brief communication

Existence of Q_γ in frog cut twitch fibers with little Q_β

Wei Chen and Chiu Shuen Hui
Department of Physiology and Biophysics, Indiana University Medical Center, Indianapolis, Indiana 46223 USA

ABSTRACT Charge movements were measured in frog cut twitch fibers with the double Vaseline-gap voltage-clamp technique. In most fibers, when a depolarizing pulse to −60 to −40 mV was applied at 13–14°C, the ON segment of a charge movement trace showed an early I_β component and a late I_γ hump component. An ongoing controversy is whether the I_γ hump component triggers calcium release from the sarcoplasmic reticulum or arises as a consequence of the release. Interestingly, a number of cut fibers showed normal I_γ components but greatly diminished, or unresolvable, I_β components. When the amount of charge associated with the current transient was plotted against the membrane potential, the steeply voltage-dependent Q_γ component appeared normal whereas the less steeply voltage-dependent Q_β component was also greatly diminished or unresolvable. These results suggest that I_γ can flow in the absence of I_β, thereby ruling out the possibility that Q_β triggers calcium release which, in turn, causes Q_γ to move. The results, however, do not rule out the positive feedback of calcium release to activate Q_γ, if calcium release is not triggered by Q_β but by Q_γ itself or by some other signal.

INTRODUCTION

Intramembranous charge movement, first observed by Schneider and Chandler in 1973, is generally accepted to be the voltage sensor for excitation-contraction coupling in skeletal muscle. On depolarization to a potential slightly above the contraction threshold, the outward current generated by the movement of the charge shows an early exponentially decaying component and a late hump component, referred to as I_β and I_γ, respectively. The charge associated with I_β was denoted by Q_β and that with I_γ by Q_γ (Adrian and Peres, 1979).

Based on the close association between Q_γ and calcium release from the sarcoplasmic reticulum, several investigators (Huang, 1982; Hui, 1982, 1983*a*,*b*; Vergara and Caputo, 1983) suggested that Q_γ could play a role in triggering calcium release from the sarcoplasmic reticulum. Alternatively, Q_γ could arise as a consequence of the release (Csernoch et al., 1989; Pizarro et al., 1990). The controversy of whether Q_γ is a cause (trigger hypothesis) or a consequence (feedback hypothesis) of calcium release has attracted a great deal of attention lately. An underlying assumption in the feedback hypothesis is that Q_β and Q_γ belong to the same pool of charge. Q_β triggers calcium release and then Q_γ moves with kinetics governed by that of calcium release. Thus, Q_γ cannot move without Q_β. There is no such restriction in the trigger hypothesis. Q_β could be the precursor for Q_γ (sequential model) or Q_γ can move entirely independent of the movement of Q_β (parallel model). The experiments to be reported in this communication were aimed at providing some new information on this controversy. It will be shown that, in some cut fibers, Q_γ can move in the presence of very little Q_β.

Address correspondence to Dr. Chiu Shuen Hui, Department of Physiology and Biophysics, Indiana University Medical Center, 635 Barnhill Drive, Indianapolis, IN 46223.

MATERIALS AND METHODS

Experiments were performed on cut fibers dissected from semitendinosus muscles of English frogs, *Rana temporaria*, cold adapted in a refrigerator at ~4°C. The procedure for dissecting and mounting cut fibers in a double Vaseline-gap chamber has been described in a preceding paper (Chandler and Hui, 1990; see also Kovacs et al., 1983). The end-pool solution (Solution A) contained 45.5 mM Cs-glutamate, 20 mM Cs_2-creatine phosphate, 20 mM Cs_2 · EGTA, 6.8 mM $MgSO_4$, 5.5 mM Cs_2 · ATP, 5 mM glucose, 5 mM Cs_2 · PIPES, and 60 μM total Ca^{2+}, pH 7.0. The center-pool solution (Solution B) contained 120 mM TEA · Cl, 2.5 mM RbCl, 1.8 mM $CaCl_2$, 2.15 mM Na_2HPO_4, 0.85 mM NaH_2PO_4, and 1 μM tetrodotoxin, pH 7.1. Fiber contraction was blocked by stretching the fiber to a sarcomere length of 3.5 μm and by the presence of 20 mM EGTA in the internal solution. All experiments were performed at a temperature of 13–14°C.

The instrumentation and experimental protocol for measuring charge movement and the method of data analysis were similar to those used by Hui and Chandler (1990). The holding potential was set at −90 mV and the CONTROL pulse was applied from −110 to −90 mV. TEST pulses were applied in an increasing order of magnitude and at the rate of one per minute. Each charge movement record was obtained by subtracting a scaled CONTROL current trace from a paired TEST current trace, and will be referred to as a TEST-minus-CONTROL current trace. The amount of ON or OFF charge was estimated from the time integral of the ON or OFF transient in a

current trace, after correcting for the sloping baseline (thin lines in the ON segments of the traces in Fig. 1).

The steady-state Q-V plot was fitted by a sum of two Boltzmann distribution functions:

$$Q(V) = \sum_{i=\beta}^{\gamma} Q_{i,max} \left\{1 + \exp\left(-\frac{V - \overline{V}_i}{k_i}\right)\right\}^{-1}, \quad (1)$$

where $Q_{i,max}$ represents the maximum amount of charge, $\overline{V}_i$ the equi-distribution potential, and k_i the voltage-dependence (or inverse steepness) factor, for $i = \beta$ or γ. Because the Vaseline seals do not have infinite resistance, charge movement in the membranes underneath the seals contribute to the total charge and was corrected by the method of Hui and Chandler (1990). In essence, the membrane segments underneath the two Vaseline seals and in the center pool were divided into grids. For each TEST pulse, the membrane potential in each grid was determined by interpolation and the amount of charge moved in each grid, weighted by a weighting function, was integrated spatially to give the total charge. Moreover, because CONTROL pulses were applied from −110 to −90 mV, a scaled amount of the CONTROL charge was inevitably subtracted from the TEST charge. This was corrected by subtracting a straight line, that intersects the Q-V curve at −110 and −90 mV, from the curve. The residual sum of squares between the measured values and the predicted values of charge was minimized by an iterative routine.

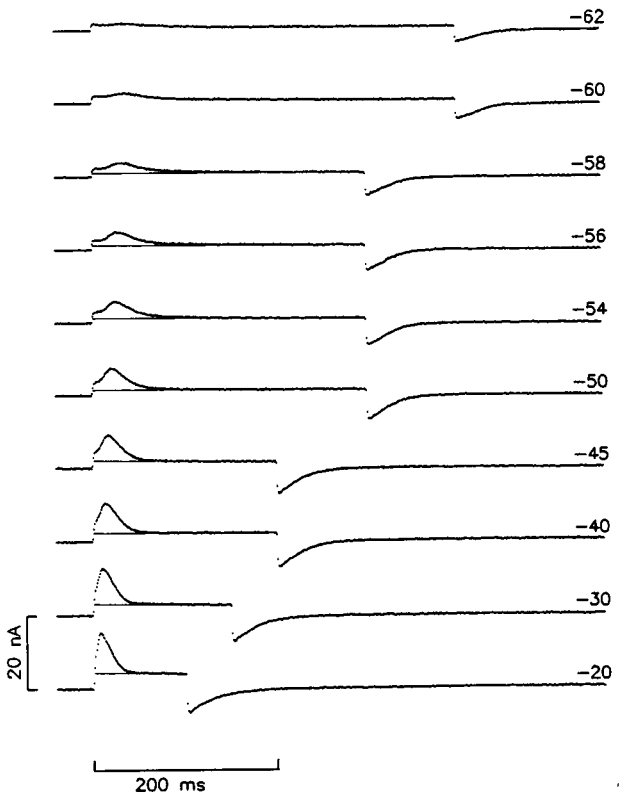

FIGURE 1 TEST-minus-CONTROL current in a cut fiber. Fiber identification: 90161. Diam = 106 μm. Sarcomere length = 3.5 μm. Temperature = 13–14°C. Saponin treatment was applied to membrane segments in both end pools at time zero. After rinsing, the solutions in the end pools were replaced by Solution A. Then, the solution in the center pool was changed to a TEA.Cl solution (Solution B). At the 20th minute, the voltage clamp was turned on and the holding potential was set at −90 mV. Traces were taken from the 61st to the 81st minutes, during which the holding current and $r_e/(r_e + r_i)$ remained constant at −14 nA and 0.992, respectively. All traces are single sweeps. Only representative traces are shown. The numbers on the right indicate the potentials in millivolts during the TEST pulses.

RESULTS

Fig. 1 shows TEST-minus-CONTROL current traces elicited by TEST pulses to the potentials indicated on the right. At the "ON" of a TEST pulse, a transient current flows outward followed by a maintained current. At the "OFF" of the pulse, a transient current flows inward and decays back to the baseline. The ON transients in these traces are different from those observed in most intact or cut fibers (see traces in the references listed above, particularly those that were recorded under identical conditions in Fig. 6 of Hui and Chandler, 1990). The ON transients from other fibers invariably showed an early I_β component, which rose to a peak within 2–3 ms and then decayed exponentially, and an I_γ component, which appeared as a bell-shaped hump in the decay phase of I_β. On the contrary, the I_β components are greatly reduced in the ON segments of the traces in Fig. 1. Instead, the ON transients show a step rise of very small amplitude followed by a prominent hump component. Thus, the amplitude of I_β in this fiber is much smaller than usual and its decay phase is probably obscured by the hump component. The I_γ hump components in the ON segments, on the other hand, appear to be quite normal. It is visible at around −62 mV. With increasing levels of depolarization, its amplitude becomes larger and its time-to-peak and half-width become shorter.

The time course of the OFF transients in the traces of Fig. 1 also appears to be slower than usual. The OFF transients in the traces has an average half-width of 23 ms, which is 2–3 times the average value in most cut fibers. As I_β was greatly reduced in this fiber, the slow OFF kinetics is consistent with the finding that the half-width of the OFF I_γ transient at −90 mV could be 5 times as large as that of the OFF I_β transient (Hui and Chandler, unpublished result).

To estimate the amount of charge associated with the ON or OFF transient in each trace, the nonlinear ionic current in the ON or OFF segment was removed by fitting a sum of exponential decay and a sloping straight line to the late phase of the transient (Chandler et al., 1976*a*) and subtracting the straight line from the total current. Some of the baselines for the ON segments are shown in Fig. 1 as thin lines. At −62 and −60 mV, the I_γ component was too broad to permit a reliable baseline

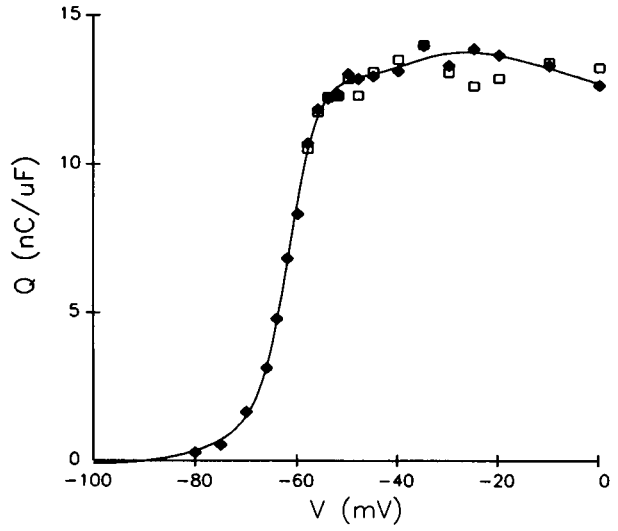

FIGURE 2 Steady-state voltage distributions of total charge in a cut fiber. Same fiber as in Fig. 1. The points were obtained from time integrals of ON transients (□) or OFF transients (◆) in TEST-minus-CONTROL current traces, some of which are shown in Fig. 1. The smooth curve was obtained by fitting Eq. 1, with CONTROL charge correction and gap correction, to the ◆. The best fit parameters are entered in the seventh row in Table 1.

fit. The baselines for the OFF segments are indistinguishable from the time axis and so are not shown.

The areas of the ON transients (□) and OFF transients (◆) from the traces shown in Fig. 1 (and others not shown) are plotted as a function of TEST pulse potential in Fig. 2. For potentials ≤ -60 mV, the ON transients were not estimated because the baselines could not be fitted reliably. For potentials > -60 mV, the amounts of ON and OFF charge were equal in each trace, within experimental error. The smooth curve was obtained by fitting Eq. 1, with corrections, to the ◆. The best fit parameters are listed in the seventh row of Table 1 under fiber 90161. The less steeply voltage-dependent component is identified with Q_β and accounts for 16.5% of the total charge in this fiber. This fraction is much smaller than the average value of 43.5% calculated from Table III of Hui and Chandler (1990), implying that this fiber was atypical in having an exceptionally small amount of Q_β. The existence of some Q_β in the *Q-V* plot is consistent with the observation that some I_β was present in the current traces, as reflected by the step rise at the beginning of the ON segment.

It should be noted that two puzzling features of the smooth curve are direct consequences of the gap correction and CONTROL charge correction described in Methods. First is that the curve dips below the voltage axis at potentials < -90 mV. In fact, the curve rises upward at potentials < -100 mV and intersects the voltage axis again at -110 mV (see Fig. 2 *B* of Hui and Chandler, 1990). Second is the negative slope at potentials ≥ -20 mV. Before CONTROL charge correction, the *Q-V* curve saturates and is flat in this voltage range. The negative slope arises from the subtraction of the straight line that represents the scaled CONTROL charge and has a positive slope. Both features will not be as noticeable if gap correction is not included (Fig. 2 *A* of Hui and Chandler, 1990).

Although fibers having such a small amount of Q_β are

TABLE 1 **Steady-state voltage distributions of Q_β and Q_γ**

1	2	3	4	5	6	7	8	9	10 (6)/(6) + (9)
Fiber	d	c_m	$\bar{V}_\beta$	k_β	$q_{\beta,max}/c_m$	$\bar{V}_\gamma$	k_γ	$q_{\gamma,max}/c_m$	
Reference	μm	$\mu F/cm$	mV	mV	nC/μF	mV	mV	$nC/\mu F$	%
87291	93	0.117	(−33.0)	(11.0)	2.2	−61.6	2.8	13.8	13.8
88041	99	0.171	−23.1	8.9	5.5	−51.7	5.9	15.8	25.7
88091	84	0.105	−39.3	7.6	3.2	−58.2	4.6	9.7	25.1
89201	78	0.111	−23.2	6.7	6.2	−52.3	4.0	15.4	28.7
94211	90	0.142	−29.6	10.9	5.0	−57.5	3.7	14.9	25.3
96291	130	0.273	(−33.0)	(11.0)	0.4	−52.5	3.6	11.7	3.3
90161	106	0.215	−37.6	6.3	3.1	−62.3	2.8	15.6	16.5
90271	124	0.393	(−33.0)	(11.0)	2.2	−55.5	2.8	8.4	20.6
90272	127	0.306	(−33.0)	(11.0)	−1.2	−52.4	3.1	12.6	−10.6
9N161	115	0.280	−32.1	10.1	3.2	−55.4	2.9	11.5	21.9
Mean						−55.9	3.6	12.9	
SEM						1.2	0.3	0.8	

Columns 1 and 2 give the fiber identifications and the values of $r_e/(r_e + r_i)$. Column 3 gives the membrane capacitance per unit length at -100 mV. Columns 4 to 9 give the best fit values of parameters in Eq. 1, with gap correction and CONTROL charge correction. Numbers in brackets were taken from the mean values listed in Table III of Hui and Chandler (1990) and were constrained as constants in the fits (see text for more detail). Column 10 gives the fractions of Q_β in the total charge.

rare, the fiber of Fig. 1 was not the only one. Out of more than a hundred fibers, we selected 10 fibers that had Q_β accounting for $<30\%$ of the total charge and the results are listed in Table 1. In columns 6 and 9, $Q_{i,max}$ is normalized by membrane capacitance and expressed in $q_{i,max}/c_m$. There were four fibers (listed under 87291, 96291, 90271, and 90272) which had even less Q_β than the fiber of Fig. 1. In the ON segments of the TEST-minus-CONTROL current traces of those fibers, the early step rise had an amplitude almost the same as the maintained pedestal current. Also, in fitting Eq. 1, with corrections, to the Q-V plots from those fibers, the fitting routine did not converge, indicating that the amount of Q_β was too small to be resolved. Each plot was therefore fitted by constraining the values of $\overline{V}_\beta$ and k_β to -33 mV and 11 mV, respectively, which are average values obtained from other fibers with a normal I_β component (Table III of Hui and Chandler, 1990). The values of $q_{\beta,max}/c_m$ from the fits were small, confirming the presence of very little Q_β. The negative value obtained for fiber 90272 could be due to scatter of the data.

The mean values of $\overline{V}_\gamma$, k_γ, and $q_{\gamma,max}/c_m$ given in Table 1 are very close to the mean values given in Table III of Hui and Chandler (1990). The differences are highly insignificant ($P > 0.5$ for $\overline{V}_\gamma$, > 0.1 for k_γ, and > 0.8 for $q_{\gamma,max}/c_m$, with the two-tailed t-test). Thus, the Q_γ component appeared to be normal in the fibers of Table 1. The mean values of the parameters for the Q_β component are not listed in the table because those values are considered to be less reliable due to the small amount of Q_β present.

DISCUSSION

A general conclusion of the results presented here is that some fibers having small (or negligible) amounts of Q_β can have normal amounts of Q_γ. The abnormality of Q_β came as a surprise and its cause is unknown. If Q_β in the T tubules could not be measured because of some abnormality of the tubules, such as detachment or closure, it is difficult to explain how a normal amount of Q_γ could be measured. Besides, the values of c_m, listed in column 3 of Table I, are all within the normal range for fibers of diameters listed in column 2. All the fibers with little Q_β appeared to be healthy, as judged by their appearances under the microscope and by their small holding currents. The sequences of runs from which the Q-V plots were obtained were all taken $\sim$1 h after saponin treatment in the end pools, same as in other fibers with normal Q_β. Although the reason for the abnormality is unknown, the results definitively rule out a tight coupling between Q_β and Q_γ that is required in a sequential model in which Q_β is the precursor for Q_γ.

On the other hand, the results are consistent with a parallel model in which Q_β and Q_γ can move independently of each other. In this case, several possibilities for the roles of Q_β and Q_γ in excitation-contraction coupling can be considered: (*a*) Either Q_β or Q_γ triggers calcium release and the other component plays no role. (*b*) Q_β triggers calcium release and Q_γ arises as a result of the release. Because of the temporal relationship between Q_β and Q_γ, the reverse order cannot be true. (*c*) Both Q_β and Q_γ are responsible for triggering calcium release. (*d*) Calcium release is triggered by a signal other than Q_β, and Q_γ arises as a result of the release. (*e*) Both Q_β and Q_γ are unrelated to excitation-contraction coupling.

In the early stage when charge movement was discovered, no I_γ hump was observed and the total charge was believed to be the trigger for calcium release (Chandler et al., 1976*a*,*b*; Adrian et al., 1976). Their charge movement traces could contain an I_γ component not manifested as a hump. Thus, the results could not be used to differentiate the roles of Q_β and Q_γ. When Q_γ was first reported, it was thought to be unrelated to excitation-contraction coupling (Adrian and Peres, 1977). Subsequently, because of the strong association between Q_γ and calcium release, Q_γ was hypothesized to be the trigger for the release (Huang, 1982; Hui, 1982, 1983*a*,*b*; Vergara and Caputo, 1983), thus supporting possibility *a*. Possibility *b* was first considered by Dr. W. K. Chandler (see Discussion sections in Horowicz and Schneider, 1981, and Hui, 1983*b*) and was recently supported by Csernoch et al. (1989) and Pizzaro et al. (1990). Possibility *c* remains open and possibility *d* could be true if calcium release is activated, for example, by IP_3 (Vergara et al., 1985), but a voltage sensor is still required to release the IP_3. Finally, there has been no evidence for possibility *e*.

The finding in this communication that Q_γ can flow in the absence of Q_β suggests that possibility *b* is unlikely. However, a positive feedback of calcium release to mobilize Q_γ is still allowed by possibility *d*. Alternatively, if Q_γ is the trigger for calcium release, it is quite likely that a positive feedback of the release can enhance the movement of Q_γ and steepen the Q_γ-V curve as a secondary effect. In other words, Q_γ could be both the trigger for and the result of calcium release. If, in the absence of Q_β, Q_γ is caused by calcium release, then Q_γ can be used to monitor calcium release indirectly and it can be concluded that Q_β plays no role in excitation-contraction coupling. If Q_γ is not caused by calcium release, then the important question is whether possibility *a* or *c* is the actual mechanism, or whether Q_β plays a role in triggering calcium release. Unfortunately, there is no information about whether the fibers of Table 1

could release a normal amount of calcium from the sarcoplasmic reticulum, because the experiments were not set up to measure calcium signal simultaneously. Assuming that the fibers could release calcium normally, then Q_β would probably not be required in the release process. If this is the case, one wonders what is the physiological role of Q_β.

Because the T tubule membrane contains many different kinds of ionic channels, it is possible that Q_β is made up of several components, one for gating sodium channels, one for gating potassium channels, and one for gating calcium channels. Perhaps part of Q_β has no known physiological role yet. It is also possible that part of Q_β is the result of the dynamic alteration in the contents of phosphoinositides, as suggested by Vergara (1987). The simple species of charge first observed by Schneider and Chandler (1973) could turn out to be a multicomponent ensemble, only part of which is relevant to excitation-contraction coupling.

This project was supported by grants from the National Institutes of Health (NS21955) and from the Muscular Dystrophy Association and American Heart Association.

Received for publication 11 May 1990 and in final form 5 September 1990.

REFERENCES

Adrian, R. H., W. K. Chandler, and R. F. Rakowski. 1976. Charge movement and mechanical repriming in skeletal muscle. *J. Physiol.* 254:361–388.

Adrian, R. H., and A. R. Peres. 1977. A gating signal for the potassium channel? *Nature (Lond.).* 267:800–804.

Adrian, R. H., and A. R. Peres. 1979. Charge movement and membrane capacity in frog muscle. *J. Physiol.* 289:83–97.

Chandler, W. K., and C. S. Hui. 1990. Intramembranous charge movement in frog cut twitch fibers mounted in a double Vaseline-gap chamber. *J. Gen. Physiol.* 96:225–256.

Chandler, W. K., R. F. Rakowski, and M. F. Schneider. 1976*a*. A non-linear voltage dependent charge movement in frog skeletal muscle. *J. Physiol.* 254:245–283.

Chandler, W. K., R. F. Rakowski, and M. F. Schneider. 1976*b*. Effect of glycerol treatment and maintained depolarization on charge movement in skeletal muscle. *J. Physiol.* 254:285–316.

Csernoch, L., I. Uribe, M. Rodriguez, G. Pizzaro, and E. Rios. 1989. Q_γ and Ca release flux in skeletal muscle fibers. *Biophys. J.* 55:88*a*. (Abstr.)

Horowicz, P., and M. F. Schneider. 1981. Membrane charge moved at contraction thresholds in skeletal muscle fibres. *J. Physiol.* 314:595–633.

Huang, C. L.-H. 1982. Pharmacological separation of charge movement components in frog skeletal muscle. *J. Physiol.* 324:375–387.

Hui, C. S. 1982. Pharmacological dissection of charge movement in frog skeletal muscle fibers. *Biophys. J.* 39:119–122.

Hui, C. S. 1983*a*. Pharmacological studies of charge movement in frog skeletal muscle. *J. Physiol.* 337:509–529.

Hui, C. S. 1983*b*. Differential properties of two charge components in frog skeletal muscle. *J. Physiol.* 337:531–552.

Hui, C. S., and W. K. Chandler. 1990. Intramembranous charge movement in frog cut twitch fibers mounted in a double Vaseline-gap chamber. *J. Gen. Physiol.* 96:257–297.

Kovacs, L., E. Rios, and M. F. Schneider. 1983. Measurement and modification of free calcium transients in frog skeletal muscle fibres by a metallochromic indicator dye. *J. Physiol.* 343:161–196.

Pizarro, G., M. Rodriguez, L. Csernoch, and E. Rios. 1990. Positive feedback in skeletal muscle E-C coupling. *Biophys. J.* 73:401*a*. (Abstr.)

Schneider, M. F., and W. K. Chandler. 1973. Voltage dependent charge movement in skeletal muscle: a possible step in excitation-contraction coupling. *Nature (Lond.).* 242:244–246.

Vergara, J. 1987. Neomycin blocks asymmetric charge movements and Ca transients in skeletal muscle fibers. *Biophys. J.* 51:199*a*. (Abstr.)

Vergara, J., and C. Caputo. 1983. Effects of tetracaine on charge movements and calcium signals in frog skeletal muscle fibers. *Proc. Natl. Acad. Sci. USA.* 80:1477–1481.

Vergara, J., R. Y. Tsien, and M. Delay. 1985. Inositol 1,4,5-trisphosphate: a possible chemical link in excitation-contraction coupling in muscle. *Proc. Natl. Acad. Sci. USA.* 82:6352–6356.