Charge movement and depolarization–contraction coupling in arthropod vs. vertebrate skeletal muscle
(excitation–contraction coupling/calciium)

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ABSTRACT Voltage-dependent charge movement has been characterized in arthropod skeletal muscle. Charge movement in scorpion (Centuroides sculpturatus) muscle is distinguishable from that in vertebrate skeletal muscle by criteria of kinetics, voltage dependence, and pharmacology. The function of scorpion charge movement is gating of calcium channels in the sarcotubular system, and depolarization–contraction coupling relies on calcium influx through these channels.

Contractile activation in muscle results from a rise in intracellular calcium ion concentration, usually triggered by depolarization of the cell membrane. Coupling of depolarization to contraction in most muscle types also requires extracellular calcium, but the controlling role of calcium is not well understood. Such calcium-dependent muscles include invertebrate skeletal muscle, vertebrate cardiac muscle, and many kinds of non-striated muscle from both groups. Skeletal muscle of vertebrates, however, is relatively insensitive to extracellular calcium (1-3), and depolarization controls release of intracellular calcium from the sarcoplasmic reticulum (SR). Depolarization is sensed by charged macromolecules in the sarcotubular system, and movement of these charged sensors generates a slow capacity current (4-6), which has been convincingly linked to regulation of calcium release (7-9). Neither the precise site nor the mechanism of charge movement (IQ) are known, but involvement of the morphological junctions between transverse tubules (T) and SR (10) is widely accepted (11-14). Because indistinguishable T–SR junctions exist in calcium-dependent muscles (15, 16), the question arises whether these muscle types show IQ like that in vertebrate skeletal muscle.

Here we describe IQ signals from an arthropod (scorpion) muscle in which depolarization–contraction coupling is abolished in the absence of external calcium (17). These signals display the properties expected for intramembrane movement of charged voltage sensors for sarcocellular calcium channels that exist at high density. The same properties distinguish arthropod IQ from its vertebrate counterpart, which underlies coupling across the T–SR junction. Our results indicate that a capacitive gating current indeed confers voltage sensitivity to depolarization–contraction coupling in both arthropod and vertebrate skeletal muscle but show also that the processes gated in the two cases are fundamentally different.

MATERIALS AND METHODS

Experiments were performed on pedipalp closer muscles (18) of Centuroides sculpturatus. Scorpion muscle was chosen because of the pronounced sensitivity of contractile activation to external calcium (17) and the well-developed SR with extensive regions of T–SR junctions (19) in this muscle type. Its small fiber size and simple T system (20) also give it favorable electrical properties. A standard three-microelectrode voltage clamp technique (21) provided a measure of membrane current density over the terminal 200–300 μm of a muscle fiber where it inserts onto the tendon-like apodeme. Control voltage and ΔV signals were digitized at 10 kHz. Test voltage pulses were followed by control pulses (stored separately) used to subtract linear currents during analysis.

Charge movement (IQ) was measured in a calcium-free medium that minimized time-dependent ionic currents and eliminated contraction. This solution contained 250 mM tetraethylammonium chloride, 7.5 mM RbCl, 10 mM MgCl2, and 10 mM Tris (adjusted to pH 7.0 with HCl). In some experiments 5 mM Cs-EGTA was included, but this did not affect results to be described here. Barium current (IBa) was recorded by omitting EGTA and adding 3 mM BaCl2. Substitution of barium for calcium also blocks contraction. Experiments with higher barium concentrations were not possible because of inability of the voltage-clamp method to adequately control membrane potential.

RESULTS

Fig. 1 compares IBa and IQ at −15 mV. Currents flowing in response to test and control pulses in the presence of 3 mM barium are shown in Fig. 1b. Subtraction of the control from test record yields the trace in Fig. 1c. Current during the pulse is characterized by a small outward transient followed by a large inward current. A rapidly deactivating tail follows the pulse.

Results from the same fiber that were obtained after removal of barium from the medium and addition of 5 mM EGTA are shown in an analogous format in Fig. 1d-f. Inward current is abolished (Fig. 1e and f). Records in Fig. 1e were also treated individually to remove time-independent ionic current (6) and then subtracted. The resultant in Fig. 1g reveals the outward transient at pulse "ON" and a faster inward "OFF" transient following repolarization. We refer to this signal, analytically generated in this manner, as voltage-dependent charge movement.

Barium-sensitive currents like that in Fig. 1 are identifiable with voltage-dependent calcium channels. Similar currents are carried by calcium and strontium ions, with ICa being roughly twice as large as IBa when each divalent cation is tested individually at identical concentration (1-1.5 mM; higher calcium concentrations support contraction, which prohibits studying ICa). These data will be described in detail elsewhere. IBa is blocked by transition element ions (Co, Ni, and the like).

Abbreviations: IQ, charge movement; IBa, barium current; GBa, barium conductance; ICa, calcium current; SR, sarcoplasmic reticulum; T, transverse tubule(s).
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Zn, Cd) and by D600 (methoxy derivative of verapamil) and is unaffected by micromolar tetrodotoxin. We have never observed sodium current or tetrodotoxin-sensitivity when working at the fiber ends, even in the presence of 250 mM NaCl.

Charge-movement measurements require that care be taken to avoid residual ionic current through activated calcium channels (22), especially in calcium-free media (23, 24). Cobalt blocks $I_{Na}$ (Fig. 2a), but addition of 5 mM cobalt to the calcium-free medium has no appreciable effect on $I_Q$ (Fig. 2b). Ionic current passing through calcium channels under these conditions and contaminating $I_Q$ records must be slight.

Fig. 3a shows a family of $I_Q$ traces covering a large activating-voltage range. Outward ON transients grow continuously in amplitude and become faster with increasing voltage. OFF transients grow in amplitude to a saturating level and have constant kinetics. Time integrals of ON and OFF responses ($Q_{ON}$ and $Q_{OFF}$) are plotted vs. activating voltage in Fig. 3b. $Q_{ON}$ and $Q_{OFF}$ moved with each pulse are approximately equal over the entire voltage range, and the points describe a saturating, sigmoidal curve.

$Q_{ON}$ vs. $Q_{OFF}$ equality is a fundamental property of a capacitive charge movement. This equality was also tested by recording OFF responses over the voltage range from −40 to −90 mV following a fixed activating pulse to 0 mV (not illustrated). $Q_{OFF}$ did not depend on the return voltage and was always within 15% of $Q_{ON}$. This test more firmly identifies our $I_Q$ signal as a capacity current.

Three additional properties of $I_Q$ in scorpion muscle suggest that it is associated with calcium-channel gating. The
same properties indicate that scorpion $I_Q$ is distinct from that related to T–SR coupling in vertebrates.

First, scorpion $I_Q$ is kinetically appropriate for gating rapidly activating calcium channels (Fig. 1 c vs. f). At 7°C, the fastest vertebrate T–SR signal from mammalian muscle is slower by a factor of 2.5–5 (for ON and OFF respectively; ref. 25); that from amphibian muscle is slower still. Differences in T–system geometry cannot account for these large kinetic differences.

Second, the voltage dependence of scorpion $I_Q$ (Fig. 3b) is appropriate for calcium-channel gating and quite different from that of $I_Q$ in vertebrate muscle. The relation between barium conductance ($G_{Ba}$) and voltage in scorpion is plotted in Fig. 3c, and the voltage dependence is similar to that in Fig. 3b. Data for $I_Q$ and $G_{Ba}$ from several experiments were fit with a simple two-state model (4, 5), and results are given in Table 1. $G_{Ba}$ has a slightly steeper voltage dependence ($k$) and more positive midpoint ($V$) than $I_Q$, but the differences are small. Published values of analogous parameters describing vertebrate $I_Q$ are also tabulated. Scorpion $I_Q$ is approximately twice as steeply voltage-dependent.

The third observation is pharmacological. Tetracaine (1 mM) drastically depresses $I_Q$ (Fig. 4a) and eliminates $I_{Ba}$ (Fig. 4b) in scorpion muscle. The strong block of these signals by this drug does not depend on activating voltage (between −80 and −110 mV). These results stand in sharp contrast to the comparatively minor effects of tetracaine on vertebrate $I_Q$ (29), at least in similarly sized fibers (30), which are most apparent over a certain activating-voltage range (31). $I_Q$ in Fig. 4a that persists in tetracaine does not resemble vertebrate $I_Q$.

Other possibilities for the functional role of charge movement in scorpion muscle are sodium- and/or potassium-channel gating. We have characterized the well-developed potassium conductance ($G_K$) in this muscle and find it to be slower and less voltage-dependent than $G_{Ba}$. These and other differences between $G_K$ and $G_{Ba}$ reinforce our identification of $I_Q$ as calcium-channel gating current and will be described elsewhere in detail. Contribution of sodium-channel gating current to our $I_Q$ records can also be discounted, because sodium current is not present at the fiber ends where these experiments were carried out (cf. ref. 32).

**DISCUSSION**

Results presented in this paper describe the voltage-dependent charge movement ($I_Q$) from an invertebrate skeletal muscle type in which contractile activation is critically
dependent on extracellular calcium. The twitching to the verterbrate $Q_T$, the tetracaine-sensitive component hypothesized to be most closely related to T-SR coupling (31, 33). This seems unlikely, because the slow delayed kinetics of $Q_T$, a striking signature of the phenomenon, bear no resemblance whatsoever to our records. Second, one might suppose that a "missing" slow component of $Q_T$ could exist that would make it verterbrate-like and that this component is critically dependent on extracellular calcium. Since our measurements were made in calcium-free medium, they might not reveal the true picture. Again, this is unlikely. $I_Q$ signals recorded in calcium-free medium and in one containing 1-1.5 mM calcium and 10 mM cobalt showed no obvious differences. Furthermore, calcium-free solutions do not cause a voltage-dependent "inactivation" of $I_Q$ at ordinary holding potentials. $I_Q$ in calcium-free medium is not affected by changes in holding potential between -70 and -100 mV, nor is the abolition of contractile activation relieved.

Thus, we conclude that no part of $I_Q$ in scorpion muscle is analogous to any part in vertebrate skeletal muscle. The function of scorpion charge movement involves calcium-channel gating.

If all of the voltage-dependent charge movement in scorpion muscle is assumed to gate calcium channels, several important channel properties can be derived. From the steepness of the $G_{Ba}$-voltage relation (Fig. 3e), it follows that six elementary charges moving completely across the membrane could gate one calcium channel. With 12.8 nC/µF of total charge, this yields $1.3 \times 10^{10}$ calcium channels per µF of membrane capacity. Maximal $I_{Ba}$ in 3 mM barium is 100 µA/µF at -10 mV (see legend to Fig. 3), and the value in 3 mM calcium would be at least 200 µA/µF (see above). Because of strong contractions and poor voltage control accompanying large $I_{Ca}$, direct measurement is impossible, but maximal $I_{Ca}$ in 5 mM calcium (physiological level) is probably 300-400 µA/µF. Dividing 350 µA/µF by the channel density yields a single-channel current of ≈0.03 pA at -10 mV.

This value is encouragingly similar to those determined from fluctuation analysis in bovine chromaffin cells: 0.09 pA in 5 mM calcium and 0.025 pA in 1 mM calcium at -12 mV (34). Although single-channel currents in these disparate preparations may simply be that different, several other factors may contribute to the smaller value calculated for scorpion channels (see ref. 35). First, peak $I_{Ca}$ might be underestimated due to current rundown. Second, total $I_Q$ might be overestimated by including minor components unrelated to calcium-channel gating. Third, six gating charges per channel is necessarily a lower limit. Finally, it is possible that some calcium channels cannot open and conduct properly, despite the operation of all or part of their gating apparatus. Such a pool of dormant channels might be subject to hormonal control like that occurring in vertebrate cardiac muscle, where β-adrenergic agonists can increase the number of conducting channels (ref. 36; cf. ref. 37). Serotonin has been suggested to have such a controlling role in crustacean muscle (38), an arthropod type closely related to scorpion.

Even if scorpion $I_Q$ serves only to gate calcium channels, it still plays an important role in depolarization-contraction coupling. Electrical excitation of a scorpion muscle fiber with a brief current pulse elicits a burst of calcium action potentials that can last for 100 msec or more (17). This discharge injects a large amount of calcium into the myoplasm from the extracellular space. A calcium influx of 300 µA/µF (see above) would raise total fiber calcium at a rate of 6 µM/msec (40 µm diameter fiber, 4 µm³/cm² capacity). This value approaches calcium-release rates cited for amphibian skeletal muscle (39, 40) and undoubtedly serves as a major source of activator calcium for a single twitch.

**Table 1.** Charge movement and barium conductance in isotonic solutions

<table>
<thead>
<tr>
<th>Muscle source</th>
<th>$Q_{max}$, nC/µF</th>
<th>$G_{max}$, mS/µF</th>
<th>$V$, mV</th>
<th>$k$, mV</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frog*</td>
<td>25</td>
<td>-30</td>
<td>12</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>26.3</td>
<td>-26</td>
<td>14.9</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>12.8 ± 2.5</td>
<td>22.9 ± 2.0</td>
<td>5.8 ± 0.7</td>
<td>This work</td>
<td></td>
</tr>
<tr>
<td>Scorpion*</td>
<td>2-3</td>
<td>-18.5 ± 2.2</td>
<td>4.2 ± 0.4</td>
<td>This work</td>
<td></td>
</tr>
</tbody>
</table>

* Rana species.
† Nonparalyzed fibers were studied at potentials negative to contraction threshold.
‡ Scorpion parameters were obtained by fits of single-channel currents as described in legend to Fig. 3b.
§ Conductance-voltage curves were constructed as described in legend to Fig. 3c.
||" $I_{Ba}$ is quite labile in these fibers and values reported here are typical of the largest conductance observed in 3 mM barium.

The charge movement was measured as described in legend to Fig. 3b. $I_{max}$ records at -15 mV in the absence or presence of 1 mM tetracaine. The recovery trace was measured 26 min after returning to the control solution. Two sweeps were averaged. Temperature, 6°C; fiber 26FE53.

**Fig. 4.** Effect of 1 mM tetracaine on $I_Q$ (a) and $I_{Ba}$ (b). (a) $I_Q$ at -10 mV in the absence (control) or presence of 1 mM tetracaine. Sixteen sweeps were averaged. Temperature was 9°C; fiber 12FE54. (b) $I_{Ba}$ records at -15 mV in the absence (control) or presence of 1 mM tetracaine. The recovery trace was measured 26 min after returning to the control solution. Two sweeps were averaged. Temperature, 6°C; fiber 26FE53.
Whether or not calcium influx is the sole activator source cannot be answered at present, and additional studies are needed (see ref. 41). It remains possible that calcium release from the SR can occur in scorpion muscle under certain conditions, such as tetanic stimulation. The ratio of tetanic/twitch force is very high and a profound post-tetanic potentiation of twitch force exists (17). Electrophysiological measurements of $I_{Ca}$ give no indication that these results are mediated by effects on calcium-channel activation. If calcium release occurs, the mechanisms underlying it are unknown. One possibility is that an internal messenger substance might regulate calcium release in scorpion muscle on this slower time scale, much as inositol trisphosphate appears to do in vertebrate skeletal muscle (42).

Thus, the nature of charge movement and the mechanism of depolarization–contraction coupling in scorpion muscle, and probably in other calcium-dependent muscle types (43), appear to be fundamentally different from the processes in vertebrate skeletal muscle. $I_{Ca}$ in arthropod skeletal muscle gates calcium channels, which rapidly inject activator for contraction. $I_{Ca}$ in vertebrate skeletal muscle reflects a different phenomenon, which regulates calcium release by the SR. If $T$–SR dyad/triad couplings with no obvious morphological differences are the actual sites of depolarization–contraction coupling in both arthropods and vertebrates, and such different mechanisms are operative, it is not surprising that structural studies have failed to yield definitive clues as to mechanisms of transmission. Whether structural parallels reflect mechanistic similarities of any other physiological processes remains to be seen.

Note Added in Proof. Recently, the tetracaine-sensitive component of charge movement in mammalian skeletal muscle has been linked to the gating of voltage-controlled calcium channels in the T system of that preparation (44, 45).

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