Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 8799–8803, November 1986
Neurobiology

Charge movement and depolarization–contraction coupling in arthropod vs. vertebrate skeletal muscle

(excitation–contraction coupling/calcium)

TODD SCHEUER* AND WILLIAM F. GILLY†

Hopkins Marine Station, Stanford University, Pacific Grove, CA 93950

Communicated by Daniel Mazia, July 28, 1986

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 sarcolemma, 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 sarcolemma, 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 (I_Q) 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 I_Q like that in vertebrate skeletal muscle.

Here we describe I_Q 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 sarcolemmal calcium channels that exist at high density. The same properties distinguish arthropod I_Q 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. Controlled 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 (I_Q) 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 $MgCl_2$, 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 (I_{Ba}) was recorded by omitting EGTA and adding 3 mM $BaCl_2$. 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 I_{Ba} and I_Q at −15 mV. Currents flowing in response to test and control pulses in the presence of 3 mM barium are shown in Fig. 1*b*. Subtraction of the control from test record yields the trace in Fig. 1*c*. 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. 1 *d–f*. Inward current is abolished (Fig. 1 *e* and *f*). Records in Fig. 1*e* were also treated individually to remove time-independent ionic current (6) and then subtracted. The resultant in Fig. 1*g* 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 I_{Ca} being roughly twice as large as I_{Ba} when each divalent cation is tested individually at identical concentration (1–1.5 mM; higher calcium concentrations support contraction, which prohibits studying I_{Ca}). These data will be described in detail elsewhere. I_{Ba} is blocked by transition element ions (Co, Ni,

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: I_Q, charge movement; I_{Ba}, barium current; G_{Ba}, barium conductance; I_{Ca}, calcium current; SR, sarcoplasmic reticulum; T, transverse tubule(s).

*Present address: Department of Pharmacology SJ-30, University of Washington, Seattle, WA 98195.
†To whom reprint requests should be addressed.

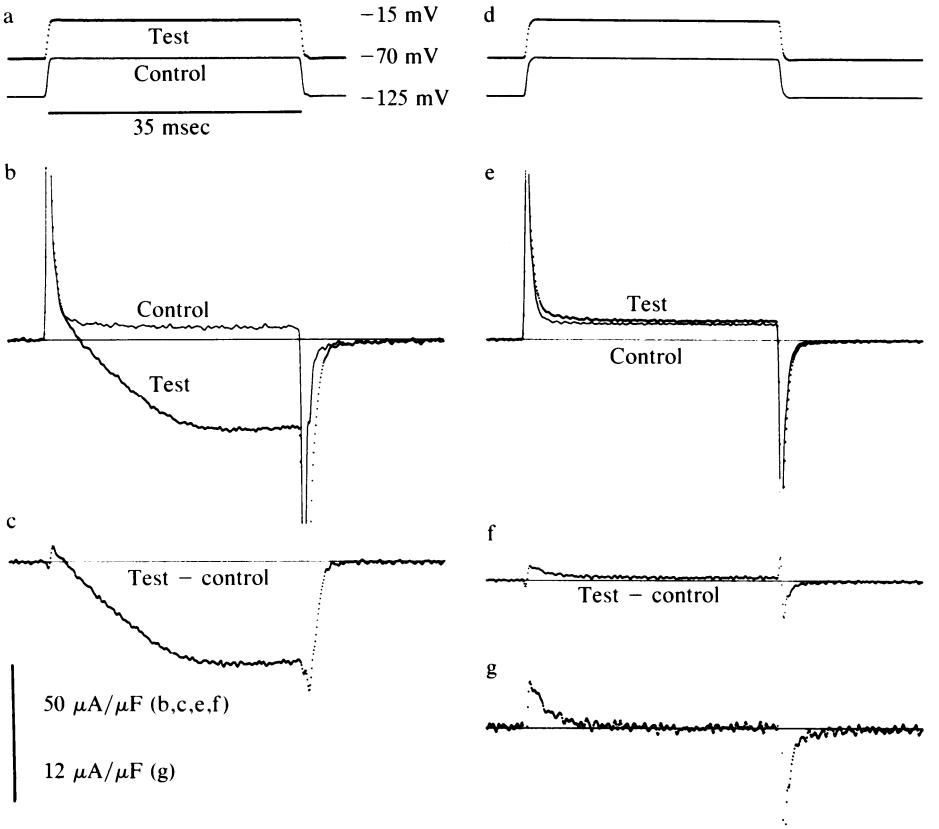

FIG. 1. I_{Ba} (*a–d*) and I_Q (*d–g*) in a scorpion muscle fiber. (*a* and *d*) Test and control voltage pulses. The test depolarization was delivered first from the normal holding potential (−70 mV). Then, 200 msec after hyperpolarization of the fiber to −125 mV, the control pulse was applied. (*b* and *e*) Currents recorded in response to test and control pulses in 3 mM barium (*b*) and in the absence of barium (*e*). Capacity current transients have been truncated. (*c* and *f*) Traces resulting from direct subtraction of control from test records. (*g*) I_Q record generated by individually correcting traces in *e* for time-independent ionic current before subtracting (see ref. 6). Eight sweeps were averaged. Temperature was 7.5°C. Fiber no. was 05FE52.

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_{Ba} (Fig. 2*a*), but addition of 5 mM cobalt to the calcium-free medium has no appreciable effect on I_Q (Fig. 2*b*). Ionic current passing through calcium channels under these conditions and contaminating I_Q records must be slight.

Fig. 3*a* 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. 3*b*. 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

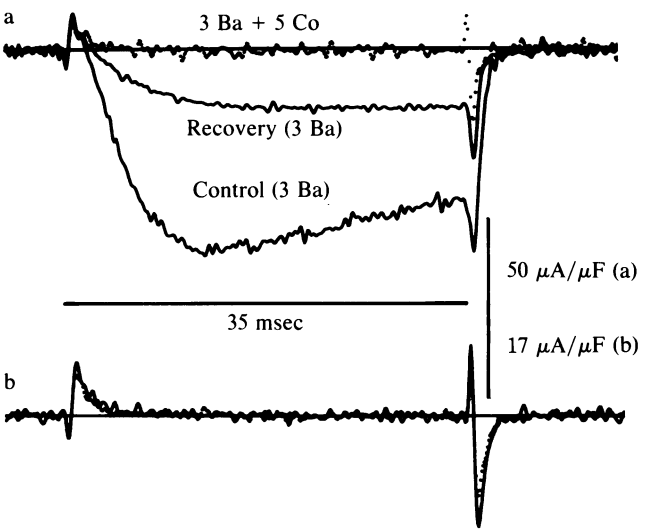

FIG. 2. Effect of cobalt on I_{Ba} and I_Q. (*a*) I_{Ba} (3 mM barium) at −15 mV before [Control (3 Ba)] and during (3 Ba + 5 Co) exposure to 5 mM $CoCl_2$ (four sweeps were averaged for each). The recovery trace [Recovery (3 Ba)] was measured 21 min after returning to the control solution (eight sweeps were averaged). (*b*) Superimposed I_Q records in the absence (solid trace) and presence (dotted trace) of 5 mM $CoCl_2$. EGTA was omitted from the cobalt solution. Sloping baselines were fit to the last 200 points of the recorded currents (not illustrated) during the pulse and subtracted from the appropriate portion of each record. A similar procedure was applied to "OFF" transients following the pulse. Sixteen sweeps were averaged. Temperature, 6.5°C; fiber 04AP52.

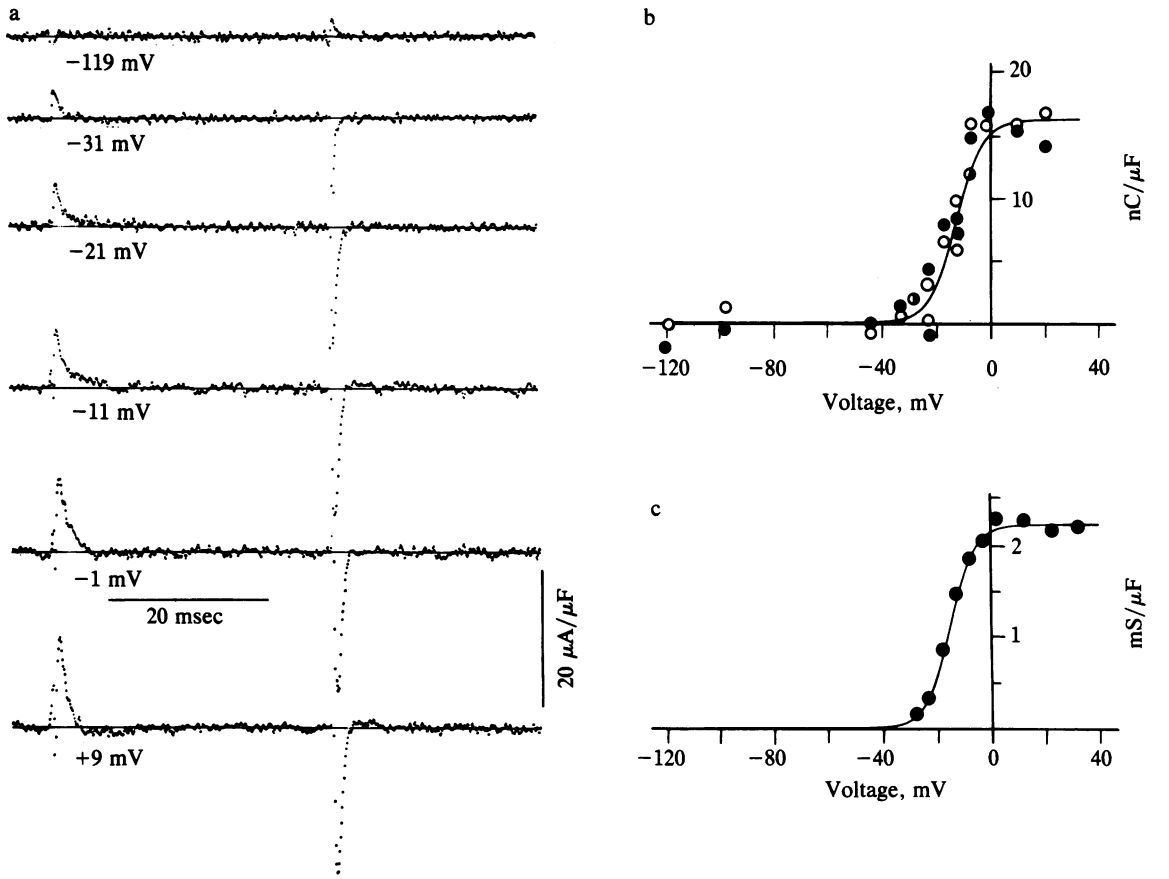

FIG. 3. Voltage-dependence of I_Q and G_{Ba} (3 mM barium). (*a*) I_Q records over a range of test pulse potentials as indicated. For records positive to −15 mV, two control pulses of half the test pulse magnitude were added together and used for subtraction. Sloping baselines were fit as described for Fig. 2*b*. Eight sweeps were averaged; fiber 01FE51. (*b*) Charge–voltage relationship. Q_{ON} (○) and Q_{OFF} (●) charge moved was determined by integrating ON and OFF transients; these values are plotted as a function of activating voltage. The solid curve is a least-squares fit to $Q(V) = Q_{max}/\{1 + \exp[(V - \bar{V})/k]\}$. Q_{max} = 16.3 nC/μF, $\bar{V}$ = −22,3 mV, and k = 4.5 mV. Same fiber as *a*. (*c*) G_{Ba}–voltage relation. The peak value of I_{Ba} (e.g., Fig. 1*c*) was used to approximate G_{Ba} by assuming an ohmic relation between I_{Ba} and driving force (apparent reversal potential = +37 mV). Current in both directions around this reversal potential is strongly blocked by nickel ions, and estimating I_{Ba} from subtraction of records obtained in the absence and presence of nickel does not significantly alter the shape of the G_{Ba}–voltage relation, particularly at very negative potentials where the curve rises most steeply. Similarly, using much more positive values of reversal potential (up to 100 mV) to calculate G_{Ba} does not seriously affect the curve in this voltage region. The solid curve was fit to $G(V) = G_{max}/\{1 + \exp[(V - \bar{V})/k]\}$, where G_{max} = 2.2 mS/μF, $\bar{V}$ = −17.8 mV, and k = 4.7 mV. Temperature, 8.4°C; fiber 06DE47. From these data, the maximal I_{Ba} that would flow at −10 mV if all the channels were open can be estimated as (2.2 mS/μF) × 47 mV = 103 μA/μF.

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. 3*b*) 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. 3*c*, and the voltage dependence is similar to that in Fig. 3*b*. 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 ($\bar{V}$) 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. 4*a*) and eliminates I_{Ba} (Fig. 4*b*) 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. 4*a* 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

Table 1. Charge movement and barium conductance in isotonic solutions

Muscle source	Q_{max}, nC/μF	G_{max}, mS/μF	$\bar{V}$, mV	k, mV	Ref.
		Charge movement			
Frog*	25	—	−30	12	26†
		—	−26	14.9	27†
	26.3	—	−34.9	16.0	22‡
Rat		—		12.3	28†
Scorpion§	12.8 ± 2.5	—	−22.9 ± 2.0	5.8 ± 0.7	This work
		Barium conductance¶			
Scorpion§	—	2–3‖	−18.5 ± 2.2	4.2 ± 0.4	This work

*Rana species.
†Nonparalyzed fibers were studied at potentials negative to contraction threshold.
‡Fibers were immobilized by internal EGTA.
§Scorpion parameters were obtained by fits of individual experiments as described in legend to Fig. 3*b*.
¶Conductance–voltage curves were constructed as described in legend to Fig. 3*c*.
‖I_{Ba} is quite labile in these fibers and values reported here are typical of the largest conductance observed in 3 mM barium.

dependent on extracellular calcium. I_Q in scorpion muscle can be distinguished from the superficially similar signal in vertebrate skeletal muscle by criteria of kinetics, voltage dependence, and pharmacological sensitivity. When compared to mammalian or amphibian I_Q, the scorpion signals recorded under nearly identical conditions are faster, more steeply voltage-dependent, and more sensitive to the local anesthetic tetracaine. These same results provide a strong link between scorpion I_Q and gating of voltage-dependent calcium channels in the sarcolemma. In each case, properties of I_Q and calcium-channel gating are well matched.

Although scorpion and vertebrate I_Q are not similar in an overall sense, an important question is whether any component of the two signals can be equated. Two hypotheses are worth considering. First, scorpion I_Q might be analogous to

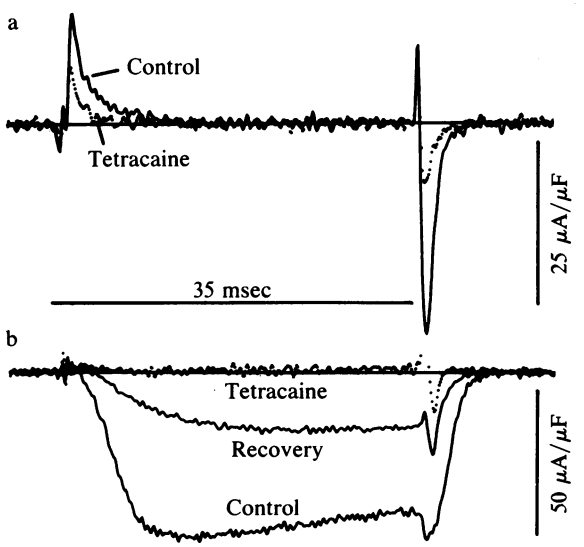

FIG. 4. Effect of 1 mM tetracaine on I_Q (*a*) and I_{Ba} (*b*). (*a*) I_Q at −10 mV in the absence (control) or presence of 1 mM tetracaine. Sixteen sweeps were averaged. Temperature was 9°C; fiber 12FE54. (*b*) I_{Ba} records at −15 mV in the absence (control) or presence of 1 mM tetracaine. The recovery trace was measured 26 min after returning to the control solution. Two sweeps were averaged. Temperature, 6°C; fiber 26FE53.

the vertebrate Q_γ, 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_γ, a striking signature of the phenomenon, bear no resemblance whatsoever to our records. Second, one might suppose that a "missing" slow component of scorpion I_Q could exist that would make it vertebrate-like and that this missing 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 scorpion 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. 3*c*), 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 1.3×10^{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 μF/cm^2 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.

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_Q in arthropod skeletal muscle gates calcium channels, which rapidly inject activator for contraction. I_Q 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).

This work was supported by National Institutes of Health Grant NS17510 and Fellowship GM09921.

1. Armstrong, C. M., Bezanilla, F. & Horowicz, P. (1972) *Biochim. Biophys. Acta* **267**, 605–608.
2. Lüttgau, H. C. & Spiecker, W. (1979) *J. Physiol. (London)* **296**, 411–429.
3. Curtis, B. A. & Eisenberg, R. S. (1985) *J. Gen. Physiol.* **85**, 383–408.
4. Schneider, M. F. & Chandler, W. K. (1973) *Nature (London)* **242**, 244–246.
5. Chandler, W. K., Rakowski, R. F. & Schneider, M. F. (1976) *J. Physiol. (London)* **254**, 245–283.
6. Adrian, R. H. & Almers, W. (1976) *J. Physiol. (London)* **254**, 339–360.
7. Horowicz, P. & Schneider, M. F. (1981) *J. Physiol. (London)* **314**, 595–633.
8. Kovacs, L. & Szucs, G. (1983) *J. Physiol. (London)* **341**, 559–578.
9. Schneider, M. F. (1981) *Annu. Rev. Physiol.* **43**, 507–517.
10. Chandler, W. K., Rakowski, R. F. & Schneider, M. F. (1976) *J. Physiol. (London)* **254**, 285–316.
11. Franzini-Armstrong, C. (1975) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34**, 1382–1389.
12. Franzini-Armstrong, C. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 2403–2409.
13. Gilly, W. F. (1981) in *The Regulation of Muscle Contraction: Excitation-Contraction Coupling*, eds. Grinnell, A. D. & Brazier, M. A. (Academic, New York), pp. 3–22.
14. Peachey, L. D. & Franzini-Armstrong, C. (1983) in *Handbook of Physiology*, eds. Peachey, L. D., Adrian, R. H. & Geiger, S. R. (Am. Physiol. Soc., Bethesda, MD), pp. 23–71.
15. Franzini-Armstrong, C. (1973) in *The Structure and Function of Muscle*, ed. Bourne, G. H. (Academic, New York), 2nd Ed., Vol. 2, pp. 531–619.
16. Franzini-Armstrong, C. (1976) in *Pathogenesis of Human Muscular Dystrophies*, Excerpta Medica International Congress Series No. 404, Proceedings of the Fifth International Conference of Muscular Dystrophy Association (Excerpta Med, Amsterdam), pp. 612–625.
17. Gilly, W. F. & Scheuer, T. (1984) *J. Gen. Physiol.* **84**, 321–345.
18. Gilai, A. & Parnas, I. (1970) *J. Exp. Biol.* **52**, 325–344.
19. Gilai, A. & Parnas, I. (1972) *J. Cell Biol.* **52**, 626–638.
20. Gilai, A. (1976) *J. Gen. Physiol.* **67**, 343–367.
21. Adrian, R. H., Chandler, W. K. & Hodgkin, A. L. (1970) *J. Physiol. (London)* **208**, 607–644.
22. Horowicz, P. & Schneider, M. F. (1981) *J. Physiol. (London)* **314**, 565–593.
23. Hess, P. & Tsien, R. W. (1984) *Nature (London)* **309**, 453–456.
24. Almers, W., McCleskey, E. W. & Palade, P. T. (1984) *J. Physiol. (London)* **353**, 565–583.
25. Simon, B. J. & Beam, K. G. (1985) *J. Gen. Physiol.* **85**, 1–19.
26. Schneider, M. F. & Chandler, W. K. (1976) *J. Gen. Physiol.* **67**, 125–163.
27. Almers, W. (1976) *J. Physiol. (London)* **262**, 613–637.
28. Hollingworth, S. & Marshall, M. W. (1981) *J. Physiol. (London)* **321**, 583–602.
29. Almers, W. & Best, P. M. (1976) *J. Physiol. (London)* **262**, 583–611.
30. Adrian, R. H. & Huang, C. L.-H. (1984) *J. Physiol. (London)* **353**, 419–434.
31. Hui, C. S. (1983) *J. Physiol. (London)* **337**, 509–529.
32. Caldwell, J. H., Campbell, D. T. & Beam, K. G. (1986) *J. Gen. Physiol.* **87**, 907–932.
33. Vergara, J. & Caputo, C. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1477–1481.
34. Fenwick, E. W., Marty, A. & Neher, E. (1982) *J. Physiol. (London)* **331**, 599–635.
35. Kostyuk, P. G., Krishtal, O. A. & Pidoplichko, V. I. (1981) *J. Physiol. (London)* **310**, 403–421.
36. Bean, B. P., Nowycky, M. C. & Tsien, R. W. (1984) *Nature (London)* **307**, 371–375.
37. Schwartz, L. M., McCleskey, E. W. & Almers, W. (1985) *Nature (London)* **314**, 747–751.
38. Kravitz, E. A., Glusman, S., Harris-Warwick, R., Livingstone, M., Schwarz, T. & Goy, M. (1980) *J. Exp. Biol.* **89**, 159–175.
39. Baylor, S. M., Chandler, W. K. & Marshall, M. W. (1983) *J. Physiol. (London)* **344**, 625–666.
40. Rakowski, R. F., Best, P. M. & James-Kracke, M. (1985) *J. Muscle Res. Cell Motil.* **6**, 403–433.
41. Melzer, W. (1982) *Eur. J. Cell Biol.* **28**, 219–255.
42. Vergara, J., Tsien, R. Y. & Delay, M. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6352–6356.
43. Atwater, I., Rojas, E. & Vergara, J. (1974) *J. Physiol. (London)* **243**, 523–551.
44. Lamb, G. D. (1986) *J. Physiol. (London)* **376**, 63–83.
45. Lamb, G. D. (1986) *J. Physiol. (London)* **376**, 85–100.