Contractile Activation in Scorpion Striated Muscle Fibers

Dependence on Voltage and External Calcium

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ABSTRACT Excitation-contraction coupling was characterized in scorpion striated muscle fibers using standard microelectrode techniques as employed in studies on vertebrate skeletal muscle. The action potential of scorpion muscle consists of two phases of regenerative activity. A relatively fast, overshooting initial spike is followed by a prolonged after-discharge of smaller, repetitive spikes. This after-discharge is accompanied by a twitch that relaxes promptly upon repolarization. Twitches fail in Na-free, tetrodotoxin (TTX)-containing, or Ca-free media. However, caffeine causes contractures in muscles paralyzed by Na- and Ca-free solutions. Experiments on muscle fibers voltage-clamped at a point with two microelectrodes in Na-free or TTX-containing media indicate that: (*a*) the strength-duration relation for threshold contractions has a shape similar to that in frog muscle, but mean values are displaced ~20 mV in the positive direction; (*b*) tetracaine exerts a parallel effect on strength-duration curves from scorpion and frog; (*c*) contractile activation in scorpion is abolished in Ca-free media; and (*d*) the contractile threshold is highly correlated with the occurrence of inward Ca current for pulses of all durations. Thus, the voltage dependence of contractile activation in scorpion and frog muscle is similar. However, the preparations differ in their dependence on extracellular Ca for contraction. These results are discussed in relation to possible mechanisms coupling tubular depolarization to Ca release from the sarcoplasmic reticulum in vertebrate and invertebrate skeletal muscle.

INTRODUCTION

Probably the most significant gap in our understanding of excitation-contraction (E-C) coupling in striated muscle concerns the mechanism by which voltage across the surface and transverse tubular (T) membranes regulates Ca release by the sarcoplasmic reticulum (SR) (see reviews by Gilly, 1981; Schneider, 1981). Presumably, this T:SR coupling process takes place via the diads and triads that constitute the only morphological link between these two membrane systems

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(Franzini-Armstrong, 1973*a*, 1975). Direct evidence for this idea is lacking, however, because experimental access to T:SR junctions has been severely restricted. In most striated muscle fiber types, including extensively studied ones such as frog twitch fibers, these junctions are buried within a structurally and electrically complex T-system (Peachey and Adrian, 1973; Valdiosera et al., 1974; Nakajima and Bastian, 1976). This feature hampers investigations designed to reveal voltage-dependent aspects of T:SR coupling by preventing direct examination of junctional electrical properties.

More direct information might be provided by studying E-C coupling in a muscle fiber with a geometrically and electrically simple T-system. Striated muscle fibers from the scorpion fulfill this requirement. Each tubule is a radially oriented cylinder that has a prominent opening to the extracellular space and makes numerous diadic junctions with the extensive SR (Gilai and Parnas, 1972). Scorpion diads are basically indistinguishable from those in frog (Franzini-Armstrong, 1973*b*, 1984; Bailey and Peachey, 1975). These features make scorpion muscle fibers well suited to electrophysiological studies of T:SR junctional transmission. Although the basic neuromuscular physiology of scorpion muscle has been reported (Gilai and Parnas, 1970) and the tubular cable properties have been analyzed in detail (Gilai, 1976), E-C coupling in this preparation has not received any attention.

This paper characterizes contractile activation in scorpion muscle and can be divided into two sections. The first constitutes a description of the action potential and the accompanying contractile activity. The second presents voltage-clamp data describing the strength-duration (S-D) relation for threshold contractions. Since the methods employed are the same as those used in experiments on vertebrate skeletal muscle, a straightforward comparison of T:SR coupling in scorpion and vertebrate striated muscle can be made. Similar measurements have not been reported for any other invertebrate muscle fiber type.

Our results indicate that the threshold membrane potential for contractile activation depends on the pulse duration in a similar way in both scorpion and frog muscle fibers. Moreover, tetracaine has parallel effects on the S-D relation in both fiber types. We also find that, in contrast to results on vertebrate skeletal muscle, voltage-dependent activation of contraction in scorpion muscle fibers is impossible in the absence of extracellular Ca ions.

Some of these results have appeared in preliminary form (Scheuer and Gilly, 1984).

METHODS

The species of scorpions used were *Uroctonus mordax*, *Vaejovis glimmei*, and *Centuroides sculpturatus*. Animals were maintained at room temperature and fed regularly on termites. No systematic differences in the physiological properties of these genera were noted. *Uroctonus* and *Vaejovis* were collected locally; *Centuroides* were supplied by Mr. Loren Honetschlager, Tempe, AZ.

All experiments were performed on long closer muscles of the pedipalps as described by Gilai and Parnas (1970). Isolated muscles were pinned out at ~1.25× slack length to the Sylgard (Dow Corning Corp., Midland, MI) floor of the experimental chamber. The distal tendon was tied to a loop of fine silk thread and either pinned down or attached to

a force transducer (see below). Experiments were performed at room temperature (19–22°C).

Voltage-Clamp Arrangement and Analog Electronics

The muscle fibers were voltage-clamped, usually at a point near the middle of their length, with a two-microelectrode technique. Intracellular voltage-sensing and current-passing microelectrodes were inserted diametrically opposite each other in a plane roughly halfway between the upper and lower fiber surfaces. The bath potential was sensed with a third microelectrode located extracellularly near the impalement site of the internal voltage electrode. The membrane potential was measured differentially between the intra- and extracellular microelectrodes.

Voltage-sensing microelectrodes were filled with 3 M KCl and current-passing electrodes with 1.5 M K-citrate. All electrodes had resistances of 10–25 MΩ and were shielded with conductive silver paint (Electrodag 416; Acheson Colloids, Port Huron, MI) to within 1 mm of their tips. This shield was insulated from the bath with 5-min epoxy and nail polish. Each voltage electrode's shield was driven at unity gain; that of the current electrode was grounded.

The voltage clamp was similar to that described by Almers (1971), but the differential amplifier measuring the membrane potential was replaced by a Philbrick (Dedham, MA) 1036 operational amplifier and the control amplifier was replaced by a National Semiconductor 5534. Command signals were rounded with a time constant of 12 μs. The total injected current was measured with a virtual ground circuit (Schneider and Chandler, 1976) whose output was filtered at 1–2 kHz.

Pulse Generation and Data Acquisition

Pulses were generated and signals were recorded using an LSI 11/23-based computer (Digital Equipment Corp., Maynard, MA) interfaced to a data acquisition system designed in the laboratory of Dr. Clay Armstrong, University of Pennsylvania, which was modified to multiplex up to four input channels. Each signal was sampled at a rate of 20 μs per point for voltage steps 1–10 ms in duration and at a rate of 40 μs per point for pulses 20–50 ms long. When three channels were multiplexed (Fig. 13), the sampling was at 30 μs per point. All data were stored on floppy disks.

Determination of Contraction Thresholds

The thresholds for contractions elicited by voltage-clamp steps were determined as described by Gilly and Hui (1980*a*) for experiments on frog muscle fibers (see Costantin, 1974, for additional details). Muscle fibers were observed with a water immersion objective and Kohler-type illumination. This yielded an optical section <10 μm thick, making it possible to focus on the upper or lower surface of the fiber in the vicinity of the intracellular microelectrodes. Sarcomere shortening was monitored in either of these regions.

The degree of visual resolution routinely obtainable with scorpion muscle is similar to that reported for whole frog muscles (cf. Plate 1 in Gilly and Hui, 1980*a*). Sarcomeres in individual muscle fibers are obvious, and their shortening in response to voltage-clamp steps can be clearly resolved. All fibers studied in this report had sarcomere lengths of <3.8 μm when mounted for the experiment. Fibers with sarcomeres nearly twice this long also exist (unpublished observations), but they were not examined.

The holding potential was −70 mV unless noted otherwise. Contractile responses were monitored visually by one experimenter without knowledge of the pulse amplitude being applied, which was chosen by the second experimenter.

For reasons that remain unclear, contractions sometimes failed totally after partial S-D

curves were obtained. The first sign of this phenomenon was a rapid drift of threshold toward unusually positive voltages. In order to control for this problem, the contractile threshold for a 20-ms pulse was periodically monitored throughout each experiment. Data from any fiber that were obtained after the 20-ms threshold had changed >10 mV from the initial determination were discarded.

Action Potential and Force Measurements

The force transducer, made from Pixie semiconductor elements (Endevco Corp., Pasadena, CA; see also Gilly and Hui, 1980*a*), was attached to the distal tendon. When twitches and tetani were measured from a whole muscle (or bundle of fibers), stimuli were applied longitudinally via two Pt plates in the bath. For most action potential recordings, a small number of fibers were selectively stimulated with a bipolar electrode made out of 70-μm-diam Pt wires spaced 150 μm apart and insulated except for their tips.

Solutions

The compositions of the saline solutions used are given in Table I. 30 mM glucose was sometimes added in force measurement experiments. This seemed to improve the longevity of the preparations. *N*-methylglucamine (NMG) solutions were prepared from a 0.5-M stock of NMG-Cl obtained by titration of the free base with concentrated HCl. This stock and all NMG-containing salines were kept frozen until the day they were used. Caffeine and tetracaine (Sigma Chemical Co., St. Louis, MO) were added to the appropriate saline on the day of the experiment. Tetrodotoxin (TTX) was obtained from Calbiochem-Behring Corp., San Diego, CA.

RESULTS

Characteristic Electrical Activity of Scorpion Muscle Fibers

Scorpion muscle fibers have a resting potential in normal saline of about −60 mV (solution A, Table I) and display a characteristic pattern of regenerative electrical activity consisting of a brief overshooting action potential followed by a prolonged afterdischarge. The initial fast spike is shown by itself in Fig. 1*A*. Stimulation was via intracellular current injection from a second electrode (lower trace), and an all-or-none action potential occurred at a threshold between −21.5 and −19.5 mV. Such impulses rise at a maximum rate of 120–130 V/s and overshoot to about +25 mV.

Fig. 1*B* shows activity recorded on a slower time base. Stimulation was via Pt

TABLE I

Solutions (mM/liter)

Solution	NaCl	NMG-Cl	KCl	$CaCl_2$	$MgCl_2$	Tris	EGTA	Resting potential (mV ± SEM)
A	250	0	7.7	5	5	10	0	−60.4±1.34; n = 49
B	0	240	7.7	0	10	10	2*	—
C	230	0	7.7	0	10	10	2*	—
D	0	240	7.7	5	5	10	0	−58.8±0.98; n = 50

* EGTA was omitted from Ca-free solutions in some experiments. These experiments are noted in the figure captions. All solutions were adjusted to a pH of 7.1 ± 0.1 pH unit with 1 N HCl or Tris base. Osmolalities of all solutions were ~500 mosmol/kg H_2O.

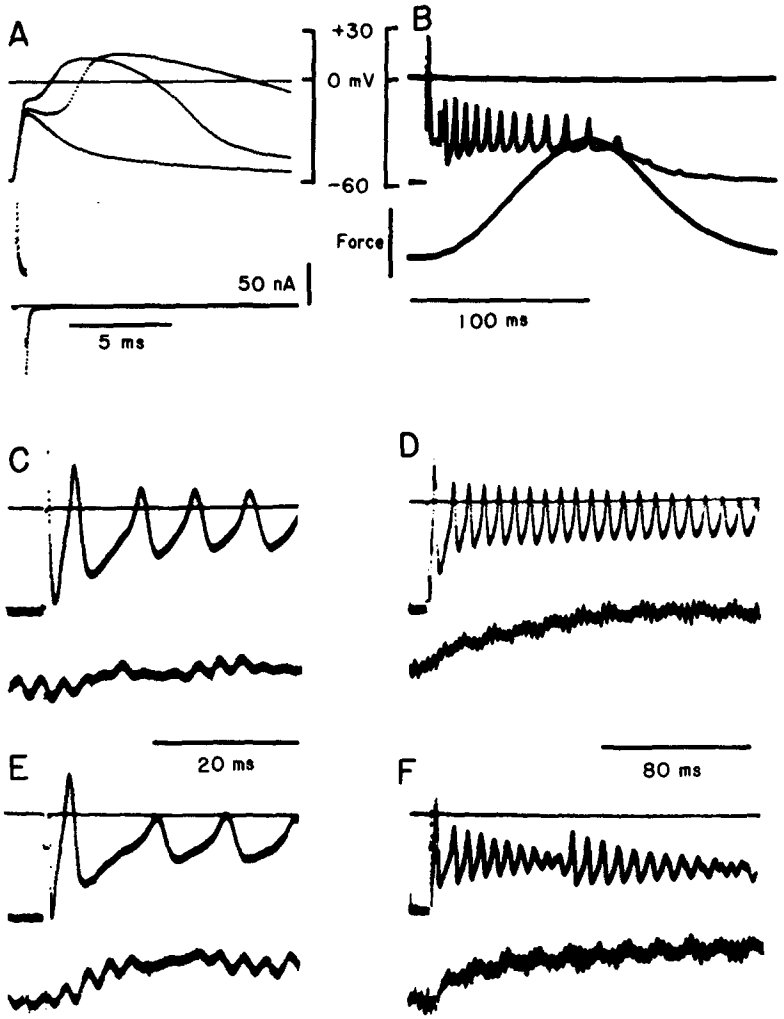

FIGURE 1. Regenerative electrical activity in scorpion muscle fibers recorded in normal saline. (*A*) One subthreshold response and two action potentials (upper traces) in response to intracellular current injections of three different intensities (lower traces). *Uroctonus mordax*. (*B*) Complete pattern of electrical activity (upper trace) associated with the twitch (lower trace). Stimulation was via large bath electrodes. *Vaejovis glimmei*. The experiment was done in laboratory of Dr. M. Morad, University of Pennsylvania. (*C–F*) Electrical (middle traces) and mechanical (bottom traces) activity illustrating the variable nature of the afterdischarge. The uppermost trace shows stimulus; the baseline marks the 0 mV level. *C* and *D* are from one muscle fiber; *E* and *F* are from another fiber in the same muscle. Stimulation was via bipolar electrode. The voltage scale is the same as in *B*. *Centuroides sculpturatus*. Force scales: (*B*) unrecorded; (*C–D*) 100 mg; (*E–F*) 50 mg.

bath electrodes, and a single brief shock resulted in a twitch of the whole muscle (lower trace). The upper trace shows a fast, overshooting action potential as described above, as well as an after-discharge of smaller repetitive spikes lasting for the duration of the twitch. An approximate correlation between the durations of twitch and after-discharge was a consistent finding.

The properties of the initial impulse are fairly consistent from fiber to fiber, but the nature of the repetitive spikes and their firing pattern are more variable. These spikes typically rise at least three times more slowly than the initial one, and may or may not overshoot. Fig. 1, *C* and *D*, shows activity from a muscle fiber, recorded on fast and slow time bases, respectively, in which the after-discharge spikes initially overshoot to about +10 mV and then decrease steadily in amplitude. Fig. 1, *E* and *F*, shows results from another fiber in the same muscle in which the repetitive spikes do not overshoot and fire in a bursting pattern.

Although details of the after-discharge vary, the features seen in Fig. 1 are typical of normal electrical activity in all species of scorpion that we examined. Repetitive small-amplitude spikes also occur in muscle fibers from *Leiurus*, an old-world species (Gilai and Parnas, 1970), but overshooting spikes were not reported.

Twitches and Tetani of Scorpion Muscle

Like the fast early action potential accompanying direct stimulation, the associated twitch of scorpion muscle fibers may also be basically all or none. The twitch force of a whole muscle is graded over a fairly small range of stimulus intensities to a maximal level (Fig. 2*A*). It seems likely that under these conditions each fiber displays an all-or-none (initial) action potential and twitch and that increasing shock strength recruits additional fibers until the entire muscle is uniformly activated. We have not yet successfully isolated single fibers to test this explanation more directly.

In all experiments to be described in which force was measured, the stimulus intensity was set higher than that needed to produce a maximal twitch as defined in Fig. 2*A*. Even with such supramaximal stimuli, however, the twitch amplitude depends on the stimulation rate. Both positive and negative staircase phenomena occur at low stimulation frequencies, but they were not studied in detail. Frequencies of 0.08–0.8 Hz gave reasonably large twitches of fairly constant amplitude over long periods of time and thus proved to be practical for most purposes.

High-frequency stimulation (>50 Hz) results in a fused tetanus. The left-most response in Fig. 2*B* shows a maximal twitch during a period of stimulation at 0.7 Hz. The 70-Hz tetanus delivered thereafter results in a considerably larger force. This twitch/tetanus procedure was quickly repeated two more times, revealing a greatly potentiated twitch and a slightly augmented tetanus. Post-tetanic potentiation of the twitch disappeared along a roughly exponential time course, with a time constant of ~5 s (not illustrated). This basic pattern was seen in every muscle thus examined.

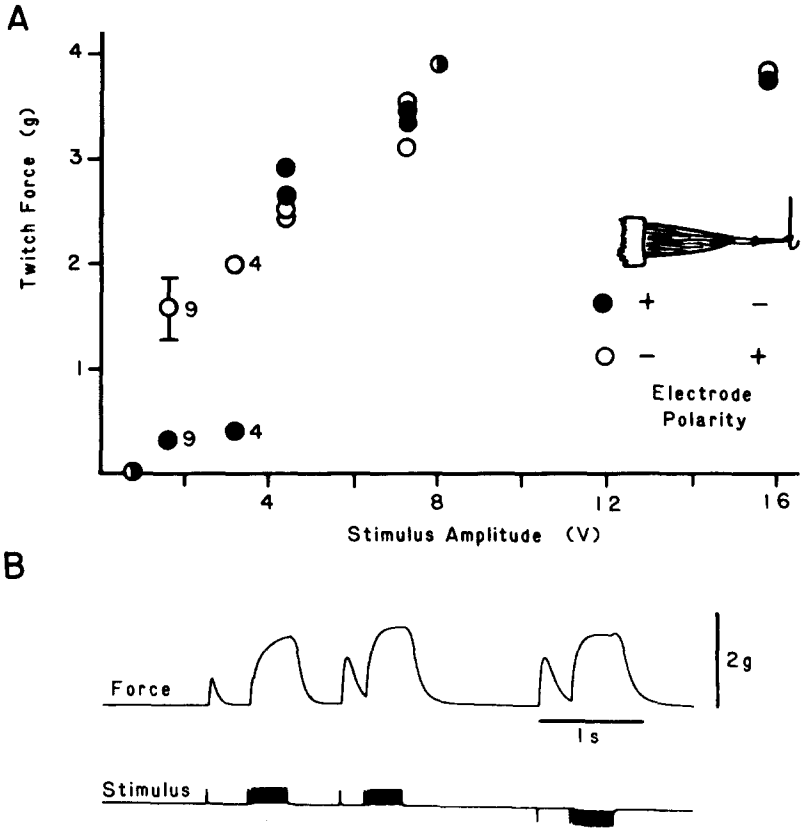

FIGURE 2. Twitches and tetani of scorpion muscle. (*A*) Twitch force plotted vs. stimulus amplitude for a muscle in normal saline (0.4-ms pulse applied via large bath electrodes). The inset indicates the correspondence between the symbol type and electrode polarity. Each symbol represents a single determination, except where indicated. Standard deviation bars are omitted where they were smaller than the symbol. Stimulation occurred once every 12 s. (*B*) Continuous record of twitches and 70-Hz tetani from a maximally excited muscle as defined in *A*. The muscle had been excited at 0.7 Hz prior to the first illustrated twitch. The post-tetanic potentiation of the twitch occurring with the second and third cases is typical, as is the increased rate of tetanic force development. Stimuli: 0.4 ms, 16 V. Solution A. 22JAM1.

Twitches Are Na Dependent and TTX Sensitive

Measurements of twitches and tetani provide a simple assay of the ionic and pharmacological sensitivities of scorpion muscle excitability. Fig. 3*A* shows that addition of 1 μM TTX to the bathing medium rapidly eliminates the twitch. Fig. 3*B* shows a twitch/tetanus series before TTX application. The same procedure after exposure to 1 μM TTX yielded the responses shown in Fig. 3*C*. Both twitch and tetanus are nearly totally blocked. Fig. 3*D* illustrates the maximal level of recovery attained after washing TTX out of the chamber.

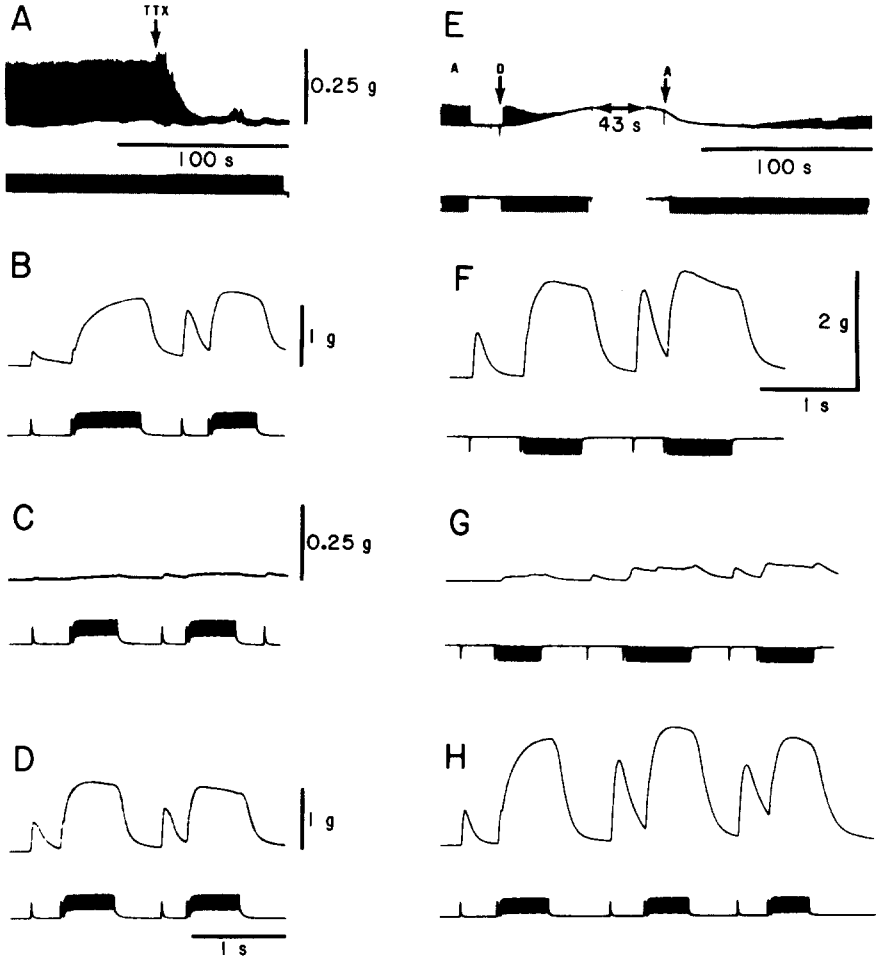

FIGURE 3. Blocking of twitches and tetani by TTX-containing and Na-free media. (*A*) Blocking of twitches (upper trace) by addition of 1 μM TTX to the bath as indicated. The lower trace shows stimuli. (*B*) Twitch/tetanus train (as in Fig. 2*B*) before application of TTX. (*C*) Responses after 4 min in TTX. (*D*) Maximal recovery was attained 45 min after washing out TTX. Stimuli: 0.4 ms, 27 V. 22JAM2. (*E*) Exposure to Na-free saline (solution D) in another muscle, as indicated, and recovery upon readmission of Na (solution A). Na withdrawal contracture was not typical (see text). (*F*) Twitch/tetanus records taken before the application of Na-free medium. (*G*) Responses after 1.2 min in solution D. (*H*) Recovery 7 min after returning to normal saline. Stimuli: 0.4 ms, 16 V in *E–G*, 27 V in *H*. 22JAM1.

Similar results were obtained when external Na was replaced by NMG (solution D, Table I). The results of such an experiment are illustrated in Fig. 3, *E–H*; the format is analogous to that in *A–D*. As indicated by the voltage-clamp results described below, abolition of the twitch in Na-free or TTX-containing media must reflect inhibition of action potential generation and conduction.

The reversible contracture that developed in the Na-free experiment in Fig. 3*E* was not a typical result. This phenomenon was observed in two separate experiments in this muscle, but was not seen in any other muscles thus studied. By analogy with cardiac (Lüttgau and Niedergerke, 1958; Chapman, 1974) and barnacle striated (Russell and Blaustein, 1974) muscle, Na withdrawal contractures in scorpion may involve Na/Ca exchange across the sarcolemma.

Electrically Stimulated Mechanical Activity Is Eliminated in Ca-free Media

A strong dependence of mechanical activation on extracellular Ca is widespread, and perhaps universal, in invertebrate striated muscle (Zachar, 1971; Hoyle, 1983). Scorpion muscle shares this feature. Fig. 4*A* shows elimination of the twitch in Ca-free saline containing 230 mM Na (solution C, Table I) and recovery following readmission of Ca. Twitch/tetanus records taken before (*B*), during (*C*), and after (*D*) exposure to the Ca-free medium indicate that abolition of mechanical activity is complete and reversible.

The elimination of twitches and tetani upon removal of extracellular Ca in scorpion muscle is clearly different than in frog muscle, where twitches (Armstrong et al., 1972) and tetani (Lüttgau and Spiecker, 1979) persist in Ca-free media. The rates of twitch failure and recovery in the Ca-free (Fig. 4*A*) and Na-free (Fig. 3*E*) experiments are similar, which is consistent with the idea that

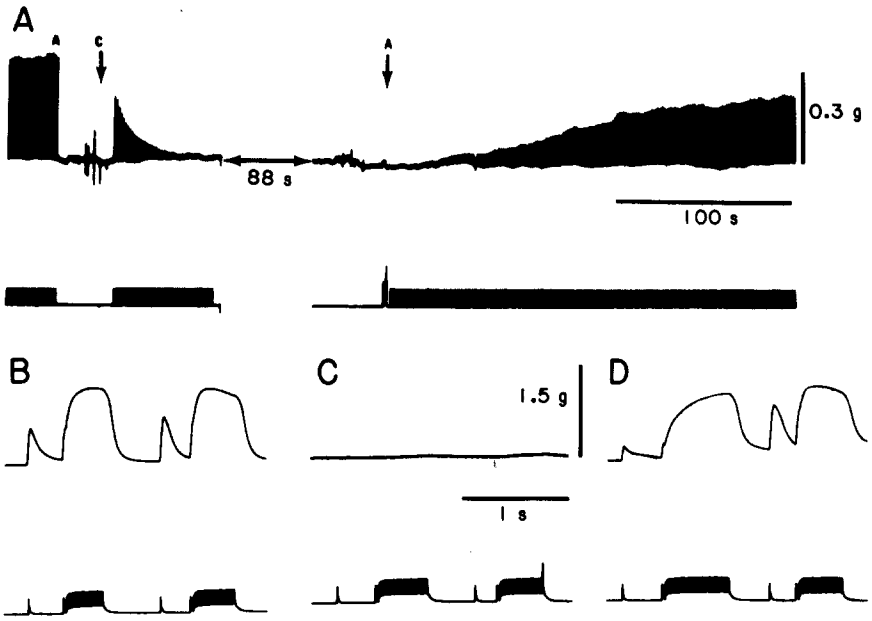

FIGURE 4. Elimination of twitches and tetani by Ca-free saline. (*A*) Twitch reduction caused by the application of Ca-free medium (solution C) and recovery upon readmission of Ca (solution A). (*B*) Twitch/tetanus responses in normal saline before the application of solution C. (*C*) Responses after 2.5 min in Ca-free medium. (*D*) Recovery 5 min after return to solution A. Stimuli: 0.4 ms, 27 V. 22JAM2.

depletion of external Ca is the direct cause of twitch abolition in scorpion. One of the major questions raised in the present report is how this effect of external Ca is mediated. Although inhibition of electrical excitability by Ca-free media might be able to account for the results in Fig. 4, voltage-clamp experiments to be described later indicate that this cannot be the ultimate cause of contractile failure.

Caffeine Causes Contractures of Scorpion Muscle in Ca-free Media

High concentrations of caffeine (>10 mM) result in a contracture nearly equal in magnitude to the maximal tetanic force (or K contracture force) in single frog muscle fibers (Lüttgau and Oetliker, 1968) by leading to a spontaneous release of Ca from the SR (see Endo, 1977). Scorpion muscle is also caffeine sensitive.

Fig. 5*A* shows twitches and tetani from a muscle in normal saline. The application of 20 mM caffeine after removing both external Na and Ca (solution B, Table I) resulted in a strong contracture (Fig. 5*B*). A total of four experiments yielded caffeine contractures that were all 50–60% of peak tetanic force recorded in normal Ringer just before washing out external Na and Ca.

Thus, caffeine can cause strong contractures in scorpion muscle. Since this effect occurs in the absence of external Ca, we conclude that a caffeine-sensitive internal store of Ca exists in scorpion muscle. Presumably, this store is the extensive SR present in these fibers (Gilai and Parnas, 1972, and unpublished observations of W. F. Gilly and C. Franzini-Armstrong on *Vaejovis*).

Voltage-Clamp Studies of Threshold Contractions: the S-D Relation

S-D curves for threshold contractions elicited by voltage-clamp pulses have provided valuable information on the voltage dependence and pharmacological sensitivity of mechanical activation in vertebrate skeletal muscle (Adrian et al., 1969; Costantin, 1974; Almers and Best, 1976). Recently, measurements of voltage-dependent charge movement (Horowicz and Schneider, 1981) and intracellular Ca transients (Kovacs and Szucs, 1983), in combination with contraction threshold determinations, have reinforced the idea that each point on an S-D curve reflects the release of sufficient Ca to raise myoplasmic free Ca to the same threshold level. Thus, the S-D curve provides a sensitive, albeit indirect, indication of the overall voltage dependence of T:SR coupling.

As a means of comparing contractile activation in scorpion muscle with that in vertebrate skeletal muscle, S-D experiments were carried out using methods identical to those used for frog muscle (Gilly and Hui, 1980*a*). Muscle fibers bathed in a Na-free saline (usually containing 1–5 μM TTX as well; solution D, Table I) were voltage-clamped at a point with two microelectrodes. The values of threshold membrane potential for contractile activation with voltage-clamp pulses of 1–50 ms duration were determined visually (see also Methods).

Typical S-D curves for threshold contraction in two different scorpion muscle fibers are shown in Fig. 6. As the pulse duration is decreased beyond 10–20 ms, the amplitude of the depolarization required to elicit a threshold contraction steadily increases. With a 0.5-ms pulse (experiment for upper curve), depolarizations to as high as +70 mV failed to activate contraction. Thus, especially with

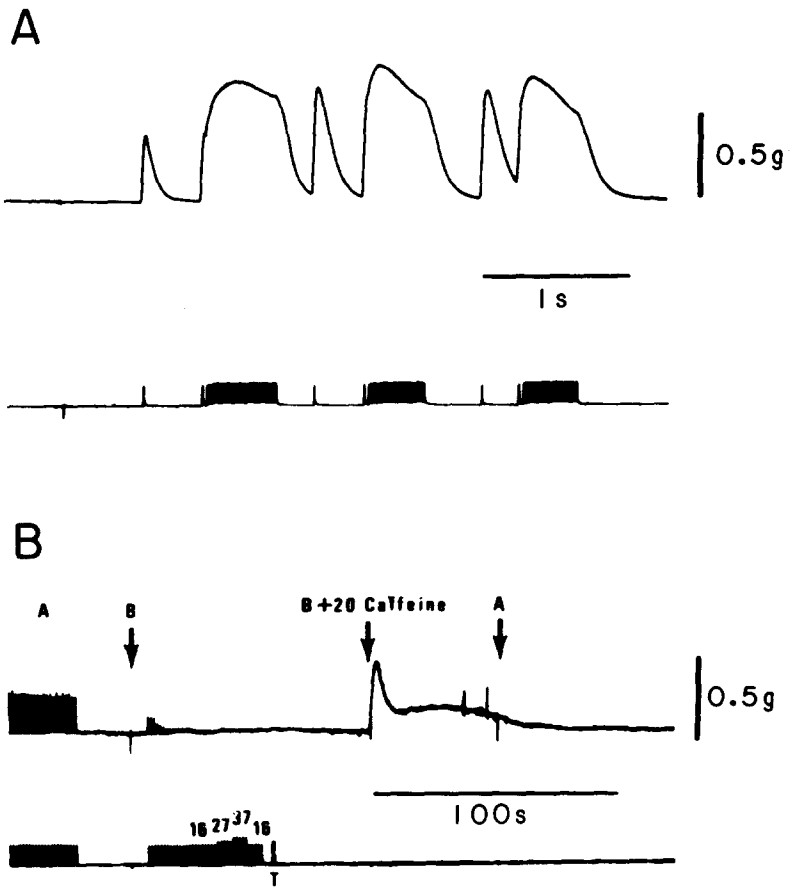

FIGURE 5. Effect of 20 mM caffeine. (*A*) Twitch/tetanus train in normal saline (solution A) before the application of caffeine. Stimuli: 0.4 ms, 16 V. (*B*) Twitches in normal saline are eliminated upon application of Ca- and Na-free saline (solution B). The application of 20 mM caffeine (in solution B) elicits a strong contracture. Relaxation accompanies the return to normal saline. Stimuli: 0.4 ms, voltages as indicated; T denotes 16 V, 70 Hz stimulation. 22JAM2.

brief pulses, the threshold depends strongly on the pulse duration. These data demonstrate the nature and approximate range of fiber-to-fiber variability in scorpion S-D curves. Curves were usually similar in shape, but they differed in their vertical position along the voltage axis.

The mean values of threshold membrane potential vs. pulse duration from 12 muscle fibers studied in Na-free saline are plotted in Fig. 7 (open circles). These values are also listed in Table II and compared with those obtained in normal saline in the presence of 1–10 μM TTX (solution A, Table I). No significant differences exist between the two populations. We therefore conclude that the failure of twitch and tetanus in Na-free media simply reflects a failure of the action potential, as suggested earlier.

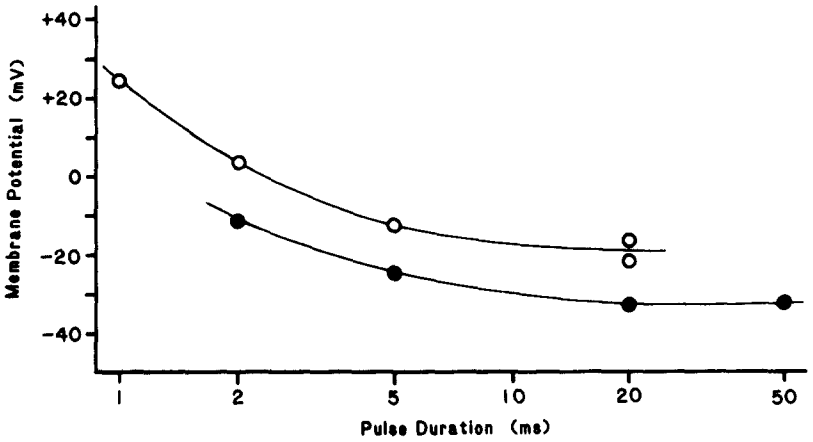

FIGURE 6. S-D curves from two different scorpion muscle fibers. Voltage steps of variable amplitude and duration were applied approximately once every 20 s. This interpulse interval was sufficient to minimize the interaction between pulses. For each duration, the minimum amplitude pulse that produced a contraction was determined. This threshold membrane potential is plotted vs. pulse duration. Data from these two fibers illustrate the approximate voltage range spanned over all experiments. (○) Fiber studied in solution D (Na-free) plus 1 μM TTX. 16DE32. (●) Fiber studied in solution D plus 2 μM TTX. 16SE31.

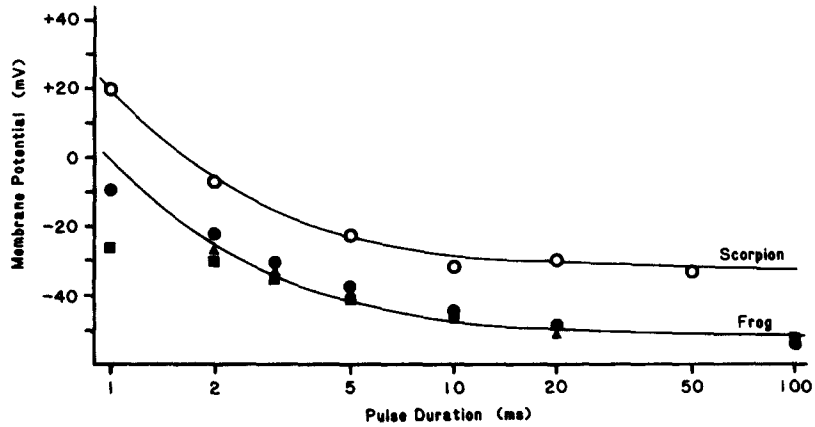

FIGURE 7. Comparison of S-D curves in scorpion and frog muscle. (○) Mean values of threshold potential (see also Table II, solution D) for activation of contraction in scorpion muscle are plotted as a function of pulse duration. The solid curves through the points were drawn by eye. Filled symbols represent published results from frog muscle. (▲) Six twitch fibers at 20°C from Fig. 3 of Gilly and Hui (1980*a*). (■) Twitch fibers at 22°C from Table I of Costantin (1974). (●) 21 slow fibers (2 fibers at 1 ms) at 20°C from Fig. 3 of Gilly and Hui (1980*a*). The curve through the filled symbols is the same curve as above, displaced 19.5 mV in the negative direction.

TABLE II

Strength-Duration Curves

Solution	Pulse duration (ms)					
	1	2	5	10	20	50
(A) Na-containing						
Contraction threshold (mV)						
Mean	23.7	−0.2	−10.2	−20.3	−27.3	−15
SEM	22.0	9.26	6.78	6.36	3.18	—
n	3	6	5	3	8	1
(D) Na-free						
Contraction threshold (mV)						
Mean	20	−6.9	−22.5	−32.0	−29.2	−33.0
SEM	—	4.3	1.9	1.7	1.6	0.3
n	2	9	10	3	12	5

S-D curves for threshold contractions in frog muscle fibers measured at similar temperatures are also plotted in Fig. 7 (filled symbols, from Table I of Costantin, 1974, and Fig. 3 of Gilly and Hui, 1980*a*). Although the threshold in frog muscle occurs at a more negative voltage at all durations, the relationship between the membrane potential and the stimulus duration has a shape similar to that in scorpion. The smooth curve through the scorpion data is also plotted after displacing it downward by 19.5 mV, and the frog points are adequately fitted. The discrepancy at 1 ms must be regarded as tentative, since determinations were made in only two scorpion and four frog fibers.

Tetracaine Interferes with Contractile Activation

As another basis of comparison between scorpion and frog muscle, S-D curves were obtained in the presence of tetracaine. This local anesthetic has profound effects on the contraction threshold in frog twitch (Lüttgau and Oetliker, 1968; Almers and Best, 1976) and slow (Gilly and Hui, 1980*b*) fibers. At a concentration of 2 mM, tetracaine drastically increases the threshold for short pulses, while having a far smaller effect for long pulses.

Scorpion muscle is similarly affected by tetracaine, and the effect of 0.5 mM tetracaine on the S-D curve is demonstrated in Fig. 8. A control curve was first determined (filled circles). Tetracaine was then added to the bathing medium and another curve was obtained (open circles). Finally, the control solution was returned to the bath and the contraction threshold was periodically monitored using 5-ms pulses during recovery (stars). Substantial recovery occurred by 11 min, at which time the experiment failed.

The contraction threshold was more positive in the presence of tetracaine at all pulse durations. However, the effect is more marked for short pulses. The threshold for 20-ms pulses is only raised ~10 mV by tetracaine, whereas the 5-ms value is elevated by nearly 40 mV. Finally, it was impossible to elicit a contraction with 2-ms pulses to potentials as positive as +70 mV in the presence of the drug.

Results similar in every regard to those in Fig. 8 were found in one other fiber successfully studied in 0.5 mM tetracaine. In another muscle fiber, 1 mM

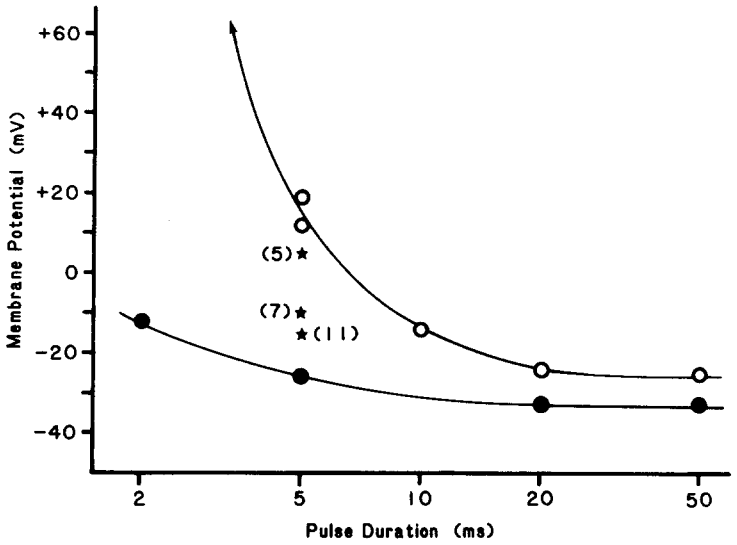

FIGURE 8. Effect of tetracaine on contractile activation. S-D curves were determined from a single fiber in the absence (●) and presence (○) of 0.5 mM tetracaine. Contraction in tetracaine could not be activated with a 2-ms pulse. Recovery was monitored using 5-ms pulses (★). The time in minutes after returning to control solution is indicated by the numbers associated with each star. The bath was rinsed with fresh, tetracaine-free solution between 5 and 7 min of recovery. Solution D plus 2 μM TTX. 16SE31.

tetracaine reversibly eliminated contraction with 20-ms pulses to as high as +30 mV, but longer pulses were not investigated. These results are similar to those obtained with 2 mM tetracaine in frog (Almers and Best, 1976).

Voltage-dependent Contractile Activation Requires External Ca

The results from S-D experiments described thus far indicate strong similarities between scorpion and frog muscle. In sharp contrast, however, stands the observation that contractile activation under voltage-clamp conditions in scorpion muscle is abolished in the absence of extracellular Ca (cf. Chiarandini et al., 1980, and Gilly and Hui, 1980*a*, for work on frog).

Exposure to Ca-free solutions (B and C in Table I) rapidly and reversibly eliminated contractile activation in response to voltage-clamp depolarizations, however large. This result was obtained in five different fibers with 20-ms pulses to membrane potentials as high as +80 mV. Including EGTA in the Ca-free solution was not necessary to abolish contractile activation. Thus, scorpion muscle, unlike frog, is critically dependent on extracellular Ca for normal contractile activation by changes in membrane potential.

Contractile failure in scorpion muscle under voltage-clamp conditions in Ca-free solutions cannot simply reflect action potential failure. Another possibility is that Ca-free media interfere with contractile activation in scorpion muscle by abolishing an inward Ca current.

Fig. 9 shows records of the total injected current (I_o) for a depolarization from

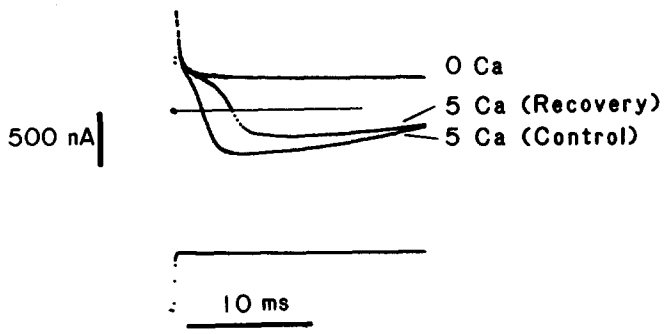

FIGURE 9. Dependence of TTX-insensitive inward current on external Ca. Total injected current (top traces) is shown in the presence and absence of Ca for a depolarization from −80 to −20 mV (bottom traces). The 5 Ca record shows control current (solution A plus 10 μM TTX). The 0 Ca trace shows that this current is eliminated in the Ca-free solution D. The recovery trace was obtained after 4 min in solution A. 10 μM TTX throughout. 10AU35.

−80 to −20 mV, a level suprathreshold for contractile activation with a 20-ms pulse. The current measured in normal saline plus TTX is clearly inward [5 Ca (Control) trace]. Removal of Ca from the bathing medium (solution C, Table I) completely eliminates this current (0 Ca trace) and abolishes contractile activation. The 5 Ca (Recovery) trace indicates that the procedure is reversible. Although the total current measured with the two-electrode point clamp does not accurately reflect membrane current from a voltage-clamped region, it does indicate that the net inward current occurs at some point along the fiber and that this current depends on external Ca ions.

Preliminary experiments with 0.5 mM Cd in the presence of 5 mM Ca indicated a total abolition of contractile activation, as with Ca-free media, and a large apparent decrease in inward current at any given voltage. In the absence of a quantitative measurement of membrane current, such experiments were not pursued. These results are consistent, however, with an important role of external Ca, possibly as a carrier of inward current, in some step of T:SR coupling in scorpion muscle.

Appreciable Inward Current Can Occur without Contractile Activation

Fig. 10 shows the results from an experiment in Na-free saline in which the voltage-dependent development of inward Ca current can be compared with the onset of mechanical activation. Voltage and I_o records for three different pulse durations are shown, and for each duration examples of subthreshold (i.e., for contraction), just-threshold (THR traces), and suprathreshold records are included. With the 2-ms pulses (Fig. 10*A*), the inward current occurred with every step shown, even those considerably negative to contraction threshold. There is no dramatic difference among the inward current traces for any of these pulses. Basically the same result is obtained with 5-ms pulses (Fig. 10*B*), but the growth of inward current with small increases in voltage becomes more apparent. With still longer steps, e.g., 10 ms (Fig. 10*C*), there appears to be very little, if any,

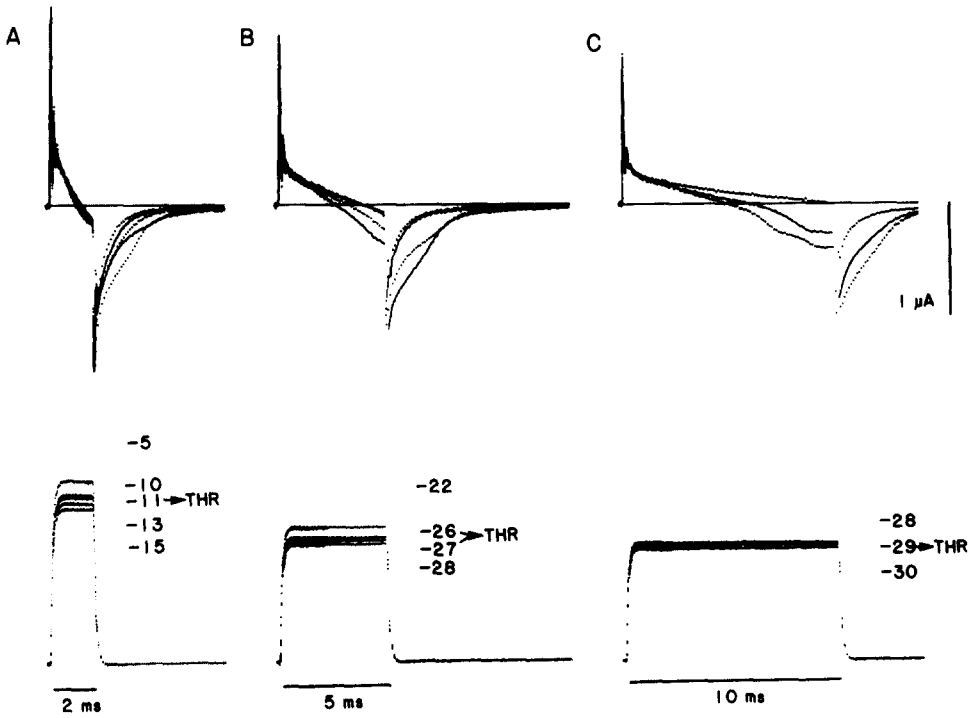

FIGURE 10. Development of inward current with voltage in relation to contraction threshold when studied with brief pulses. The contraction thresholds and the total current (top traces) were recorded in response to pulses (bottom traces) of three different durations: 2 (*A*), 5 (*B*), and 10 ms (*C*). Membrane voltages during the pulses (in millivolts) are noted adjacent to the voltage traces. THR indicates the contractile threshold. Current traces grow in amplitude, both during and after the pulse, in response to voltage steps of increasing size. Solution D plus 2 μM TTX. 29OC32.

inward current at −30 mV, whereas at −29 mV the inward current and contraction are clearly activated. Thus, the results indicate that for pulses up to 10 ms long, the contractile threshold is always more positive than the voltage at which the net inward (Ca) current first appears.

Because the net current is a measure of total outward as well as inward Ca current, the true voltage at which inward current begins to activate must be slightly more negative than the traces shown in Fig. 10 would indicate. Whatever that exact voltage might be, the conclusion remains that inward current occurs at a more negative voltage than does contractile activation.

One problem with the point-clamp method used here does bear on this apparent correlation. Both longitudinal and radial transmembrane voltage gradients exist, and the region of fiber from which current is measured is therefore not defined. With a negative slope conductance, as must underlie the inward Ca current, it is not certain that inward current is flowing in the superficial region where the fiber is voltage-clamped and where contraction is being monitored.

An associated difficulty is that membrane potential might not be well controlled where contraction is being monitored. Thus, either longitudinal or radial voltage gradients could lead to a situation where the contraction threshold was first reached at a site distant from the intracellular voltage electrode. Judging by the nature of the contractile responses, this does not seem likely. Sarcomere shortening can be resolved sufficiently well to ensure that contraction actually occurs only in the clamped region of the fiber around the microelectrodes. Threshold contractions are typically symmetrical about the electrodes, just as in TTX-poisoned frog muscle (unpublished observations). We have never seen any obvious sign of radial escape from voltage control as occurs in frog muscle when tubular Na currents are present (Costantin, 1970). Both the small diameter of scorpion fibers and the simple tubular geometry tend to make this problem much less severe than in frog.

Longitudinal voltage gradients can also be independently checked to demonstrate adequate control with a second intracellular voltage-sensing electrode. The results from such a control experiment are shown in Fig. 11 for pulses just below (*A*) and just beyond (*B*) the contractile threshold. The experiment was carried out in normal saline without TTX in order to provide the worst possible voltage nonuniformities associated with inward Na and Ca currents. The top pair of traces in each panel shows the controlled voltage, V_c, and that measured independently near the site at which contraction was monitored (V_2). With either of the command pulse amplitudes, V_c and V_2 are similar. The difference traces

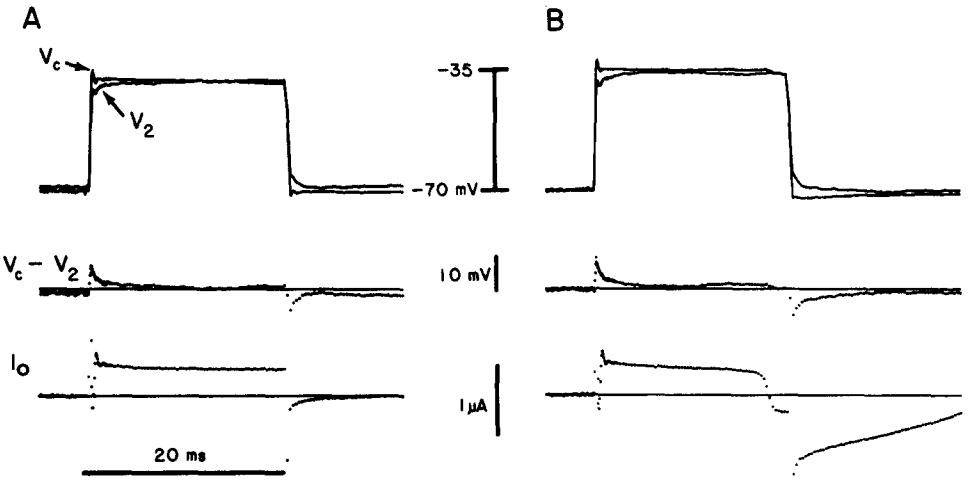

FIGURE 11. Test of voltage nonuniformities near the contraction threshold. The voltage was recorded with a third intracellular microelectrode near the site where contraction was monitored, ~40 μm downstream from the clamped point. Records were obtained in response to voltage pulses from −70 to −38 mV (*A*) and −35 mV (*B*), just subthreshold and just suprathreshold for contraction, respectively. The upper row shows voltage recordings of the controlled voltage (V_c) and the voltage near the site where contraction was monitored (V_2). Records in the second row ($V_c - V_2$) are point-by-point subtractions of the two voltage traces. The bottom row shows total injected current, I_o. Solution A, no TTX. 17JA31.

($V_c - V_2$), obtained by subtraction, indicate that the average amplitude of V_2 is within 1–2 mV of the controlled value, even in the presence of the inward current (I_o, bottom traces) that accompanied the stronger depolarization.

On the basis of control experiments like this one, we feel that our voltage measurements accurately reflect the situation where contractile activation is

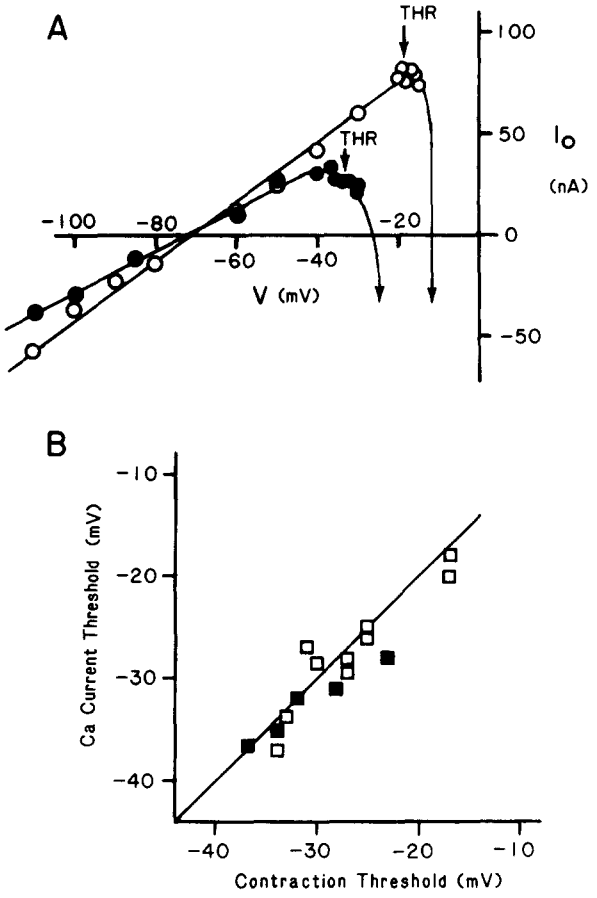

FIGURE 12. Correlation between "threshold" voltages for inward current and contractile activation. (*A*) Total current (I_o)-voltage relationship determined with 20-ms pulses in two fibers with substantially different contraction thresholds. Currents were measured at the end of the pulse when no time dependence was detected. For traces where the currents varied with time, the value recorded was the most negative point on the trace. The voltage determined for the contraction threshold in each fiber is indicated by the arrows (THR). The curves through the points were drawn by eye. Solution D. (●) 02DE38, 5 μM TTX. (○) 06DE32, 1 μM TTX. (*B*) The threshold for contractile activation is plotted vs. the threshold for inward current. The threshold for inward current was taken as the point where the slope of the I_o-V curve, such as those shown in *A*, equalled 0. Solid squares were measured in solution A (Na-containing) plus 1–5 μM TTX. Open squares were measured in solution D (Na-free), both with and without TTX.

being observed. At present, we cannot quantitate the amount of Ca current in this region, however, and this remains an important question.

Correlation of Thresholds for Contraction and Inward Ca Current

As discussed in conjunction with Fig. 10, the rapid increase in the inward current with voltage near the contraction threshold becomes more prominent with longer pulses. In an attempt to test further the idea of a correlation between "threshold" for the inward current and for contraction with long pulses, the following approach was taken (cf. Costantin, 1968; Adrian et al., 1972). A series of 20-ms voltage steps was given over the membrane potential range of −110 to −20 mV, and the steady state value of total current was plotted vs. voltage to form an I_o-V curve that defines input resistance. In some cases, when the transient inward current was obvious, the most negative I_o value was used. Deviations from linearity of the I_o-V curve indicate the presence of voltage-dependent conductances, and activation of inward current causes the slope conductance to change from positive to negative. "Threshold" for the inward current was arbitrarily defined to be the voltage where $dI_o/dV = 0$.

At voltages near contraction threshold, the I_o-V curve in scorpion muscle bends negative because of the appearance of inward current, and examples of two curves are shown in Fig. 12*A*. Contraction thresholds are indicated by the THR arrows. Thus, as with shorter pulses, a sign of inward current either occurs at more negative potentials than the contraction threshold or occurs at the identical threshold voltage. The two curves chosen for Fig. 12*A* emphasize this point; the thresholds for inward current are nearly 20 mV apart, and the contraction threshold follows this pattern.

The threshold for inward current was thus determined for a total of 15 fibers in which I_o-V curves were obtained over a broad voltage range and in which the contraction threshold in response to a 20-ms pulse was determined. Contraction threshold vs. inward current threshold is plotted in Fig. 12*B*. The parameters clearly show a strong correlation.

DISCUSSION

This paper describes electrical and contractile activity in an interesting and potentially useful type of striated muscle fiber. The results described here also allow the most direct comparison to date of T:SR coupling in invertebrate and vertebrate striated muscle. Despite the phylogenetic distance spanned by scorpions and frogs, skeletal muscle fibers from both orders have nearly identical T:SR diad junctions (Gilai and Parnas; 1972; Franzini-Armstrong, 1973*a*, *b*, 1984). Thus, one might expect the mechanisms underlying T:SR coupling also to be alike (cf. Franzini-Armstrong, 1976; Gilly and Hui, 1980*a*, *b*). Our results only partially bear out this expectation, however. Despite strong similarities in the apparent voltage dependence and the tetracaine sensitivity of T:SR coupling in scorpion and frog, some step in scorpion muscle appears to be fundamentally different in its absolute requirement for (extracellular) Ca. Our voltage-clamp experiments on scorpion thus support similar suggestions concerning external Ca in crayfish muscle based on K contracture experiments (Zacharová and Zachar, 1967; see also Zachar, 1971).

Functionally, scorpion muscle behaves more like frog twitch muscle than typical invertebrate muscle and responds to an all-or-none, TTX-sensitive action potential with a brisk twitch. The regenerative electrical activity is considerably more complex than that in frog, however. Scorpion muscle has both voltage-dependent Na and Ca permeabilities with fairly rapid kinetics. On the basis of the action of TTX (Figs. 3 and 9), Na and Ca channels appear to be distinct. This is also suggested by the nature of the firing pattern in scorpion muscle. It is likely that the initial all-or-none action potential is largely carried by Na since conduction is blocked in Na-free or TTX-containing media. The Ca current observed in Na-free and TTX-containing media probably contributes to the repetitive spikes constituting the after-discharge. Similar repetitive spikes can occur in Na-free and TTX-containing solutions (unpublished data).

Although the initial overshooting impulse alone would probably exceed threshold for contractile activation, electrical activity during the after-discharge is probably important to full development of the twitch. During this activity, a considerable amount of Ca might enter the fiber, but in the absence of quantitative voltage-clamp data, it is presently uncertain whether enough Ca could enter to activate the myofibrils directly.

On the basis of our results with caffeine, we conclude that the SR in scorpion muscle can release Ca in response to this drug. Given this and the strong morphological similarities between scorpion and frog muscle, we assume for the present purposes that scorpion SR can also release Ca in response to a depolarization of the surface and tubular membranes. The remainder of this discussion will focus on the comparative details of how surface and tubular membrane potential changes might be coupled to Ca release by the SR in scorpion vs. frog muscle.

In vertebrate skeletal muscle, a step in T:SR coupling is believed to involve a voltage-dependent charge movement that occurs within the tubular membrane in the vicinity of the T:SR junctions (Schneider and Chandler, 1973). This process confers voltage sensitivity to contractile activation. S-D curves for threshold contractions in voltage-clamped muscle fibers were first used by Adrian et al., (1969) to assess this voltage dependence indirectly. Recently, an important correlation between threshold contractions and charge movement has been established. Horowicz and Schneider (1981) found that threshold contractions are accompanied by a fixed amount of charge movement, regardless of the pulse duration. Furthermore, Kovacs and Szucs (1983) have shown that the signal from intracellular antipyralazo III is also of a fixed amplitude for any pulse that produces a threshold contraction. Thus, with pulses of varied duration, a certain amount of charge movement can be found that, in some as yet unknown way, leads to release of a sufficient amount of Ca to exceed threshold for contractile activation. S-D curves in vertebrate muscle must therefore reflect the overall voltage dependence of at least the charge movement and Ca release processes.

The S-D curves reported here for scorpion muscle bear a strong resemblance to those in a variety of vertebrate skeletal muscle types (Costantin, 1974; Gilly and Hui, 1980*a*; Dulhunty, 1980). Based on the arguments above, this could be taken to mean that a mechanistically similar voltage-dependent process mediates

T:SR coupling in both muscle types. This idea is in line with the similarities in diad structure. Unfortunately, charge movement measurements have not been reported from any type of invertebrate muscle, and it is thus possible that scorpion muscle lacks charge movement altogether. In this case, the similarity in S-D curves might be fortuitous, and the scorpion curve would necessarily reflect the voltage dependence of some other process involved in T:SR coupling.

Similarly, our observations of tetracaine's effect on the S-D curve imply that some mechanism closely regulating Ca release from the SR may also be alike in scorpion and frog muscle. Almers and Best (1976) found that tetracaine caused a profound inhibition of Ca release in frog muscle without seriously affecting charge movement (but see Hui, 1983). They concluded that tetracaine paralyzes frog muscle by blocking a Ca release channel in the SR membranes (cf. Almers, 1977). Again, any similarity between results on scorpion and frog could be simply fortuitous, but a common tetracaine-sensitive Ca release mechanism is equally plausible.

These similarities between scorpion and frog are intriguing in light of the absolute requirement for extracellular Ca ions in the T:SR coupling of scorpion muscle and the apparent lack of any such requirement in frog skeletal muscle. Several possibilities present themselves in trying to account for this profound difference in the otherwise similar preparations.

One explanation is that contractile activation in scorpion fails in Ca-free media because Ca in the SR is depleted. Two arguments speak against this idea. (*a*) Caffeine remains effective in causing strong contractures when applied to muscles paralyzed by Ca-free solutions. (*b*) Rates for failure and recovery of contraction in the Ca-free voltage-clamp experiments were similar to those using measurements of twitches and tetani to monitor the rate of extracellular space washout in Na-free or Ca-free media (Figs. 3 and 4).

A second idea assumes that T:SR coupling is qualitatively alike in frog and scorpion muscle, and that the failure of contraction observed in Ca-free media for the latter results from a shift in the voltage dependence for "activation" or "inactivation" of E-C coupling (Hodgkin and Horowicz, 1960). This is also not likely to be correct, for two reasons. (*a*) A broad range of voltages and durations were explored in our voltage-clamp experiments and no sign of contractile activation was even seen in Ca-free media. (*b*) Our holding potential (as negative as −80 mV) should have been sufficiently negative to avoid a total failure of contraction caused by a shift of inactivation to abnormally negative voltages in Ca-free solutions (Lüttgau and Spiecker, 1979).

More interesting and realistic explanations assign an important role to extracellular Ca ions in the T:SR coupling process of scorpion muscle. One obvious possibility is that a coupling-related charge movement does exist in scorpion muscle and that this process is reversibly blocked by removal of tubular Ca. Thus, T:SR coupling in scorpion and frog muscle might fundamentally differ only in the presence or absence of such a Ca-dependent regulatory site on the molecular structures underlying charge movement in the T-membranes. This is a testable hypothesis, and such experiments are in progress.

Another possibility is that Ca influx may be the important factor. The TTX-

insensitive Ca channels in scorpion muscle seem to have rapid enough kinetics to play such a role. Voltage dependence of the Ca channels also appears suitable. Finally, reversible elimination of inward Ca current by Ca-free media or reduction by cadmium ions is accompanied by a failure of contractile activation.

Two general ideas come easily to mind to explain how Ca influx could be important. The first is that Ca ions enter the vicinity of the diadic feet linking T and SR membranes and influence (e.g., catalyze) an event in these structures that is a prerequisite for Ca release. Although an intracellular site of action for Ca exists in this scheme, the regulatory mechanism might be similar to that proposed above where Ca served as an extracellular co-factor for charge movement. For example, an intracellular Ca ion might be required to operate the linkage between charge movement and Ca release in a scheme like that postulated by Chandler et al., (1976). The second idea is that Ca influx directly initiates regenerative release of Ca from the SR. Such a "Ca-induced Ca release" process exists in skinned muscle fibers, but its relevance to contractile activation in intact fibers remains to be established (see Endo, 1977).

Although our results are consistent with the possibility that Ca influx is important to T:SR coupling in scorpion muscle, the correlations we find between the inward current and contractile activation may be coincidental. The Ca current is obviously important to scorpion muscle in supporting the electrical after-discharge underlying the twitch. Thus, from a teleological viewpoint, it would be surprising if Ca current and contractile activation were not matched fairly well in time and voltage dependencies even if Ca influx played no role other than sustaining regenerative depolarization.

Whatever the mechanism might be, the fact that T:SR coupling in scorpion muscle requires extracellular Ca stands as the only major qualitative difference between our results on scorpion and those on vertebrate skeletal muscle. The discrepancy is unlikely to be due to any peculiarity of scorpion muscle. Voltage-clamp experiments on other anthropod muscle fibers, in which release of internal Ca is thought to be important, also indicate a marked dependence on external Ca (Caputo and Dipolo, 1978; but see also Atwater et al., 1974). This is true for vertebrate cardiac muscle as well (see Fozzard, 1977), a muscle type that also has T:SR junctions structurally similar to those in scorpion muscle. It is also unlikely that a failure of T:SR coupling in Ca-free media simply reflects more effective Ca depletion in the scorpion's simple T-system. Both the other above-mentioned muscle types showing external Ca sensitivity have very complex systems of restricted clefts and transverse tubules. The efficacy of tubular Ca depletion in experiments on vertebrate skeletal muscle has been questioned (Miyamoto and Racker, 1982), however, and evidence for Ca influx playing a role in T:SR coupling in frog muscle has recently been presented (Eisenberg et al., 1984; see also Eisenberg et al., 1983).

In summary, our results show that the voltage dependence of contractile activation and the sensitivity of the process to tetracaine are similar in skeletal muscle fibers from scorpion and frog. Our results also indicate a necessary role for external Ca in scorpion muscle. We cannot presently identify this role, nor can we definitely indicate what relevance our observations have to vertebrate

striated muscle, either skeletal or cardiac. Since scorpion and frog skeletal muscle have T:SR diadic junctions that are practically indistinguishable, the idea of fundamentally similar mechanisms of T:SR coupling in both muscle types remains appealing. It may be that the vertebrates have evolved some relatively minor specialization in the T:SR coupling pathway that serves to buffer the process from variations in plasma levels of Ca more effectively than in skeletal muscle fibers from other taxa. Further studies of scorpion muscle fibers and of vertebrate fiber types with experimentally accessible T:SR junctions should help clarify exactly how the coupling processes differ.

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