The generation of electric currents in cardiac fibers by Na/Ca exchange

MULLINS, L. J. The generation of electric currents in cardiac fibers by Na/Ca exchange. Am. J. Physiol. 236(3): C103-C110, 1979 or Am. J. Physiol.: Cell Physiol. 5(2): C103-C110, 1979.—The presence of a detectable Ca current during the excitation of a cardiac fiber implies that the Ca lost during the resting interval of the duty cycle must also be detectable. Ca outward movement appears to be effected by Na/Ca exchange when more Na enters than Ca leaves per cycle, thus making the mechanism electrogenic. Since Na/Ca exchange can move Ca either inward or outward depending on the direction of the electrochemical gradient for Na, a potential exists where there is no electric current generated by the Na/Ca exchange mechanism, i.e., a reversal potential $E_R$. Cardiac fibers appear to have a reversal potential that is about midway between their resting membrane potential and their plateau. Carrier currents both inward and outward are therefore generated during cardiac action potentials. The implications of the conditions stated above are explored.

membrane potential; cardiac action potential; reversal potential; squid giant axon

A WIDELY ACCEPTED GENERALIZATION is that membrane channels of various sorts convey ion movements in a downhill direction, thus leading to the generation of bioelectric currents. Accompanying such a process is the need for a battery charger in the form of a Na/K pump driven by metabolism (ATP) that will restore the ionic gradients upon which the above bioelectric behavior depends. In the case of cardiac muscle fibers, it would appear that there are two inward and four to five outward currents involved in electric excitation (12) so that, if anything, one might like to propose a simplification of this array of currents. The purpose of this review is, however, to suggest the existence of yet another current, one that is generated by the movement of $Na^+$ and $Ca^{2+}$ in opposite directions across the cell membrane via a carrier-mediated process.

In a squid giant axon, Na channel flux can have a magnitude of $30 \text{nmol/}(\text{cm}^2\cdot\text{s})$, Na pump flux $30 \text{pmol/}(\text{cm}^2\cdot\text{s})$, and Ca pump flux $30 \text{fmol/}(\text{cm}^2\cdot\text{s})$ or ratios of $10^{10}:1$. Thus, one might conclude that Ca pumping can hardly influence bioelectric behavior because even the Na pump has at best a modest effect on membrane potential. Such an analysis ignores, however, the fact that certain cells both require a large Ca flux to carry out their physiological activities and have a repetitive sort of bioelectric activity so that flux balance must be maintained over periods of the order of one second.

A countertransport of Na for Ca across the membrane of cardiac cells was proposed by Reuter and Seitz (32) as a means for pumping Ca out of these cells by a mechanism only indirectly dependent on metabolism. Under this scheme the Na/K pump, driven by ATP, would produce a Na electrochemical gradient across the cell membrane and this gradient would be used to energize the outward transport of Ca. Further support for this arrangement was provided by Blaustein and Hodgkin (7), who showed that Ca efflux in squid axons poisoned with CN was manyfold that of influx and that Ca efflux depended on $Na_\infty$, thus suggesting Ca efflux did not depend on ATP. More detailed studies (1) showed that if $Na_\infty$ was made low and $Na_\infty$ high (i.e., reversing the Na concentration gradient across the membrane), then Ca influx was large and Ca efflux small, while there was also a Ca,-dependent Na efflux whereby 3-5 Na emerged per Ca entering. These findings strongly suggested that the Na/Ca exchange system is reversible and can move Ca in a direction opposite to that of the Na electrochemical gradient. Further studies (25) showed that hyperpolarization by externally applied currents increased Ca efflux about $e$-fold/25 mV change in $E_m$ while depolarization had the opposite effect. Baker and McNaughton (4) showed that the Ca,-dependent Na efflux (presumably reflecting Ca influx by the carrier) was also sensitive to membrane potential and that Na efflux increased with depolarization. Thus efflux is given by $(k \exp -EF/RT)$, influx by $(k \exp EF/RT)$ or the flux ratio by $(\exp -2EF/RT)$; this is an expression appropriate for the movement of two positive charges (or $4 Na^+ = 1 Ca^{2+} = 2^+$). These sensitivities of the Ca fluxes to membrane potential imply that the carrier is an electrogenic mechanism (i.e., moves
unequal quantities of electric charge per cycle), although in a sense opposite to that of the electrogenic Na pump. If \( r \) is defined as the coupling ratio \( Na/Ca \), then electrogenicity sets a minimum value of 3 for \( r \) as indeed Blaustein (6) has proposed. There are two difficulties with this value for the coupling ratio: first, it does not explain why both influx and efflux are changed e-fold by a 25-mV change in membrane potential (a ratio of 3 could exist if each flux were only half as sensitive to membrane potential or all the sensitivity could be put on either the influx or the efflux), and second, a ratio of 3 gives, for even the most generous estimates of \( E_m (\sim 75 \text{ mV}) \) and Na ratio (=10), a \( Ca \) ratio of \( 2 \times 10^4 \), which is one-fifth of the measured \( Ca \) ratio in squid axons (15). Furthermore, since there are substantial passive leaks of \( Ca \) into squid axons, \( Ca \) cannot approach its equilibrium value but must be substantially higher. Arguments relating to the thermodynamics of transport (23) and its kinetics (24) therefore set a minimum value of 4 for \( r \), and this value is used in the following discussion, which is also valid for all values of \( r \geq 3 \).

Countertransport is not confined to \( Ca \) alone because there is abundant evidence that Mg fluxes across the squid axon membrane depend on \( Na \), and influx is inhibited by \( Na \) (2, 13, 26). There is also evidence (33) that \( H^+ \) efflux depends on \( Na \) in snail neurons. The stoichiometry of the above processes has not been looked at; hence it is impossible to say whether they might contribute electric currents to the membrane, but the possibility of such an action ought to be kept in mind.

**Membrane Current Produced by Na/Ca Exchange**

The operation of any electrogenic ion pump involves the production of membrane current. The most familiar and widely accepted electrogenic mechanism is the Na/K pump; this produces a hyperpolarization that in the steady state can make the membrane potential approach, but not exceed \( E_K \) (27). The pump depends on \( Na_0 \), \( Na_i \), and ATP; in rather well-defined ways. It differs from the Na/Ca system mainly in two respects: 1) it is not thermodynamic and, following the usual assumption of static mechanisms of the cell, \( E_{Na} \) is also fixed, and if \( Y_L \) changes not at all, \( I \) shall first consider how the system behaves if \( E_{Na} \) is fixed and later consider the effect of variations in both \( E_m \) and \( E_{Na} \).

From the discussion above, it can be concluded that the Na/Ca exchange system can move \( Ca \) in either direction across the membrane and that such movement will involve the flow of current since the mechanism is inherently electrogenic. The direction that \( Ca \) is driven depends on the direction of the Na electrochemical gradient and can be most easily summarized by saying that if

\[
n \varepsilon F (E_{Na} - E_m) > \varepsilon F (E_{Ca} - E_m)
\]

where \( n \) is the coupling ratio \( Na/Ca \) and \( z \) is ion valence, then \( Ca \) is driven out of the cell by Na entering; and if the Na electrochemical gradient is less than the \( Ca \) electrochemical gradient, then \( Ca \) will move into the cell in exchange for internal Na. Thus the direction of movement of \( Ca \) depends on three variables, \( E_{Na} \), \( E_{Ca} \), and \( E_m \). The expression above implies that when the Na and Ca electrochemical gradients are exactly in balance, then for \( r = 4 \), \((2E_{Na} - E_{Ca} - E_m = 0) \). Under physiological conditions where \( E_{Na} \) and \( E_m \) are fixed by the homeostatic mechanisms of the cell, \( E_{Ca} \) is also fixed, and if \( n \) times the Na electrochemical gradient is exactly equal to the \( Ca \) electrochemical gradient, it is clear that \( E_m \) is a potential where there is zero membrane current through the Na/Ca exchange mechanism. In effect this is a reversal potential for the exchange mechanism. There will be an outward movement of \( Ca \) (and an inward current) if \( E_m \) is more negative than \( E_{Ca} \), where \( E_R \) is the reversal potential defined as \( E_R - 2E_{Na} - E_{Ca} \). Membrane current and charge movement associated with \( Ca^{2+} \) will always have equal magnitudes and opposite signs in the Na/Ca exchange system because a single cycle of the mechanism moves 4 \( Na^+ \) in one direction and 1 \( Ca^{2+} \) in the other. Thus the net charge movement is 2 but in a direction opposite to that of the \( Ca \) movement.

There are several ways of thinking about the Na/Ca exchange mechanism. One is to suppose it to be a voltage-dependent device that provides a reversal potential for \( Ca \) movement that is less positive than \( E_{Ca} \). Another is to suppose that it transforms \( Ca^{2+} \) from a univalent cation to a divalent anion insofar as the generation of membrane current is concerned.

This review will be concerned with showing that three variables (\( E_m \), \( E_{Ca} \), and \( E_{Na} \)) interact so as to control the direction of Ca movement across the membrane of a cardiac fiber by a carrier-mediated process—one that is entirely unrelated to Ca movement through slow inward channels. Because, during an action potential, \( E_m \) can change with a time constant of the order of a millisecond (the membrane \( RC \) product), whereas \( E_{Ca} \) changes with a time constant in the range of 10-100 ms, and (at constant heart rate) \( E_{Na} \) changes not at all, I shall first consider how the system behaves if \( E_{Ca} \) is fixed and later consider the effect of variations in both \( E_m \) and \( E_{Na} \).

The equation on which the reversal potential is based is

\[
[Ca]_o \left( \frac{[Na]}{[Na]_o} \right)^n \exp \left( -\frac{2\varepsilon F}{RT} \right)
\]

This has the advantage of reminding the reader that Ca fluxes depend on five variables (ATP is a further variable that affects the magnitude of Ca flux), and especially that \([Ca]_o \), a parameter involved in contractions, is affected by the internal and external \([Na]\).

**Applicability of Na/Ca Exchange to Cardiac Cells**

As noted above, Ca fluxes in a giant nerve fiber are so small relative to those of the Na pump, and in turn these are 1/1000th of those of bioelectric activity, that it is impossible to ascribe any significant role in bioelectric activity to Ca movements. In cardiac Purkinje fibers, how-
however, it is easy to measure a slow inward current that flows during depolarization that is at least in part carried by Ca\(^{2+}\) (30). The fact that slow inward current does not appear to reverse at plausible values for \(E_{Ca}\) has led Reuter and Schulz (31) to propose that the slow inward current channel is imperfectly selective to Ca and carries appreciable Na. This means that the reversal potential for the channel will lie between \(E_{Na}\) and \(E_{Ca}\). The suggestion to be made in this review, however, is that variation in \(Na_{o}\) involved in testing the Reuter and Schulz proposal leads to changes in a carrier-mediated Na/Ca exchange and thus produces a current that subtracts from \(I_{Ca}\). The existence of such a current obviates the need for the proposed imperfectly selective channels. Although channels for monovalent cations are often imperfectly selective between Na and K, for divalent cations any Ca gained during the depolarizing cycle must be lost during the recovery phase of the third of the cardiac cycle, and because in the steady state perfectly selective between Na and K, for divalent cations Na inward across the cell membrane and that these ions competed for passage. An additional suggestion by these authors was that the carrier in its loaded state is capable of charging a pump driven by ATP in an uncoupled mode is then a postulate for the scheme. Such a pump will necessarily have the electrical consequences outlined above. Another consequence of an electroneutral coupling is that for values of \(Ca_{o}\) < 20 \(\mu\)M the Na/Ca exchange mechanism would pump Ca inward rather than outward. In the discussion that follows, it appears simpler to ascribe all the free energy change involved in Ca pumping to the Na/Ca exchange system operating with a 4 Na/Ca coupling mode.

Figure 1 has been drawn to diagram the following arbitrary assumptions: 1) the cardiac action potential is a square wave of \(E_{m}= -80 \rightarrow 0\) lasting one-third of the duty cycle; 2) \(E_{R} = -40 mV\). It is recognized that \(E_{R}\) will change as \(E_{Ca}\) changes, but for didactic purposes this change is considered later. These assumptions result in a loss of Ca via the Na/Ca carrier that is twice the gain, implying that Ca gain via carrier \((Q_{Ca})\) is equal to Ca gain via channels \((Q_{Ca})\). Actual values for entry and loss of Ca via the Na/Ca exchange mechanism depend, of course, on the value of \(E_{R}\) and on how phase and amplitude relationships of the action potential relate to \(E_{R}\).

**Evidence for Na/Ca Exchange in Cardiac Fibers**

Observations that increases in \(Ca_{o}\) tend to promote, and increases in \(Na_{o}\) tend to inhibit cardiac contractility have a long history. The first quantitative expression of this effect was by Wilbrandt and Koller (35). An extension of the experimental information and a variation of the model was made by Lüttgau and Niedergerke (21), who suggested that a carrier existed that could convey 1 \(Ca\) or 2 \(Na\) inward across the cell membrane and that these ions competed for passage. An additional suggestion by these authors was that the carrier in its loaded state might be negatively charged so that reductions in membrane potential would increase the delivery of Ca to the cell interior.

The transformation of this scheme for Ca entry into Na/Ca countertransport was made by Reuter and Seitz (32), who showed clearly that many of the properties of Ca influx vs. Na, were also present for Ca efflux and that it was possible to envisage a single mechanism that would move Ca inward or outward. At equilibrium the ratio of Ca concentrations across the cell membrane would, according to this scheme, be equal to the square of the Na concentrations across the fiber, or about 100. Now the actual concentration ratio across the cell membrane is more like 10\(^3\) and the concept of equilibrium with respect to Ca in a cardiac fiber is a difficult one to visualize given the great quantities of this ion sweeping in with each action potential and the even greater quantities of Ca released by the sarcoplasmic reticulum (SR) and then reaccumulated with each beat of the heart. Clearly what is required for a functional cardiac fiber is a Na/Ca coupling ratio substantially greater than 2 so that Ca pumping can reach values of Ca, appropriate to a relaxed muscle fiber, or a pump that is driven by ATP rather than the Na electrochemical gradient. The evidence for Na/Ca exchange is quite compelling, whereas that concerning a 2:1 stoichiometry is at best quite weak. It would seem therefore that an electrogenic mechanism for Na/
Ca exchange such as that demonstrated in squid axons also exists in the cardiac fiber. Such an arrangement does not readily lend itself to a competition between Na and Ca for carrier sites because there are more sites for Na than for Ca. The concept of competition between Na and Ca was never very firmly established experimentally and what evidence there was could be otherwise interpreted. Measurements of Ca efflux in squid axons (9) showed that, over a 100-fold range of Cai, there was the same fractional reduction in Ca efflux as Na was increased, a result not compatible with a direct competition of Na and Ca for sites. Further, Blaustein (6) has made it clear that Na/Ca exchange is a simultaneous rather than a consecutive transfer of Na and Ca. Taken together, these findings are not compatible with the original Wilbrandt and Koller idea and are more simply explained by supposing that Na and Ca have separate sites on the carrier.

One of the predictions of a simple competition for a site by Na and Ca is that Ca entry should be given by 

\[ [\text{Ca}^2+]_{\text{site}} = [\text{Ca}^2+]_{\text{site}}^0 \times \frac{[\text{Na}^+]_{\text{site}}}{[\text{Na}^+]_{\text{site}}^0} \]

and the fact that experimental data appear to fit this relation is used to support the notion of a stoichiometry of 2 Na/Ca. The actual form of the equation for Ca influx (depends on E_m, Cai, and Na, but assuming these to be constant) appears to be 

\[ [\text{Ca}^2+]_{\text{site}} = (1 + [\text{Na}^+]_{\text{site}} + [\text{Na}^+]_{\text{site}}^2 + [\text{Na}^+]_{\text{site}}^3 + [\text{Na}^+]_{\text{site}}^4 + C) \]

where C is a constant (24). The actual fit to data is equally good and a curve of the above relation varies roughly as \([\text{Na}^+]_{\text{site}}^2\), but the assumptions underlying the equation are quite different. Kinetic data on the activating or inhibiting effects of Na on Ca fluxes (17) are never accurate enough to discriminate between a 2- or 4-power function and ought not to be so used. Another test for stoichiometry in cardiac fibers has been to depolarize them with elevated \([K^+]_o\) and look for changes in Ca flux. In squid axones this clearly results in a decrease in Ca efflux, implying that the exchange is rheogenic, whereas in cardiac fibers (19) there was little change in flux. In view of the complications both of Ca flux measurement and of other actions of \([K^+]_o\), it seems safer to look at more indirect measures of possible electrogenic Na/Ca exchange. It has been shown in frog atrial fibers (18) that tension depends on Na, when the fiber is clamped to extreme levels of depolarization where Ca channel current can be expected to be small. One must suppose, therefore, that the requirement for Na is so that Ca, can enter via Na/Ca exchange. Were this exchange electroneutral the mechanical response should not continue to increase with large positive values of membrane potential. Another finding (16) is that Ca current declines with extreme depolarizations but tension continues to increase, implying that there are mechanisms in the fiber that can provide more Ca even though one is approaching \(E_{Ca}\). In the case of frog fibers (with only a vestigial SR) Na/Ca exchange is strongly implicated in Ca entry; in the case of other fibers it is, of course, more difficult to discriminate between internal release and actual Ca entry.

A final argument is that in addition to the sensitivity of Ca fluxes to changes in membrane potential in squid axons, a value of 4 Na/Ca is necessary to explain the low value that \([\text{Ca}^2+]_{\text{site}}^0\), normally has in axons. In cardiac muscle an equally low value for Ca, (or an even lower one) is necessary for muscle relaxation. In squid, Requena et al. (29) have shown that a normal Ca, can be maintained in the virtual absence of ATP so this finding again supports the notion of a 4 Na/Ca coupling.

**Determination of \(E_R\) in Cardiac Cells**

Estimates of both \(E_{Na}\) and \(E_{Ca}\) are difficult in cardiac cells because of the anatomic complexity of the tissue. Values for Ca, can, however, be set by a knowledge of the sensitivity of contractile systems to this ion and these suggest that Ca, must be of the order of 50 nM or less. With a Ca, of 3 mM, this yields a value of \(E_{Ca}\) close to +140 mV. Although values for \(E_{Na}\) in cardiac fibers are subject to some uncertainty, the fact is that in a wide variety of tissues where accurate values for Na, are available, the ratio Na,/Na, is 10 or less. Estimates of a ratio substantially larger than this have been made by Ellis (15a) and Lee and Fozzard (20) on noncontracting cardiac cells using appropriate microelectrode techniques. A difficulty with accepting such values as representative of the working cardiac fiber is that one expects substantial Na entry both through Na and possibly the Ca channels; this flux will inevitably raise Na, Measurement of the dependence of \(E_m\) on \(K_o\) in cardiac tissue, coupled with action potential amplitudes, suggests that rather conventional values for Na, and \(P_{Na}/P_{Na}\) ratios may apply. Analytical Na values for cardiac tissue suggest that \(E_{Na} = 50 \text{ mV}\) may be a fair estimate. The use of the above values means that \(E_R = (2E_{Na} - E_{Ca})\) or 2 (50 mV) - 140 mV or -40 mV, a potential about midway between the resting potential of the fiber and the potential at the plateau of the action potential.

**Ca Movement during the Action Potential**

If we reconstruct the events involved in the movement of Ca\(^{2+}\) during an action potential they would proceed somewhat as follows.

Depolarization leads to:

1) Na entry via Na channels (inward current)
2) Inactivation of Na channels
3) Ca entry via Ca channels (inward current)
4) Ca entry via Na/Ca exchange (outward current)
5) Inactivation of Ca channels
6) Apparent inactivation of Na/Ca exchange

Events 3 and 4 above occur during the plateau of the action potential; both involve the entry of Ca from the external medium but in one case the current is inward, and in the other the current is outward. If the currents were of equal magnitude, and identical in time course, a voltage clamp measurement would show no inward current, yet there would still be a large Ca entry.

**Inactivation of Ca Entry**

Steps 5 and 6 above will bring about a cessation of Ca entry (and of current flow). If we assume that process 5 is the more rapid, then an inward current during the plateau will be followed by an outward current. It is not obvious why the Na/Ca exchange or carrier current should inactivate and indeed the term apparent inactivation was used above to denote this. The sequence of
events listed above ignores the release of Ca by the SR in response to membrane depolarization. If this is included then during the plateau Ca also rises as a result of this release. Because studies of skinned muscle fibers show that tension goes from zero to maximum when [Ca] is varied about 20-fold, this means that the Ca; assumed for a fiber at rest of 50 nM will rise to 1 μM and that $E_{Ca}$ will shift from +139 mV to +101. In turn, this means that $E_R$ will shift from −40 to zero (a value close to that at the action potential plateau). Thus the outward current from Na/Ca exchange flowing during the plateau will apparently inactivate as $E_{Ca}$ decreases. The current flowing through the Ca channels will also be affected by the change in $E_{Ca}$ occasioned by the SR Ca release but this effect should be relatively minor because it should vary as 101/139 or 0.73 of its initial value.

Clearly all of the foregoing cannot be examined critically without a series of assumptions about events taking place during the action potential cycle in a cardiac fiber. Following the assumption of Fozzard (16), all cardiac cells will be considered to be alike even though one recognizes their differences. This means that they are considered to have a resting potential of −80 mV and an action potential of 80 mV amplitude that is virtually a square wave lasting for one-third of the cardiac cycle, as shown in the third trace of Fig. 2. A further assumption is that all the rise in [Ca]; during contraction is contributed by the SR during one-half of the plateau even though one recognizes that substantial increases in [Ca]; are made by Ca entering from outside. This change in Ca; produced by the SR is shown in the top trace of the figure, and the Carrier current produced by the operation of Na/Ca exchange has been calculated on the assumption that $E_{Na}$ does not change while the arrangement shown earlier in Fig. 1 relating the charge movement via the Ca channels and the Na/Ca exchange has to be maintained. A further assumption is that Na/Ca exchange current is not gated (i.e., instantaneous) while $I_{Ca}$, the Ca channel current, is a slow current and one that inactivates incompletely during the action potential. The final trace is the sum $I_{Ca} + I_C$ and is presumably what might be measured as slow inward current in a voltage clamp.

**Current-Voltage Relations of Na/Ca Exchange**

$E_R$ is a thermodynamic concept, but the current-voltage relations of the Na/Ca countertransport depend on model considerations. On the simplest of such assumptions (23), current is proportional to sinh $\Delta E_R F / RT$ where $\Delta E_R$ is the displacement of the membrane potential from $E_R$.

Calcium can enter a cell via the following well-documented modes: 1) Na channels, 2) Ca channels, 3) membrane leaks, or 4) Na/Ca exchange, but any measurable efflux of Ca can only take place via Na/Ca exchange. This because the flux ratio for passive Ca movement (influx/efflux) is of the order of 107.

If Ca influx via mechanisms 1-3 above is denoted as leak $m_l$ and carrier influx of Ca is $m_c$, then for the steady state $m_l + m_c = m_{in}$, the efflux of Ca via the carrier. Because $m_{in}$ must be larger than $m_c$, the system cannot be at equilibrium with respect to the carrier, or [Ca], is always higher than values calculated from equilibrium considerations.

**Long-duration voltage clamps** to, for example, zero membrane potential, may present some ambiguity in interpretation because [Ca], can change rapidly and this affects primarily $I_C$ (Fig. 2). For reasonably short clamps and in tetrodotoxin-treated fibers the sinh $\Delta E_R$ relationship mentioned above should be valid.

One of the awkward findings of investigators seeking to ascribe an experimentally measured current to Ca has been the fact that sometimes such currents reversed at potentials only modestly positive (20–40 mV) rather than at plausible values for the Ca equilibrium potential. These considerations are important in understanding the relations between $I_{Ca}$ and $I_C$ in cells having Ca channels, as shown in Fig. 3. This figure has a rather conventional $I_{Ca}$ (peak) line for $E_{Ca}$ of +140, together with values for $I_C$ with $E_R = −40$ mV and a sinh dependence of $I_C$ on the displacement of the membrane potential from $E_R$. When these currents are summed, the reversal potential is +25

![Fig. 2. An action potential of the same sort as assumed in Fig. 1 is shown in the third trace from the top of this figure. It is assumed that this is the controlling event that initiates both the release of calcium from the SR, as shown in the top trace under the heading of $E_{Ca}$ and the change in the carrier current $I_C$ shown in the second trace, as well as the initiation of a calcium channel current that is gated and is shown in the fourth trace. The carrier current at the resting potential is assumed to be Na moving inward upon depolarization of the membrane to a potential of 0; the current is assumed to reverse and be equal and opposite without any gating delay. The dashed line on the $E_{Ca}$ trace is the assumed value of $E_{Ca}$. The decline of $E_{Ca}$ from 140 to 100 as a result of SR Ca release causes $E_R$ and $I_C$ to become zero. The return of $E_{Ca}$ to −80 while $E_{Ca}$ is +100 yields a larger inward $I_C$. The re-uptake of calcium by the SR brings $I_C$ back to its original value. $E_{Ca}$ assumed to increase in a gated manner and to inactivate at a rate ½ that at which the current rose. The current during this interval of time that might be experimentally measured is shown in the bottom trace as the sum of $I_{Ca} + I_C$; this rises in a positive direction with a delay. At earlier times than those plotted, the current is positive but may well be merged with fast currents that are imperfectly resolved in the voltage clamp.](http://ajpcell.physiology.org/)

Downloaded from http://ajpcell.physiology.org/ by 10.220.33.5 on April 13, 2017
mV, which is clearly in the range of potentials where alleged Ca currents sometimes reverse.

**Stability of \(E_{Ca}\)**

One of the difficulties in making any sorts of measurements of Ca fluxes, or concentrations, is the recognized ability of the interior of cells to buffer Ca concentration changes. In the squid giant axon it has been possible to show rather clearly the following (10): 1) there is little Ca in mitochondria at normal levels of \([Na]\), and Ca buffering by mitochondria only becomes significant when \([Ca]\) is 1 \(\mu\)M or greater; 2) buffering of Ca that is CN insensitive (presumably by Ca binding proteins) is such that only 1/20th of the entering Ca goes to increase \([Ca]\). A second aspect of the buffering problem is the rate at which Ca is buffered intracellularly. Present techniques for measuring \([Ca]\) are not fast enough to look at the speed of buffering, but it does seem likely that on the time scale of bioelectric events (milliseconds) not much buffering takes place.

In spite of the foregoing, there are substantial reasons for supposing that Ca entry via bioelectric activity changes \(E_{Ca}\) just inside the membrane. This is so because Ca is so small compared with Ca entry. In squid axon, Na influx is 40 pmol/(cm\(^2\) s) while Na, is 40 mM, a ratio of 10\(^{-5}\), whereas Ca influx is 40 fmol/(cm\(^2\) s) and Ca, is 40 mM, or a ratio of 10\(^{-8}\). A conclusion is that Ca influx is capable of changing Ca 1000-fold more easily than Na influx is able to change Na. Given the much lower mobility of Ca compared with Na, it is clear that \(E_{Ca}\) is a much more labile entity than \(E_{Na}\) if bioelectric activity is to be considered.

**Stability of \(E_{Na}\)**

One can argue that Na pumping establishes both \(E_{Na}\) and \(E_{m}\). Because these are the determinants of \(E_{Ca}\), the behavior of the Na/Ca exchange system is determined by Na pumping. Slowing the rate of Na pumping by, for example, partial ouabain poisoning or lowered \([K]_o\), will lead in the steady state to an increase in \([Na]\), and hence to decreases in the steady-state values of both \(E_{Ca}\) and \(E_{Na}\). Because, as noted above, the leakage fluxes of Ca tend to keep Ca higher than the value specified by equilibrium, the principal effect of an increased Na, is to promote Ca inward movement since this is facilitated by increases in \([Na]\), independent of the change in \(E_{Na}\) so produced. As a first approximation, the value of \(E_{R}\) will not change because changes in \(E_{Na}\), are slow enough so that \(E_{Ca}\) will follow them, but the current developed for a given displacement of \(E_{Na}\) from \(E_{R}\) will be affected for outward currents (Ca moving inward). If Na pumping were hyperpolarizing the membrane, the loss of this potential would further increase \(E_{Ca}\). On the other hand, if Ca pumping (and its associated Na entry) were depolarizing the membrane, one would expect more depolarization in a ouabain-treated fiber.

Decreases in \([Na]\), have two sorts of effects, 1) the instantaneous effect where \(E_{Na}\) is made negative and hence \(E_{R}\) moves to extremely negative values of membrane potential, and 2) the steady-state solution, where \([Na]\) becomes extremely small and \(E_{Na}\), tends to return to its original value. In the former case there is a large inward Ca movement and outward (hyperpolarizing) current at the resting membrane potential. In the latter case, currents both inward and outward are extremely small because \([Na]\) on both sides of the membrane is extremely small.

As an example of these sorts of changes, Fig. 4 has been drawn to show the effect of a change of \([Na]\), such that \(E_{Na}\), changes by 25 mV on the parameter \(2(E_{Na} - E_{Ca} - E_{m})\). This change produces an instantaneous decrease in the value of the parameter, which is equivalent to saying that Ca will enter the fiber and \([Na]\), will decrease until the influx of Na balances the pump rate. If, as is often the case, Na efflux is a linear function of Na, then at the new steady state the value of \(E_{Na}\), will be the same as it was initially so the parameter will return.
to a value of zero as shown in the diagram. Restoring [Na], to its original value will produce a substantially higher value for $E_{Na}$, because now [Na], is considerably lower than it was originally, and one has the effect of making the value of the parameter large and positive or, stated differently, this change will produce transient loss of Ca that will end when the value of the parameter reaches zero. Note that the asymmetry of this response is in fact due to changes in [Na] produced by changes in [Na], but the fiber does not continue to gain Ca when Na_o is reduced and, in fact, the gain is only a transient.

A second sort of change that one can make is to change the membrane potential rather than $E_{Na}$. This is also shown in Fig. 4. Here the change is symmetrical effect because hyperpolarization does not change [Na] on either side of the membrane and merely results in a loss of Ca, to a point that the change in $E_{Ca}$ is equal to the change in membrane potential imposed by hyperpolarization. At this point the parameter again reaches a value equal to zero and removal of hyperpolarization then allows Ca entry to the point where the value of $E_{Ca}$ again balances that of $E_{Na}$.

These considerations are particularly important in evaluating the proposition that Ca influx is controlled by [Ca]_i/[Na] because often the effect of decreased Na, on Na, is not considered. The relationship is amazing complex because Na efflux as a function of Na, can be expected to be an S-shaped curve with physiological values of Na, somewhat below the position of maximum slope. Decreasing Na, by means of a decrease in Na, means that one now needs to consider the relationship between Na, and Ca influx. This is another S-shaped curve with physiological values of Na, again below the point of maximum slope.

A return of Na, from low values to a normal one has effects that are the reverse of those described above. The instantaneous effect is a depolarization of the membrane potential and a decrease in Ca, and in the steady state, Ca fluxes come into balance as Na, rises to its normal value.

**Effects of Metabolism**

The effects of metabolism on cardiac ion currents has been recently reviewed by Carmeliet (11). In addition to the effects that can be conventionally ascribed to Na transport (changes in $E_m$ because of electrogenic Na pumping) it is important to recognize that although nerve excitation has been clearly shown to be independent of recovery process, a measure of the difference between nerve fibers and cardiac fibers is the assumption made in this review that Na/Ca exchange contributes significantly to bioelectric phenomena. A consequence of this is that ATP can be expected to affect Na/Ca exchange as shown by DiPolo (14a) for squid axons. These measurements show that both Ca influx and efflux via the Na/Ca exchange system is much reduced if ATP is absent from the cell. These measurements mean that I_ will be substantially affected by modest reductions in ATP even though the effect of ATP on Ca fluxes is a catalytic one rather than that of a substrate on an ion pump. There is therefore no reason to postulate that Ca influx via Ca channels is sensitive to metabolism because both Ca influx and efflux via the Na/Ca exchange can be substantially affected, and thus effect the changes in Ca movement seen by metabolic inhibition.

The opportunity for indirect effects of metabolism on Ca fluxes also exists because changes in Na, produced by a lack of Na pumping can be expected to affect 1) $E_{Na}$ and hence $E_{Ca}$, and 2) the magnitude of Ca influx and hence the associated outward current independent of effects on $E_m$. The reason for specifying two separate effects of Na, on carrier currents lies in the distinction that must be drawn between 1) thermodynamics which specifies the direction but not the magnitude of counter-transport fluxes, and 2) kinetics which, depending on a particular model, yields expressions for the magnitude of the net flux (a power function of Na, and Ca, for levels below saturation). This point has been addressed experimentally by Requena (28), who measured Ca efflux from squid axons at a constant Na,/[Na]_o ratio of 5.5. Efflux was 3.5-fold larger when Na,/[Na]_o = 110/20 than when it was 440/80 and it was vanishingly small when the ratio was 27/5. Such results can be explained by the known activating effect of Na, and of the inhibitory effect of Na, (24).

I am indebted to Dr. Paul DeWeer for his comments on an early version of this manuscript and to Drs. D. C. Gardshy and P. F. Cranefield for many helpful comments regarding the presentation.

This study was aided by National Institutes of Helath Grant ROI NS 5846-12 and National Science Foundation Grant PCM 76-17364.

**REFERENCES**


L. J. Mullins
Department of Biophysics
University of Maryland School of Medicine
Baltimore, Maryland 21201