

# An Alternative Interpretation of Charge Movement in Muscle

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## INTRODUCTION

The systems which link the electrical activity of skeletal muscle with the contraction of its filaments have received a great deal of attention in the last 30 years. The main structures involved have been described, and the function of many of the structures is known, at least in outline form. The surface membrane of skeletal muscle fibers is invaginated into a set of inner membranes that form the *transverse tubular system* (T system). The invaginated tubules of the T system conduct some of the current produced by the action potential into the interior of the fiber. The current flowing into the tubules spreads the action potential from the surface membranes to the inner membranes and initiates inward sodium current across the tubular membranes. There is another membrane system called the *sarcoplasmic reticulum* (SR) linked to the tubular membrane at the T-SR junction. The SR is a specialized form of the endoplasmic reticulum found in almost all cells, specialized to store and release calcium on the command of the T system. The calcium released from the SR is the chemical signal that allows the cross bridges to cycle and contraction to occur.

This paper discusses the possibility that the signal, which both transmits information from T system to SR and which initiates calcium release, is electrical. In particular, we consider the hypothesis (1) that ions can move and thereby carry electrical charge and current from the lumen of the T system to the lumen of the SR and (2) that the signal, which initiates calcium release, is a change in the potential within the lumen of the SR. A full version of this work has recently been published by Mathias and colleagues (12).

The hypothesis of electrical coupling between T system and SR has a long history. Electrical coupling between cells is an important physiological mechanism, mediated by a specialized structure called a *gap* junction. It was natural, therefore, for early papers of Peachey and Porter (15) and Peachey (14) to pursue the analogy between the T-SR junction and gap junctions, suggesting that the T system might communicate with the SR by ionic current flow across the T-SR junction.

Evidence for electrical coupling was sought by a variety of techniques. The structure of the T-SR junction was examined to see if it resembled the gap junctions that are known to allow ionic current flow from one cell to the next. Early micrographs by Franzini-Armstrong (9) showed a structure different from that of gap junctions, although more recent micrographs by Somlyo (17) and Eisenberg and Gilai (7) show a structure that is not so different. Measurements were made of the flux of tracer molecules, with the hope that the measurements would elucidate the intracellular compartments accessible to ions [see references in Neville (13)]. Attempts were made to demonstrate the movement of extracellular markers into the SR, most recently by Franzini-Armstrong and colleagues (10). Electrical measurements were made from *resting* muscle fibers by Valdiosera and his colleagues (18) while looking for a contribution from the SR. None of these experiments provided evidence for electrical coupling between the T system and the SR.

In the last few years electrical measurements of high resolution have been made on voltage-clamped fibers. Measurements were first made by Schneider and Chandler (16) on muscles in the range of potential in which they normally contract. These measurements described nonlinear displacement currents of the type called *gating* currents in squid axon. The nonlinear displacement currents in skeletal muscle were found to have several distinctive characteristics: They are present in fibers treated with pharmacological blocking agents thought to block almost all ionic currents; they vary with potential, being small at normal resting potentials, increasing with moderate depolarization, and finally saturating at large depolarizations; and they have a time dependence quite distinct from that of ionic currents. In particular, the integral of the transient current following a depolarization (in fibers treated with blocking agents) is found to approximate the integral of the transient current following a repolarization to the initial holding potential. These properties are characteristic of displacement currents produced by the movement of membrane-bound charges. They are not properties of any known ionic current. Therefore, these experimental findings are considered evidence for the existence of membrane-bound charges, charges that sense voltage and thereby control the voltage-dependent properties of muscle fibers.

The nonlinear displacement currents measured by Chandler and colleagues (5, 6) and Adrian and Almers (1, 2) shared many characteristics of the hypothetical activator of contraction that had been previously postulated by Hodgkin and Horowicz (11) and in more detail by Adrian and colleagues (4).

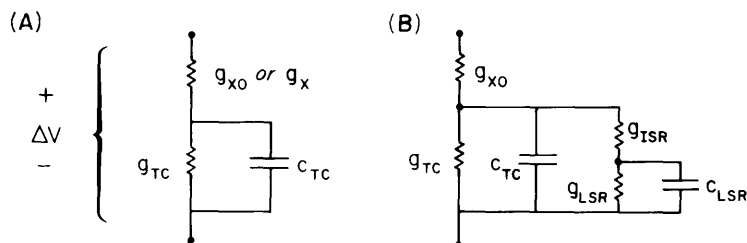
These currents were too slow to be gating currents for ionic conductances involved in the action potential. It was natural then to identify the nonlinear charge movements with the movement of a membrane-bound macromolecule and to seek a role for that macromolecule as the activator of contraction, namely the initiator of calcium release.

There is another hypothesis, however, that can describe the qualitative properties of excitation-contraction (E-C) coupling and the quantitative properties of charge movement. The rest of this paper will present an alternative model that uses a transient ionic current flow across the T-SR junction to link the potential change across the T system with the potential change across the membranes of the SR. Charge movement, in this model, is the charging of the linear capacitance of the terminal cisternae (TC) membranes. Calcium release in the alternative model is supposed to arise in a voltage-activated mechanism in the TC membrane.

Several questions about this model need to be answered, given the previous work in the field. How can the electrical model account for electrical data from resting fibers? What is the structural basis of such a model? How can the model quantitatively explain the charge movement data? How can the SR be an "intracellular" compartment and still be electrically connected with an extracellular compartment, the lumen of the T system? And finally can the alternative model account for E-C coupling?

The answers to these questions lie in the role of structural complexity in the measurement of electrical properties from tissues like skeletal muscle. Such complexity is characteristic of most tissues and cells and the attendant problems have a scope beyond the questions considered here [see Eisenberg and Mathias (8) for some general discussion]. Structural complexity ensures that the potentials measured, and the currents applied, to a skeletal muscle fiber are not across a single membrane but rather across a combination of membranes. The structural complexity allows an alternative explanation of charge movement. That explanation necessarily involves a certain complexity, since it assumes particular properties for each of the structures known to be present. We consider such complexity to be a natural, not an awkward, feature of any model of E-C coupling, which tries to deal with the mechanism of T-SR communication and the mechanism of calcium release. We feel it likely that each structure (i.e., the T-SR junction, terminal cisternae of the SR, or longitudinal tubules of the SR) has a role in E-C coupling. Therefore, we expect a complete model of E-C coupling to be at least as complex as the structure that produces it.

Analysis of the complete model is aided by consideration of the simple circuit shown in Figure 1, which we call the *linear* model. This model represents just the T-SR junction and terminal cisternae. Structural complexity appears in this circuit as a single resistor (described as the conductance  $g_x$ ) in series with resistance-capacitance network, a typical membrane model. The time and voltage-dependent conductance  $g_x(t, \Delta V)$  is supposed to be rep-



**Figure 1.** Simple circuit models. (A) shows the “linear” circuit model used to isolate the implications of  $g_x$  for measurements of transient currents. Note that the *only* voltage- or time-dependent component in this circuit is  $g_x$ . (B) illustrates a more complex version of the linear circuit, which includes the longitudinal sarcoplasmic reticulum. This figure was taken from reference (12) with permission of the authors.

representative of the properties of the T-SR junction in skeletal muscle. The membrane shown is supposed to be the membrane of the TC of the SR. In this simple model the membranes of the TC are linear circuit elements, having resistance and capacitance independent of time or voltage.

The linear model is certainly too simple to be expected to describe a muscle fiber. It fulfills useful criteria, however:

1. It can approximate the voltage dependence of charge movement over a limited range of voltage.
2. It produces equal amounts of ON and OFF charge when the standard voltage clamp protocols are simulated.
3. It can produce a voltage change in the TC large enough to trigger a calcium-release mechanism.
4. The circuit parameters necessary to fit the data are consistent with the probable properties and known amounts of TC membrane.
5. The complexity of the properties of  $g_x$  necessary to produce agreement with experimental data is not too great.

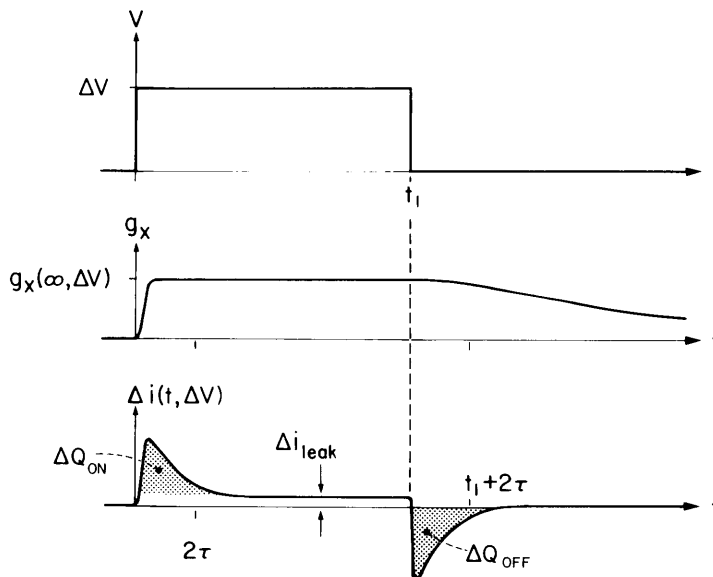
Similar criteria have been previously applied to a number of simple circuit models by Chandler and colleagues (5). The circuit models analyzed by those authors did not satisfy the criteria very well and were therefore considered unsatisfactory models of charge movement. We have used models of similar topology but with quite different properties of the components. Such circuit models can describe many of the features of charge movement.

The properties of the conductance  $g_x(t, \Delta V)$  assumed in these calculations are shown in Figure 2. The conductance of the T-SR junction is supposed to increase rapidly with depolarization to a value of the order of  $500 \mu\text{mho}$  for the amount of T-SR junction associated with  $1 \text{ cm}^2$  of outer surface for a fiber of radius  $40 \mu\text{m}$ . The conductance is supposed to decrease slowly upon repolarization to the original potential.

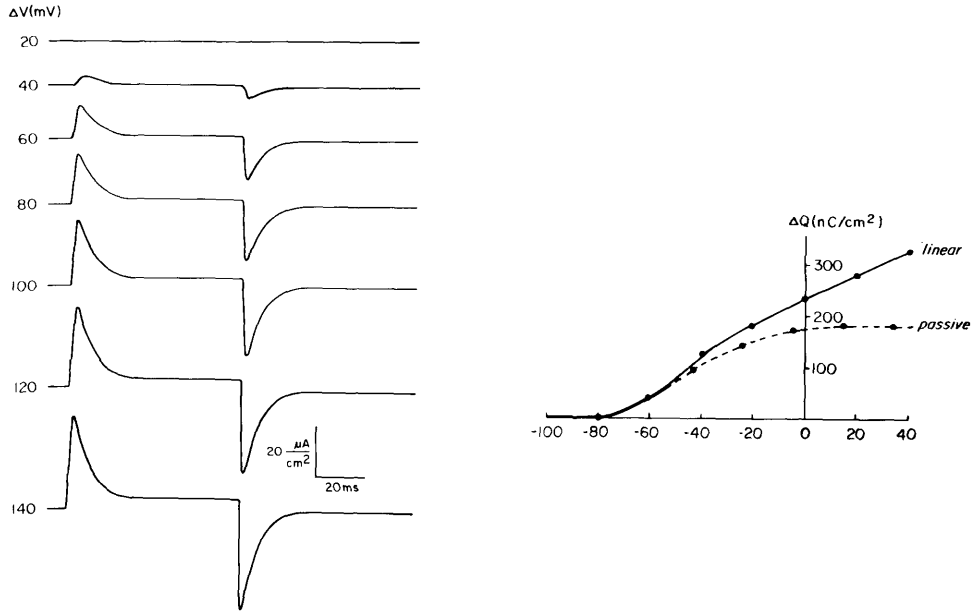
The results of the calculations for the linear model are shown in Figure 3. The calculated nonlinear displacement currents, which are the result of the

same sequence of pulses used to determine charge movement experimentally, are qualitatively similar to experimental results, although clear saturation of charge movement is not seen, nor is there asymmetry in the time course of the currents at the ON and OFF of the pulse. The quantity of charge movement at the ON and OFF of the pulse are as equal as they are found to be in muscle. The parameters used to describe the TC membrane are not unreasonable.

The reason that the linear circuit gives acceptable equality of charge movement as a consequence of a voltage-dependent resistance (with no voltage-dependent capacitance or bound charge movement) is easy to see. The time- and voltage-dependence chosen for  $g_x$ —in particular its rapid increase with depolarization—ensures that the great majority of the ON charge movement occurs after the conductance  $g_x$  has reached its steady value. Similarly, the slow decline of  $g_x$  with time after repolarization implies that most of the OFF charge movement will occur before  $g_x$  has declined too much from the value reached during the preceding depolarization. Since the charge movement at the ON and the OFF of the pulse are through circuits with



**Figure 2.** The time-dependence of the postulated conductance from T system to SR and the resulting transient current. The record labelled  $g_x(t, \Delta V)$  shows the assumed time dependence following a depolarization of  $\Delta V$ . The conductance  $g_x$  reaches a steady value called  $g_x(\infty, \Delta V)$ ; for other values of  $\Delta V$ , the time-course is almost identical but, of course,  $g_x(t = \infty)$  changes. The nonlinear transient current, resulting from the addition of currents from depolarizing and hyperpolarizing pulses  $\Delta V$ , is shown as  $\Delta i(t, \Delta V)$ , with the steady level of current  $\Delta i_{\text{leak}}$  determining the nonlinear leak conductance defined by  $\Delta g_{\text{eff}} \equiv \Delta i_{\text{leak}} / \Delta V$ . The integrals of the transient currents, indicated by stippled areas, determine the nonlinear charge movements  $\Delta Q_{\text{ON}}$  and  $\Delta Q_{\text{OFF}}$ . This figure was taken from reference (12) with permission of the authors.



**Figure 3.** The asymmetry currents produced by the linear model. The upper panels show the nonlinear currents produced for a set of  $\Delta V$ . Note that each record is the sum of the currents produced by a depolarizing and hyperpolarizing pulse. The lower panel shows the integrals of the transient charge (i.e., the stippled area of Figure 2) for both linear and passive models. This figure was taken from reference (12) with permission of the authors.

almost the same parameters, it is clear that the charge movements should be nearly equal.

The simplicity of the linear circuit model is useful because it allows analytical expressions for the main parameters of charge movement. In particular, it is easy to compute the effective capacitance of the TC, effective capacitance being measured experimentally from integrals of transients [see Adrian and colleagues (3) and a generalized discussion in Eisenberg and Mathias (8)]

$$\text{Effective capacitance of TC} = \left( \frac{g_x}{g_x + g_{TC}} \right)^2 c_{TC}$$

Note that the effective capacitance depends on the conductances in the circuit as well as the capacitance. Thus, even in a model (like our linear model) in which all the capacitors are independent of potential, the measured (namely, effective) capacitance will depend on potential, as a result of the potential dependence of the conductances.

Nonlinear charge movement can be described as an increase in capacitance with depolarization. The equation above shows that the increase in  $g_x$  postulated previously would produce an increase in capacitance, i.e., charge

movement. The increase in a single conductance like  $g_x$  with depolarization is not sufficient, however, to produce the saturation in charge movement seen experimentally. The curve relating charge movement and potential for the linear model is asymptotic to a linearly increasing charge-voltage curve, not to a horizontal line. In this regard the linear model is an inadequate representation of a muscle fiber. The equation above shows, also, that a change in  $g_{TC}$  will produce a change in effective capacitance. The *passive model* introduced later in this paper will include a voltage dependence in  $g_{TC}$  and thus will produce saturation in charge movement.

The linear model permits approximate expressions for the time-constant  $\tau$  of the charge movement, namely,

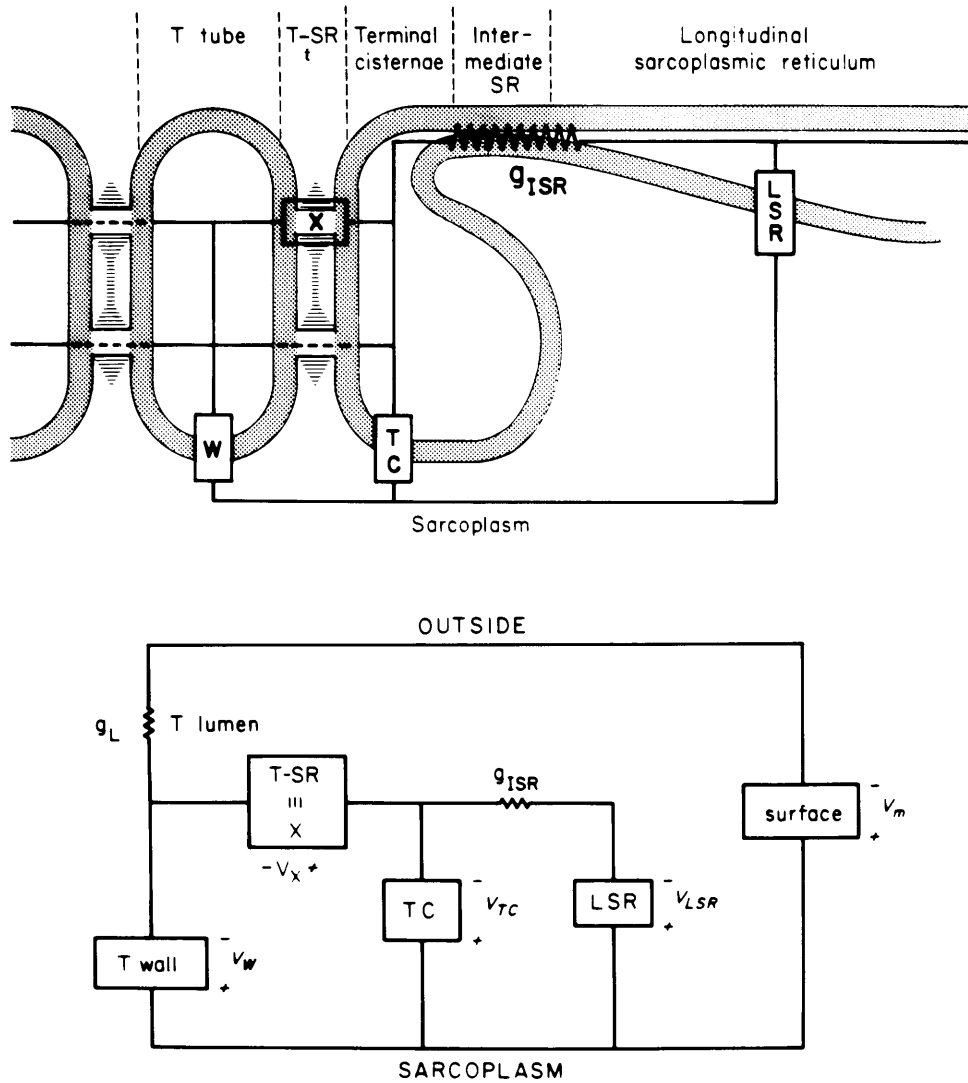
$$\tau = \frac{C_{TC}}{g_x + g_{TC}}$$

Note the little freedom available in the specification of the time constant in the linear model. Once the properties of the TC are assumed to be independent of potential, then the only variable which can adjust the time course of the charge movement is  $g_x$ . If  $g_x$  has the time course postulated (see Figure 2), it will have nearly the same value during the ON and OFF response: The time course of the charging and discharging transients will be nearly the same. The property attributed to  $g_x$  to give equality of charge movement thus implies symmetry in the ON and OFF transients. Since experimental observations show considerable asymmetry in the transients, it is clear that the linear model is not an acceptable representation of a muscle fiber. On the other hand, we shall show that a more complex voltage- and time-dependence in the properties of  $g_x$  can produce the required asymmetry.

#### PASSIVE MODEL

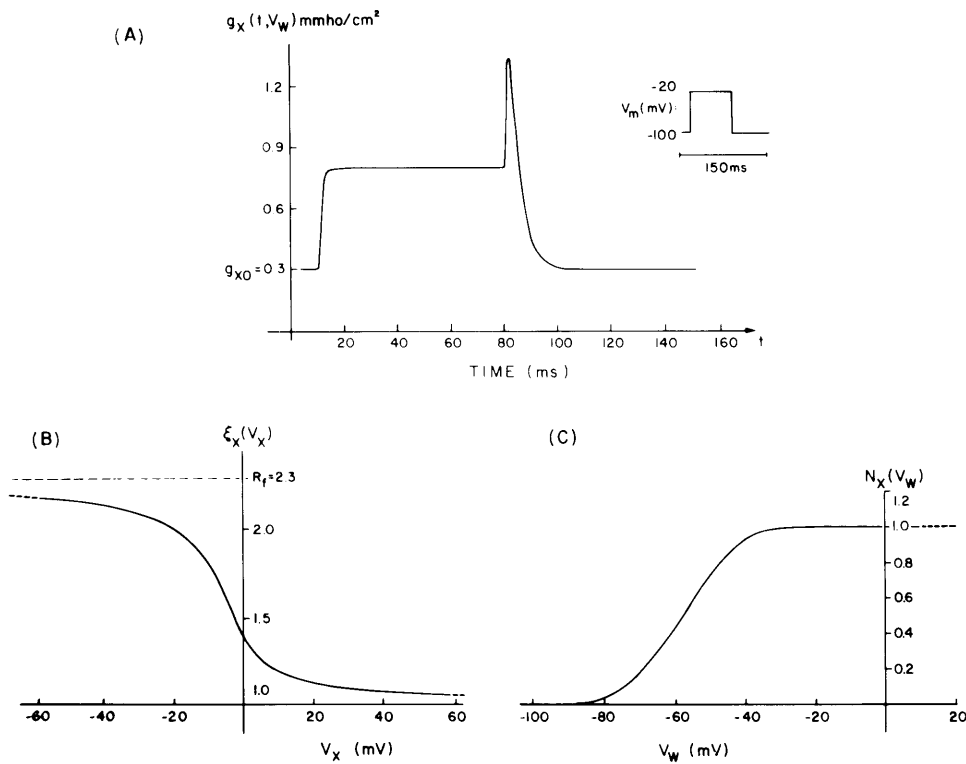
The linear model just described fails to describe the saturation or time course of the charge movement measured from skeletal muscle. And it does not include the entire sarcoplasmic reticulum. The passive model, shown in Figure 4, includes the longitudinal tubules of the SR and assumes more complex properties for the conductance  $g_{TC}$  of the membranes of the TC and for the conductance  $g_x$  linking the T system and the SR. In particular, the longitudinal tubules are supposed to be isolated by a substantial resistance, and  $g_{TC}$  is supposed to rectify in a manner reminiscent of the potassium conductance of nerve axons.\* The conductance  $g_x$  is assumed to have a more complex behavior, as illustrated in Figure 5, in which it has both instantaneous and steady-state rectification. The instantaneous rectification is assumed

\*The calculations were performed without including time-dependence in the properties of  $g_{TC}$ . The potassium conductance of squid axons or skeletal muscle is sufficiently fast compared to charge movement that its kinetics would have little effect.



**Figure 4.** The passive circuit model. The upper panel shows a circuit model of the T system, TC, and SR of skeletal muscle. The properties of specialized structures are indicated by boxes, identified in more detail in Mathias and colleagues (12). Noteworthy features of the model are the presence of a conductive path  $g_x$  for ionic current flow from the lumen of the T system to the TC and the presence of a substantial resistance  $1/g_{ISR}$  isolating TC from the longitudinal SR. The lower panel shows the circuit representation of a complete fiber, now including the luminal conductance of the T system and the properties of the surface membrane. In our computations the T system is treated as a lumped circuit element; distributed properties are approximated by including  $g_L$ . This approximation will not be particularly accurate at very short times or during an action potential. This figure was taken from reference (12) with permission of the authors.

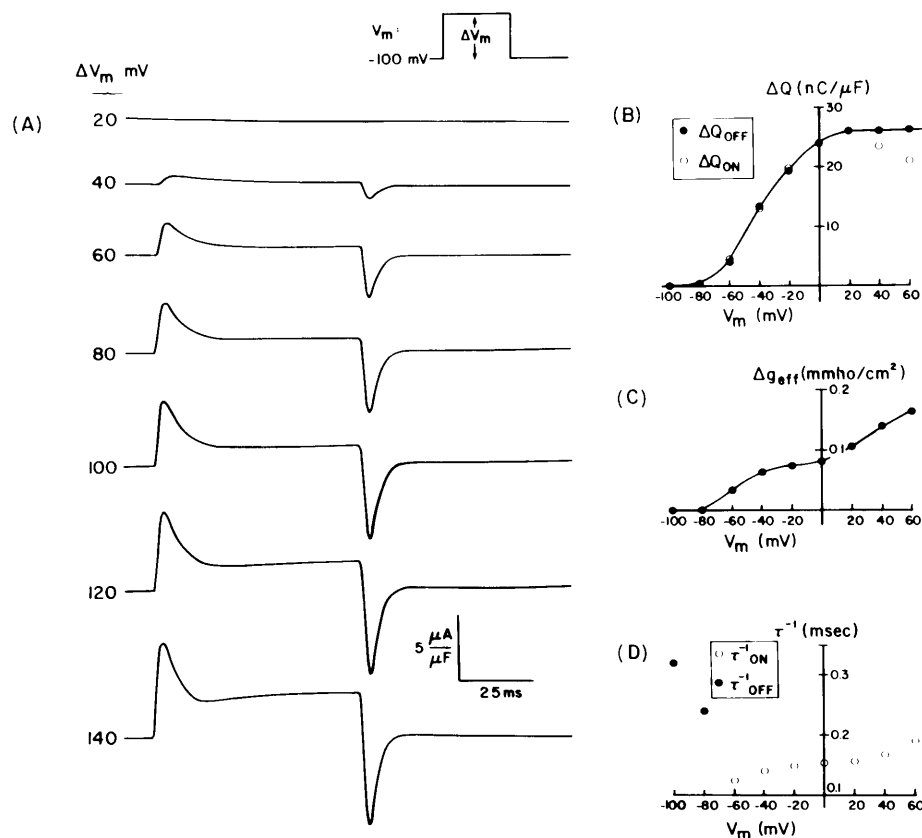




**Figure 5.** The time- and voltage-dependence of the ionic pathway postulated from T system to SR. (A) shows the time-course of the conductance  $g_x$  for the step of potential (of 70 msec duration and height  $\Delta V$ ) illustrated symbolically. Note the rapid increase in the conductance, and the rather slower decline (more evident in Figure 2). A jump in the conductance occurs when the pulse is turned off because of instantaneous rectification which depends, in effect, on the direction of current flow. (B) and (C) illustrate the functions which describe the instantaneous and time-dependent rectification of  $g_x$ , respectively. This figure was taken from reference (12) with permission of the authors.

to allow inward current to flow more easily than outward current, producing the fast transient current shown at the OFF of the pulses shown in Figure 6. With these assumptions, it is possible to predict records of nonlinear displacement current which are quite similar to those recorded experimentally (Figure 6). The charge movement at the ON and OFF of the pulse are reasonably equal; the charge movement saturates with increasing depolarization, and the time-course of the charge movements is quite similar to that observed.

There are probably other ways to introduce time- and voltage-dependent conductances into the passive model and to get good agreement with experimental data. It may be possible, for example, to assume a conductance  $g_x$  with quite simple properties, perhaps even independent of voltage and time,



**Figure 6.** The predicted nonlinear transient currents. These records are to be directly compared with the experimental results of Chandler and colleagues (5, 6) and Adrian and Almers (1, 2). The currents shown in (A) are the sum of the currents produced by the pulse shown (of 70 msec duration and height  $\Delta V$ ) and a pulse of the same size in the hyperpolarizing region. Since the circuit elements are assumed to be independent of voltage in the hyperpolarizing region, the summation emphasizes the nonlinear components of current. (B) illustrates the integrals of the transients shown in (A), computed after subtraction of a constant baseline. The area represented by this procedure is illustrated in Figure 2. Note the rough equality of ON and OFF charge and the saturation of charge movement. Because of the slope of the baseline at depolarizations to +20 mV and +40 mV, we underestimate the value of  $\Delta Q_{ON}$ . The solid line is therefore drawn through the filled circles representing  $\Delta Q_{OFF}$ . (C) shows the amount of nonlinear ionic current (graphically identified in Figure 2) accompanying the nonlinear transient current. Note the increase in nonlinear ionic current which accompanies the saturation of nonlinear charge movement. (D) illustrates the estimated time-constants of the nonlinear transient currents. This figure was taken from reference (12) with permission of the authors.

and to get good agreement with data by introducing a sufficiently complex nonlinear generalization of  $g_{TC}$ , a generalization including several time- and voltage-dependent conductances, each in series with an equilibrium potential. We have not attempted to investigate the range of models possible, but we have considered a simple "active" model that includes calcium release.

## ACTIVE MODEL

An active model was constructed by replacing  $g_{TC}$  with a circuit element containing two Hodgkin-Huxley conductance systems, one for calcium and one for a counterion with an equilibrium potential opposite to that for calcium. We found that the calcium current associated with the large release of calcium necessary to saturate the binding sites on the thin filament would severely distort the current measured in a voltage clamp, unless it was balanced by an almost equal and opposite current *at all potentials and times*. Such a countercurrent could be part of an electrically silent calcium-release mechanism, electrically silent in the same sense that chloride transport in the red blood cell is electrically silent. In particular, if the calcium-release mechanism *required* calcium release to be accompanied by an almost equal (within 1%) and opposite movement of a countercurrent, it would be possible to have massive calcium release with only a small contribution to the observed current. An electrically silent mechanism of this sort could still depend on voltage in two ways. First, it might be controlled by a voltage sensor in the SR membrane, much as sodium conductance is apparently controlled by a voltage sensor in an axon membrane. Second, an "electrically silent" calcium-release mechanism might still have a net current associated with calcium movement. Such a current would be small compared to the flux of calcium but could still be large compared to the net current flow of other ions. In this way the conductance for calcium ions produced by a (mostly) electrically silent mechanism could confer significant electrical properties on the system.

## GENERAL CONCLUSIONS FROM THE ANALYSIS OF THESE MODELS

A few general conclusions arise from the analysis of these models of ionic current flow from T system to SR:

1. Models of electrical coupling, which are to describe charge movement as measured in frog muscle, seem to require a substantial electrical isolation between the terminal cisternae and longitudinal tubules of the SR. This property is *not* required to produce substantial voltages in the SR; it is only required to fit the amount and time-course of the charge movement reported in frog. It would be interesting then to measure charge movement in preparations that apparently have a single compartment of SR to see if its amount and time-course can be explained by models including ionic current flow into the SR.

2. The coupling conductance  $g_x$  is a very small number, when written as the property of the structures linking the T system and the SR. This conductance is sufficiently small that the lumen of the SR is as isolated from the extracellular space as is the sarcoplasm itself! Thus, we view the T-SR junction as quite distinct from a gap junction, although it might have a common molecular heritage.

3. Models of excitation-contraction (E-C) coupling involving electrical coupling from T system to SR require that calcium release be, in large measure, electrically silent as described earlier in the paper.

#### SUMMARY

The circuit models presented here can explain most of the properties of charge movement measured in skeletal muscle. But this fact does not mean that our models are correct either in general or in detail. Even if the general idea of electrical coupling is correct, it is unlikely that the properties we have attributed to the conductances  $g_x$ ,  $g_{TC}$ , or  $g_{LSR}$  are correct. Conductances with quite different properties can probably explain measured charge movement. Furthermore, such conductances can be expected to explain many of the detailed properties of excitation-contraction coupling being presented in this volume, properties that were unknown to us when the present model was constructed. Finally, it seems likely that the charge movement observed in skeletal muscle has multiple origins, whereas in our model it has only one. Some of the charge movement in muscle probably arises in the movement of membrane-bound charges associated with ionic conductances, some part as the movement of macromolecules at the T-SR junction, and perhaps one part as the result of current flow into the sarcoplasmic reticulum.

A result of our analysis, which appears generally valid, is that structural complexity can give rise to currents which mimic gating currents. Experiments have been proposed by Mathias and colleagues (12) to distinguish between the different possible causes of charge movement. The possibility of ionic current flow from T system to SR is appealing to us, because many of the structural complexities required to make such current flow explain the properties of charge movement in skeletal muscle are known to exist. But the appeal of a model is not relevant to its validity. That is an experimental question. The experiments stimulated by these models are likely to cast useful light on the mechanism of excitation-contraction coupling whatever that mechanism is.

#### ACKNOWLEDGMENT

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## DISCUSSION

*Krasne:* One of your suggestions is that the terminal cisternae would have a voltage-dependent conductance (cation conductance). As you no doubt know, Dr. Christopher Miller, who works on SR vesicles, has fused these to bilayers and has found cation conductances; he has also found that these seem to be voltage-dependent (*J. Membr. Biol.*, 1978, **40**: 1-23). Have you looked at any of his data on the voltage dependences or kinetics to see if that kind of a channel is consistent with what you are postulating would exist in the terminal cisternae? And Question 2: He has found a number of blockers for this channel. Do you know if anyone has tried putting something like cesium in the fiber, or substances that could block that conductance, for example, sulfhydryl reagents? Would you consider that feasible for seeing if these affect the apparent charge movement?

*Mathias:* Someone should study the blockers. There is too much ambiguity concerning the state of Miller's preparation to allow the calculations of kinetics or voltage dependence. It would be interesting to see the effect of some of the blockers on the saturation of charge movement. It would be a very good experiment.

*Rakowski:* You alluded to one experimental protocol that might differentiate between your proposed model and a model based on membrane charge, namely, if you depolarize a fiber so as to open the electrical access to the SR, and then—ON the OFF—go to successively more and more hyperpolarized voltages, you would predict that if there were conductance connecting the SR and the T system, the OFF transients should get larger and larger. I have done that experiment, and the result is inconsistent with your model. That is, if you depolarize a fiber and get an ON-charge transient and make successively larger hyperpolarizing steps at the OFF, the OFF area tends to remain constant, although there is some slight change. Would you say that that experiment disproves your model? Or is there another explanation?

*Mathias:* Yes, at least in part. As the conductance,  $g_x$ , is made to rectify, making the transients on the OFF faster, you can also turn it off faster, because the charge movements are over. If the conductance turns off faster, the more you hyperpolarize, the less that effect would be. So you would tend not to get too much more charge movement. The second way is to assume that charging of the terminal cisternae is only some fraction of the total charge movement. Then it would not be as dramatic an increase as you might otherwise expect.