P. (1960). Potassium contractures in single muscle fibres. *J. Physiol.* **153**, 386–403.

KOVACS, L. & SCHNEIDER, M. F. (1978). Contractile activation by voltage clamp depolarization of cut skeletal muscle fibres. *J. Physiol* **277**, 483–506.

KUFFLER, S. W. & VAUGHAN WILLIAMS, E. M. (1953*a*). Small-nerve junctional potentials. The distribution of small motor nerves to frog skeletal muscle, and the membrane characteristics of the fibres they innervate. *J. Physiol.* **121**, 289–317.

KUFFLER, S. W. & VAUGHAN WILLIAMS, E. M. (1953*b*). Properties of the slow skeletal muscle fibres of the frog. *J. Physiol.* **121**, 318–340.

LÄNNERGREN, J. (1967). The effect of calcium on potassium contractures of single slow muscle fibres of *Xenopus laevis*. *Acta physiol. scand.* **70**, 16–25.

LÄNNERGREN, J. (1975). Structure and function of twitch and slow fibers in amphibian skeletal muscle. In *Basic Mechanisms of Ocular Motility and Their Clinical Implications*. ed. LENNERSTRAND, G. & BACH-Y-RITA, P. pp. 63–84. New York: Pergamon Press.

LÜTTGAU, H. C. (1963). The action of calcium ions on potassium contractures of single muscle fibres. *J. Physiol.* **168**, 679–697.

MILEDI, R., PARKER, I. & SCHALOW, G. (1977*a*). Calcium transients in frog slow muscle fibres. *Nature, Lond.* **268**, 750–752.

MILEDI, R., PARKER, I. & SCHALOW, G. (1977*b*). Measurement of calcium transients in frog muscle by the use of arsenazo III. *Proc. R. Soc.* B **198**, 201–210.

MILEDI, R., STEFANI, E. & STEINBACH, A. B. (1971). Induction of the action potential mechanism in slow muscle fibres of the frog. *J. Physiol* **217**, 737–754.

NASLEDOV, G. A. (1965). Correlative study of certain morphological and functional features of muscle fibers. *Fedn. Proc.* (Translation Suppl.) **24**, T1091–1095.

NASLEDOV, G. A. (1969). Interrelationship between membrane potential level and contraction in tonic muscle fibers. *Fiziol. Zh. SSSR* **55**, 569–575.

NASLEDOV, G. A., MANDELSTAM, J. E. & RADZJUKEWICH, T. L. (1972). A study of excitation-contraction coupling in frog tonic muscle fibres of *Rana temporaria*. *Experientia* **28**, 1305–1306.

NASLEDOV, G. A. & THESLEFF, S. (1974). Denervation changes in frog skeletal muscle. *Acta physiol. scand.* **90**, 370–380.

NASLEDOV, G. A., ZACHAR, J. & ZACHAROVA, D. (1966). The ionic requirements for the development of contracture in isolated slow muscle fibres of the frog. *Physiologia bohemoslov.* **15**, 293–306.

OOTA, I., KOSAKA, I. & NAGAI, T. (1976). Role of superficially membrane-bound calcium on excitation-contraction coupling in frog skeletal muscle. *Jap. J. Physiol.* **26**, 117–126.

OOTA, I., TAKAUJI, M. & NAGAI, T. (1972). Effect of manganese ions on excitation-contraction coupling in frog sartorius muscle. *Jap. J. Physiol* **22**, 379–392.

PAGE, S. G. (1965). A comparison of the fine structure of frog slow and twitch muscle fibres. *J. cell Biol.* **26**, 477–497.

PEACHEY, L. D. (1968). Muscle. *Ann. Rev. Physiol.* **30**, 401–440.

PODOLSKY, R. J. & COSTANTIN, L. L. (1964). Regulation by calcium of the contraction and relaxation of muscle fibers. *Fedn Proc.* **23**, 933–939.

PORTZEHL, H., CALDWELL, P. C. & RÜEGG, J. C. (1964). The dependence of contraction and relaxation of muscle fibres from the crab *Maia squinado* on the internal concentration of free calcium ions. *Biochim. biophys. Acta* **79**, 581–591.

SAKAI, T., KURIHARA, S. & YOSHIOKA, T. (1974). Action of manganese ions of excitation-contraction coupling of frog skeletal muscle fibres. *Jap. J. Physiol.* **24**, 513–530.

SANDOW, A., PAGALA, M. K. D. & SPHICAS, E. (1975). Excitation-contraction coupling: effect of 'zero'-Ca^{2+} medium. *Biochim. biophys. Acta* **404**, 157–163.

SHANES, A. M. (1961). Calcium influx in frog rectus abdominus muscle at rest and during potassium contracture. *J. cell comp. Physiol.* **57**, 193–202.

SMITH, R. S. & OVALLE, W. K., Jr. (1973). Varieties of fast and slow extrafusal muscle fibres in amphibian hind limb muscles. *J. Anat.* **116**, 1–24.

STEFANI, E. & CHIARANDINI, D. J. (1973). Skeletal muscle: dependence of potassium contractures on extracellular calcium. *Pflügers Arch.* **343**, 143–150.

STEFANI, E. & STEINBACH, A. B. (1969). Resting potential and electrical properties of frog slow muscle fibres. Effect of different external solutions. *J. Physiol.* **203**, 383–401.

STEFANI, E. & UCHITEL, O. D. (1976). Potassium and calcium conductance in slow muscle fibres of the toad. *J. Physiol* **255**, 435–448.

TAYLOR, S. R., RÜDEL, R. & BLINKS, J. R. (1978). Calcium transients in isolated amphibian skeletal muscle fibres: detection with aequorin. *J. Physiol.* **277**, 291–323.

ZHUKOV, E. K. (1967). Three processes underlying calcium level in the contractile system of muscle fibers. *Fiziol. Zh. SSSR* **53**, 1393–1399.

EXPLANATION OF PLATES

PLATE 1

Identification of fibre types under the microscope. This shows an optical section through the mid-planes of neighbouring slow (top) and twitch pyriformis muscle fibres near their femoral ends. The picture was taken with a whole muscle mounted for an experiment, and this degree of resolution was routinely obtained. Twitch fibres were recognized by the longitudinal striations, rows of lipid droplets, and straight Z-lines. Slow fibres characteristically lacked these internal structures and had relatively poorly aligned sarcomeres.