"Creep Currents" in Single Frog Atrial Cells May Be Generated by Electrogenic Na/Ca Exchange

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ABSTRACT The objective of these experiments was to test the hypothesis that the "creep currents" induced by Na loading of single frog atrial cells (Hume, J. R., and A. Uehara. 1986. *Journal of General Physiology.* 87:833) may be generated by an electrogenic Na/Ca exchanger. Creep currents induced by Na loading were examined over a wide range of membrane potentials. During depolarizing voltage-clamp pulses, outward creep currents were observed, followed by inward creep currents upon the return to the holding potential. During hyperpolarizing voltage-clamp pulses, creep currents of the opposite polarity were observed: inward creep currents were observed during the pulses, followed by outward creep currents upon the return to the holding potential. The current-voltage relations for inward and outward creep currents in response to depolarizing or hyperpolarizing voltage displacements away from the holding potential all intersect the voltage axis at a common potential, which indicates that inward and outward creep currents may have a common reversal potential under equilibrium conditions and may therefore be generated by a common mechanism. Measurements of inward creep currents confirm that voltage displacements away from the holding potential rapidly alter equilibrium conditions. Current-voltage relationships of inward creep currents after depolarizing voltage-clamp pulses are extremely labile and depend critically upon the amplitude and duration of outward creep currents elicited during preceding voltage-clamp pulses. An optical monitor of mechanical activity in single cells revealed (*a*) a similar voltage dependence for the outward creep currents induced by Na loading and tonic contraction, and (*b*) a close correlation between the time course of the decay of the inward creep current and the time course of mechanical relaxation. A mathematical model of electrogenic Na/Ca exchange (Mullins, L. J. 1979. *Federation Proceedings.* 35:2583; Noble, D. 1986. Cardiac Muscle. 171–200) can adequately account for many of the properties of creep currents. It is concluded that creep currents in single frog atrial cells may be attributed to the operation of an electrogenic Na/Ca exchange mechanism.

INTRODUCTION

In the preceding paper (Hume and Uehara, 1986b), it was shown that Na loading of single frog atrial cells produced changes in membrane currents that closely resembled the "creep currents" originally described in some mammalian preparations after exposure to strophanthidin (Lederer and Tsien, 1976) or low-K solutions (Eisner and Lederer, 1979). In this paper, the properties of these creep currents are further characterized in an attempt to test the hypothesis that these changes in membrane current may be generated by an electrogenic Na/Ca exchanger. Our results indicate that: (a) reversal potential measurements of creep currents provide data that are consistent with the Na/Ca exchange hypothesis; (b) creep currents may be important in the control of tonic tension in amphibian heart; and (c) the behavior of creep currents in Na-loaded frog atrial cells can be simulated using a recently developed mathematical model of electrogenic Na/Ca exchange in the heart (Mullins, 1979, 1981; Noble, 1986). Preliminary reports of these results have been presented (Uehara and Hume, 1985; Hume and Uehara, 1986a).

METHODS

The technique for enzymatic dispersion of single frog atrial cells and the voltage-clamp technique have been previously described (Hume and Giles, 1981, 1983). Data acquisition and analysis and the composition of solutions were the same as in the preceding article (Hume and Uehara, 1986b). Unless otherwise noted, all solutions contained tetrodotoxin (TTX; 3×10^{-6} M) and experiments were carried out at 22°C.

In some experiments, mechanical activity was monitored by measuring changes in light intensity (Purves et al., 1974; Kass, 1981) using a photodiode (BPX 63, Siemens Components, Inc., Santa Clara, CA) placed directly over part of the image of the cell, which was displayed on the screen of a television monitor.

RESULTS

An example of the changes in membrane currents that are produced by Na loading of single atrial cells by exposure to the Na ionophore monensin (10^{-5} M) is shown in Fig. 1A. The top traces are superimposed membrane currents elicited by 200-ms voltage-clamp pulses to −150, −40, and +20 mV before and after 15 min exposure to monensin (10^{-5} M); the middle traces are voltage; the bottom traces are the subtracted difference current (Δi) induced by Na loading. As shown in the preceding article (Hume and Uehara, 1986b), an outward creep current (Δi) is induced by Na loading during depolarizing pulses (to −40 and +20 mV), followed by an inward creep (tail) current upon the return to the holding potential. In this experiment, hyperpolarizing voltage-clamp pulses were also applied before and after exposure to monensin, which revealed additional changes in membrane current produced by Na loading that were not studied previously. In response to hyperpolarizing voltage-clamp pulses, Na loading appears to produce changes in membrane currents (Δi) of the opposite polarity to those observed in response to depolarizing voltage-clamp pulses. During the pulse to −150 mV, an inward creep current was observed, followed by a slowly relaxing outward creep (tail) current on the return to the holding potential. The changes in peak current (open circles) and in current at 200 ms (X's) during both

depolarizing and hyperpolarizing voltage-clamp pulses are plotted as a function of voltage in panel *B*. Also plotted is the peak amplitude of the inward and outward (tail) currents (solid circles) observed on the return to the holding potential (−90 mV).

All three curves intersect the voltage axis at the holding potential, and voltage-clamp pulses in either direction away from the holding potential result in time-dependent changes in membrane current both during and after the pulses. The data in panel *B* might be interpreted in the context that any displacement in
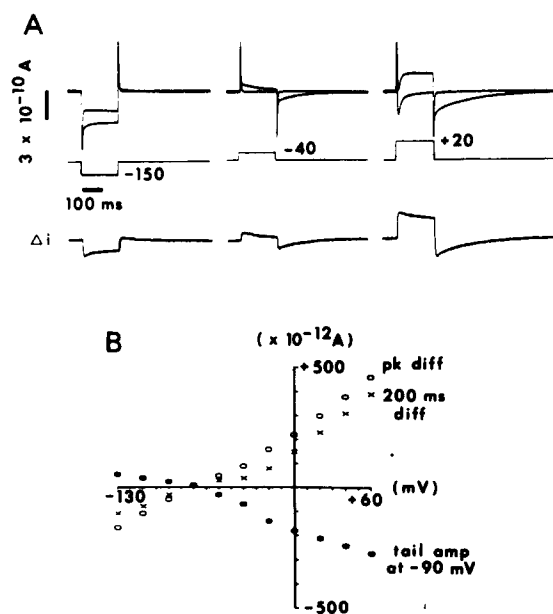

FIGURE 1. Changes in membrane current induced by Na loading of single frog atrial cells. (*A*) Superimposed membrane currents elicited by voltage-clamp pulses to −150, −40, and +20 mV before and after 15 min exposure to 1×10^{-5} M monensin; the middle traces are membrane voltage; the bottom traces are the subtracted difference currents (Δi; monensin − control) induced by Na loading. (*B*) Current-voltage relationships for the difference current induced by Na loading (peak difference current: open circles; difference current measured at 200 ms: X's) are plotted. The peak amplitude of the inward (tail) current after depolarizing voltage-clamp pulses or of the outward (tail) current after hyperpolarizing voltage-clamp pulses are plotted as solid circles. The holding potential was −90 mV.

potential away from the holding potential represents a shift away from some equilibrium condition, and it is only after the return to the holding potential that the same equilibrium conditions are re-established (see Discussion). The fact that all three curves intersect the voltage axis at the same potential may indicate a common reversal potential for both inward and outward currents (at equilibrium) whether they occur during or after voltage displacements. Furthermore, a common reversal potential may indicate that all of these current changes are mediated by a common mechanism.

The data in Fig. 1 raise the question of whether the creep current equilibrium potential happens to coincide with the holding potential by chance or whether the holding potential directly influences the creep current equilibrium potential. To determine whether the equilibrium potential for the creep currents is dependent upon the membrane holding potential, creep currents induced by Na loading of atrial cells were compared at two different holding potentials in the same cell. Current-voltage relationships were first determined under control conditions at $V_h = -110$ and -50 mV; cells were then exposed to monensin (3×10^{-5} M) for 10 min at $V_h = -80$ mV, and current-voltage relationships were determined again with $V_h = -110$ and -50 mV in the continued presence of monensin. Difference current-voltage relationships at each holding potential are plotted in Fig. 2. It can be seen that the equilibrium potential for the monensin-
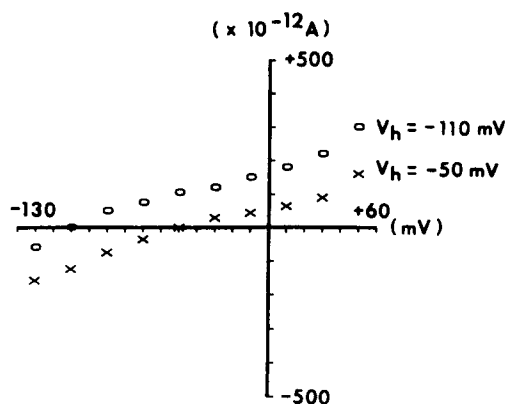

FIGURE 2. Dependence of creep current equilibrium potential on membrane holding potential. 200-ms voltage-clamp pulses were applied to voltages from −130 to +50 mV (in 20-mV increments) from two holding potentials ($V_h = -110$ and -50 mV) under control conditions and then after Na loading by exposure to monensin (3×10^{-5} M) for 10 min. The current-voltage relationship obtained in control was subtracted from that obtained after Na loading at either $V_h = -110$ mV (open circles) or $V_h = -50$ mV (x's) to give difference current-voltage relationships. Same procedure as described in Fig. 1.

induced creep currents is directly determined by the membrane holding potential. This experiment verifies that changes in membrane potential in Na-loaded atrial cells directly influence the equilibrium conditions associated with the creep currents. (These results also argue against the possibility that creep currents may be generated by a $[Ca]_i$-activated, nonspecific membrane channel; see Discussion.)

We now will consider whether experiments aimed at examining the stability of inward creep currents induced by Na loading of single atrial cells provide results that are also consistent with the predicted nonequilibrium conditions after voltage displacements away from the holding potential.

Reversal Measurements of Inward Creep Currents

Fig. 3 shows typical results from an experiment in which we attempted to determine the reversal potential of the inward creep current in an Na-loaded

atrial cell. The top row of current traces was obtained under control (C) conditions. A 200-ms voltage-clamp step was applied from a holding potential to +20 mV and followed by either a return to the holding potential (−80 mV) or a second voltage-clamp step to −50, −10, or +50 mV. An inward Ca current was activated by the initial depolarization to +20 mV; very little change in the time-dependent current occurred during the second voltage-clamp step, except at +50 mV, where activation of the delayed rectifying outward K^+ current occurred. After 15 min exposure to monensin (10^{-5} M), an identical voltage-clamp protocol was applied (M). The difference current (Δi) induced by Na loading is shown in the third row of traces. Na loading induced an outward creep current during the step depolarization to +20 mV, followed by an inward creep current (arrow)
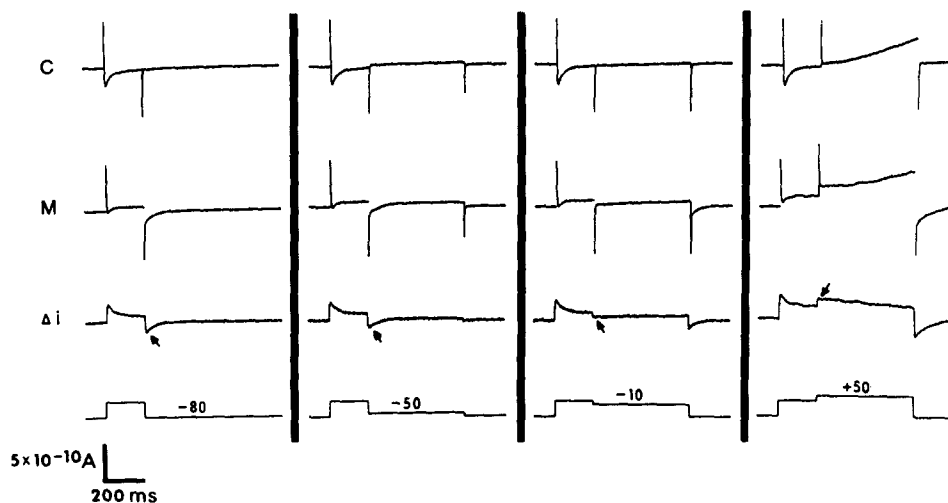

FIGURE 3.   Determination of the reversal potential of inward creep current after a prior depolarization. The top row of current traces was recorded under control conditions (C). 200-ms voltage-clamp steps were applied from the holding potential (−80 mV) to +20 mV and followed either by a return to the holding potential or by a second clamp step to −50, −10, or +50 mV. The middle row of current traces was recorded in response to the same protocol after 15 min exposure to monensin (10^{-5} M). The third row of traces shows the digitally subtracted difference currents (Δi) induced by Na loading. The bottom row of traces is the membrane voltage records. All solutions contained TTX (3×10^{-6} M).

when the potential was returned to −80 mV. The amplitude of the inward creep current was reduced when the membrane was repolarized to −50 mV. Repolarization to −10 mV caused the inward creep current to disappear (flat current trace), which suggests that −10 mV represents the reversal potential for the inward creep current. When the potential was changed to +50 mV, the creep current reversed direction (see arrow) and resembled the outward creep current activated during the preceding depolarization to +20 mV. This current record, however, is complicated by the activation of the time-dependent K^+ current at +50 mV that overlaps with the creep current. Despite this complication, the conclusion that a genuine reversal of the creep current occurs is justified, since

a genuine reversal in current direction was previously observed at potentials negative to the range of activation of i_K (e.g., Figs. 1 and 2).

The data in Figs. 1 and 2 show that the creep current equilibrium potential was strongly influenced by the membrane holding potential. It is somewhat surprising that after the depolarizing voltage-clamp pulse to +20 mV, the creep current equilibrium potential seemed to shift from the holding potential (−80 mV) to a level close to −10 mV. This result suggests that the creep current equilibrium potential is rather unstable and can be modified during the experi-
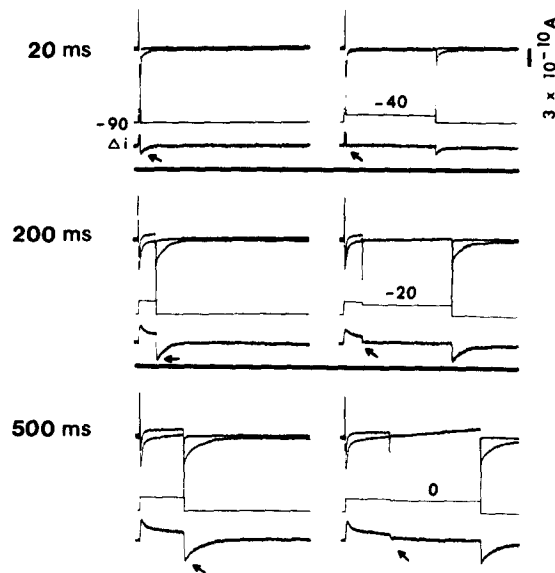

FIGURE 4. Dependence of inward creep currents on the duration of preceding voltage-clamp pulses. The top traces are superimposed currents elicited before and after exposure to 1×10^{-5} M monensin; the middle traces are membrane voltage; the bottom traces are the difference currents (Δi) induced by Na loading. In the left column, voltage-clamp pulses to +10 mV were applied for durations of 20, 200, and 500 ms. After these pulses, the potential was stepped back to the holding potential (−90 mV). Inward creep currents (Δi) induced by Na loading are highlighted by arrows. In the right column, identical voltage-clamp pulses to +10 mV were followed immediately by a 1-s pulse to the potentials indicated (−40, −20, and 0 mV). These were the experimentally determined reversal potentials for inward creep currents after pulses of 20, 200, and 500 ms duration. See text for further details.

mental protocol. A different protocol was used in the experiment shown in Fig. 4 to examine the stability of the inward creep current equilibrium potential. Superimposed current traces obtained before and after exposure to monensin (10^{-5} M) are shown along with the corresponding voltage traces (middle traces; controls and monensin are always compared with similar clamp protocols) and the difference current (Δi) induced by Na loading (bottom traces). The records shown in the left column show the subtracted inward creep currents (arrows) measured after repolarization of the membrane potential to −90 mV following

a depolarizing voltage-clamp pulse to +10 mV of 20, 200, or 500 ms duration. The amplitude of the inward creep current became larger as the duration of the preceding depolarization was prolonged (similar to the results shown in Fig. 5 in the preceding article). The records shown in the right column show the experimentally determined reversal potentials for the inward creep currents (Δi traces flat; see arrows) after the depolarizing voltage-clamp pulse to +10 mV for durations of 20, 200, or 500 ms. There was a progressive positive shift of the reversal potential of the inward creep current as the duration of the preceding pulse was increased. The reversal potential after the 20-ms pulse occurred at −40 mV; the reversal potential shifted to −20 and then to 0 mV after the 200- and 500-ms pulses, respectively.

(x 10⁻¹²A)

+500

-130   +60   (mV)
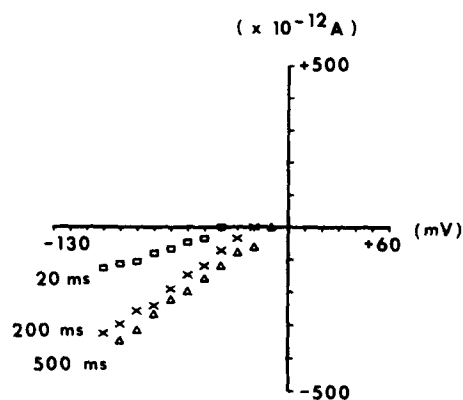
20 ms

200 ms

500 ms

-500

FIGURE 5. Current-voltage relationship of inward creep currents as a function of the duration of preceding voltage-clamp pulses. Data are from same experiment as in Fig. 4, using a similar protocol. The prepulses to +10 mV were applied for durations of 20, 200, or 500 ms. These were followed immediately by 1-s pulses to potentials ranging from −120 to 0 mV, applied in 10-mV increments. The peak amplitude of the inward creep current is plotted as a function of the potential of the second voltage-clamp pulse. Notice that as the duration of the prepulse was prolonged, the current-voltage relationship of the inward creep current shifted in the depolarizing direction along the voltage axis.

In this particular experiment, in addition to determining the reversal potentials for the inward creep currents, we measured the peak amplitude of the inward creep current as a function of potential (a second set of voltage-clamp pulses after the depolarizations to +10 mV was applied over the range of −110 to 0 mV in 10-mV increments). The peak amplitude of the inward creep current as a function of membrane potential is plotted in Fig. 5. The duration of the prepulse to +10 mV was either 20 (squares) or 200 (X's) or 500 (triangles) ms. This figure shows that the voltage dependence of the inward creep currents induced by Na loading shifted in a positive direction along the voltage axis as a function of the duration of preceding depolarizing voltage-clamp pulses to a constant potential. This result strongly suggests that the driving force for the inward creep currents is significantly modified during the time course of the preceding voltage-clamp pulse.

The experimental results shown in Figs. 4 and 5 clearly indicate a considerable degree of time-dependent instability of the reversal potential for the inward creep currents. The experiment described in Fig. 6 tested whether the reversal potential of the inward creep current also depends upon the voltage of the
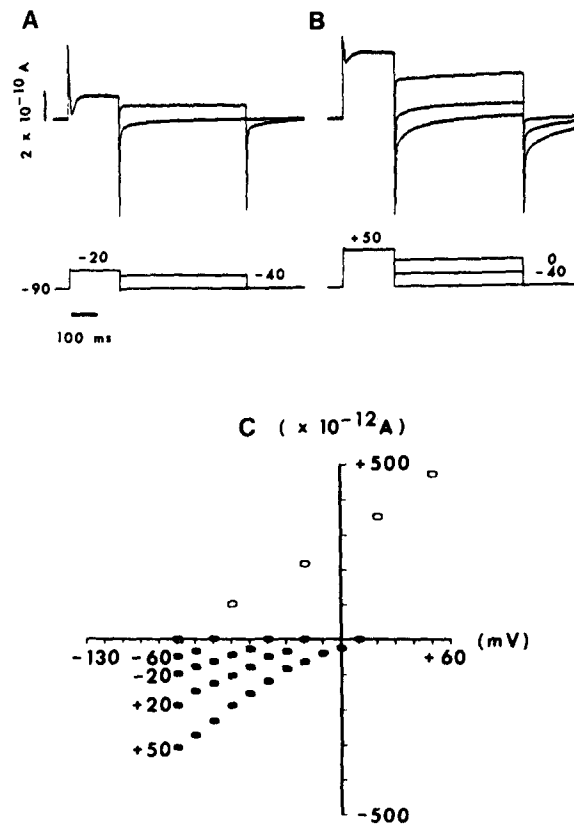

FIGURE 6. Dependence of inward creep currents on the potential of the preceding voltage-clamp pulses. The experimental protocol was similar to that used in Figs. 4 and 5; reversal potentials and current-voltage relations for inward creep currents were determined after prepulses, in this case, of constant duration but of variable amplitude. Cell were preloaded with Na by prior exposure to 1×10^{-5} M monensin. (A) Membrane currents elicited by a 200-ms pulse to -20 mV, followed by either a repolarization to -90 or a 1-s pulse to -40 mV (reversal potential). (B) Membrane currents elicited by a 200-ms pulse to $+50$ ms, followed by either a repolarization to -90 mV or a 1-s pulse to either -40 or 0 mV. (C) Current-voltage relationship of inward creep currents as a function of the potential of the preceding voltage-clamp pulses of constant duration (solid circles). The amplitude of outward current, measured at 200 ms, during the prepulses is also plotted (open circles).

prepulse. In this experiment, repetitive 200-ms pulses to a constant potential were applied to a cell after exposure to monensin (10^{-5} M) for 15 min. Immediately after each of these prepulses, a second 500-ms pulse to potentials ranging from -80 to $+20$ mV was applied, and the peak amplitude of the inward creep

currents was measured. The amplitude of the prepulse was then changed and the protocol was repeated. In this particular cell, families of inward creep currents were obtained after 200-ms prepulses to -60, -20, $+20$, and $+50$ mV. Fig. 6A shows two superimposed current traces elicited by two prepulse steps to -20 mV, followed by a repolarization either to the holding potential (-90 mV) or to -40 mV for 500 ms. When the cell was repolarized to -90 mV, an inward creep current was observed; repolarization to -40 mV, however, was accompanied by a flat current record (reversal potential). Fig. 6B shows results obtained after 200-ms prepulses applied to $+50$ mV. A large inward creep current was observed on repolarization to -90 mV and, in contrast to the results obtained when the prepulse was to -20 mV (A), when the potential was stepped to -40 mV after the prepulse to $+50$, an inward creep current was elicited. The reversal potential for the inward creep current was $+10$ mV in comparison with -40 mV after the depolarization to -20 mV. Therefore, there appears to be a positive shift of the reversal potential for the inward creep current as the prepulse potential is made more positive.

The peak amplitude of the inward creep currents (solid symbols) as a function of potential (of the second voltage-clamp pulses) is plotted in Fig. 6C. Prepulses of a constant duration of 200 ms were applied to -60, -20, $+20$, and $+50$ mV. The current-voltage relationship of the inward creep currents shifted in a positive direction along the voltage axis as the voltage of the prepulse was made more positive. The amplitude of the outward creep current induced by monensin (difference current obtained by subtraction from control) measured at 200 ms is also plotted (open symbols). As observed in earlier experiments, there was an increase in the magnitude of the outward creep current induced by Na loading as the voltage of the prepulse was applied to progressively more positive potentials (compare with Fig. 1B). It appears that the larger the magnitude of the outward creep current elicited during depolarizing voltage-clamp steps, the greater the positive shift of the current-voltage relationship of the inward creep current that follows the depolarization. The data in Figs. 4 and 5 indicate that as the duration of the preceding outward creep current was prolonged, there also was a positive shift of the current-voltage relationship for the following inward creep currents. These results indicate that the driving force for the inward creep currents is significantly modified by the outward creep current induced by Na loading.

The experimental data presented in Figs. 4–6 demonstrate that both the current-voltage relations and the reversal potentials of the inward creep currents induced by Na loading of atrial cells are very unstable and are critically dependent upon both the potential and duration of the preceding clamp steps. These experiments tend to substantiate our earlier interpretation of the current-voltage relations in Fig. 1: that displacements in potential away from the holding potential produce a shift from one set of equilibrium conditions to another. The time- and voltage-dependent changes in current, which we have called creep currents, seem to be intimately related to changes in ion concentration gradients and might therefore be responsible for the establishment of new equilibria after displacements in potential.

In the preceding article (Hume and Uehara, 1986*b*), it was shown that a reduction of $[Na]_o$ produced differential changes in the outward and inward

creep currents induced by Na loading. The outward creep currents were increased and inward creep currents (measured at the holding potential) were significantly reduced. We decided to study the sensitivity of the creep currents to changes in $[Na]_o$ further by examining the influence of a reduction of $[Na]_o$ on the reversal potential of the inward creep current. Fig. 7 shows results from an experiment in which the cell was examined under control conditions and exposed to monensin (10^{-5} M) for 15 min in normal Ringer's solution. Then the
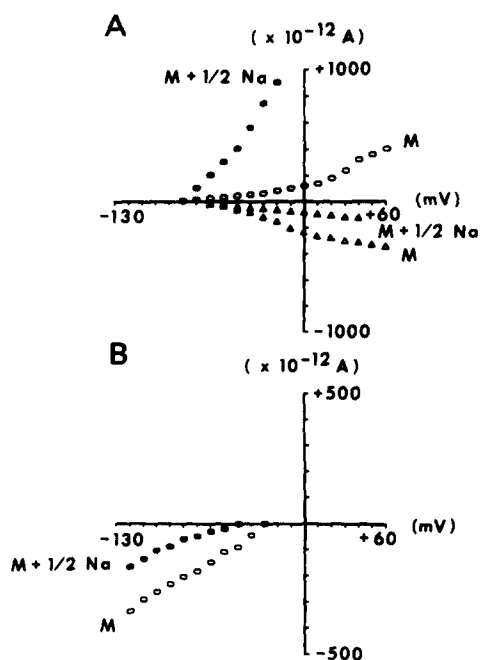

FIGURE 7.   Re-examination of the influence of $[Na]_o$ on outward and inward creep currents induced by Na loading. The cell was preloaded with Na by prior exposure to monensin (1×10^{-5} M). (A) Current-voltage relationship of the difference current and the peak amplitude of inward creep current induced by monensin in normal Ringer's solution (M) and after changing to 50% Na Ringer's solution (M + ½ Na). The protocol was similar to that used in Fig. 1. (B) Current-voltage relationship of inward creep current after a 200-ms prepulse before (open circles) and after (solid circles) addition of 50% Na Ringer's solution. The voltage-clamp protocol was similar to that used in Figs. 4–6; however, the prepulse duration and voltage were constant before and after changing to 50% Na Ringer's solution.

solution was changed to a 50% Na Ringer's that also contained monensin (10^{-5} M). As in Fig. 8A of the preceding article, 50% reduction of $[Na]_o$ increased the amplitude of the outward creep currents at all potentials and reduced the amplitude of the inward creep currents recorded on repolarization back to the holding potential. In this experiment, we also measured the current-voltage relationship for the inward creep current at both concentrations of $[Na]_o$ using the same protocol that was used in Fig. 6. That is, 200-ms pulses to 0 mV were

immediately followed by 500-ms pulses to potentials ranging from −120 to −10 mV. The current-voltage relation for the inward creep current obtained in 50% Na Ringer's (M + ½ Na) was significantly shifted in the hyperpolarizing direction along the voltage axis in comparison with that obtained in normal Ringer's solution (M). This negative shift of the reversal potential by ~20 mV explains the reduced amplitude of the inward creep current that was recorded at −90 mV (arrow) after the depolarizing pulses shown in Fig. 7*A*.

Relationship Between Creep Currents and Mechanical Activity

Several techniques have been devised for directly measuring tension changes in small, isolated cell preparations (Brady et al., 1979; Tarr et al., 1979; Warshaw and Fay, 1983). However, there are several practical limitations that preclude the routine measurement of tension from single myocytes. These include the accurate measurement of extremely small (micrograms) changes in tension, the separation of small changes in tension from background mechanical interference, and the attachment of a transducer to the ends of a single cell without damaging it. For these reasons, optical methods have recently been used to assess changes in mechanical activity in small cardiac preparations (Krueger et al., 1980; Kass, 1981; Roos and Brady, 1982; Mitchell et al., 1983). Our interest in comparing changes in membrane current with changes in mechanical activity in single atrial cells relates to: (*a*) the dramatic changes in contractile activity produced by Na loading of atrial cells as assessed by visual examination, (*b*) the similarity between the voltage dependence of the outward creep current induced by Na loading and the known voltage dependence of tonic tension in frog myocardium (Goto et al., 1971; Morad and Orkand, 1971; Vassort and Rougier, 1972), and (*c*) the possible relationship between mechanical relaxation and the inward creep current induced by Na loading, both of which occur after depolarizing voltage-clamp pulses back to the holding potential.

We used a photodiode placed directly over part of the image of an atrial cell displayed on the screen of a television monitor to assess the changes in light intensity that accompany changes in mechanical activity. It was our hope that with this technique, it would be possible to make qualitative comparisons between changes in membrane current and changes in mechanical activity simultaneously during voltage-clamp experiments. Fig. 8 shows the results of an experiment in which the time course of the contraction accompanying an atrial action potential was monitored optically. In *A*, the photodiode was placed over a portion of the cell that appeared to adhere to the bottom of the recording chamber. Although inverted, the signal from the photodiode is similar in both shape and time course to twitches that have routinely been measured from frog myocardium (for comparison, see Horackova and Vassort, 1979*b*; Klitzner and Morad, 1983). A recent comparison between tension measurements and optical monitoring of contraction under isometric conditions in cardiac Purkinje fibers has shown that the latter method gives a relatively accurate measure of the time course of contraction (Kass, 1981). The record shown in *B* was obtained from the same cell. In this case, however, the photodiode was placed over a different portion of the cell, which did not adhere to the bottom of the chamber. In this case, the

output of the photodiode is obviously different from the waveform shown in *A*. The time course of the contraction in this case may be contaminated by movement artifacts or may reflect conditions that are nonisometric. In subsequent experiments, we restricted our measurements to areas of cells that appeared to adhere to the bottom of the chamber. Any experiments in which optical signals displayed obvious movement artifacts (such as those in *B*) were rejected. Furthermore, we assumed that the adherence of the cell to the bottom of the chamber increased the likelihood of approaching isometric conditions.

To assess further the usefulness of this technique, we examined whether this optical monitor of contraction accurately reflected the known voltage dependence of tension during voltage-clamp experiments. It is well established that two
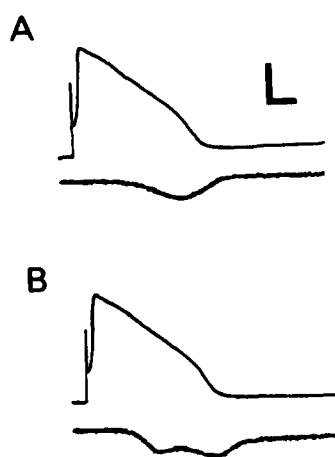

FIGURE 8.   Action potentials and optical signals accompanying mechanical activity in single frog atrial cells. The top traces in both *A* and *B* are action potentials elicited from the same atrial cell in response to intracellularly applied stimuli of 1.0 ms duration using a bridge circuit (not shown). The bottom traces are optical signals (in arbitrary units) from a photodiode (filtered at 50 Hz) that monitored the changes in light intensity that accompanied mechanical activity at two different regions (*A* and *B*) of the same cell. The vertical calibration is 50 mV; the horizontal calibration is 100 ms. TTX was absent in this experiment.

types of tension responses can be elicited in frog atrium: phasic contractions that accompany action potentials or short-duration voltage-clamp steps, and tonic contractions that are elicited by long-duration voltage-clamp depolarizations (see Chapman, 1983, for review). The two types of tension have different voltage dependences and are believed to be generated by different mechanisms. Phasic tension appears to be closely related to the magnitude of i_{Ca} and shows a voltage dependence similar to that of i_{Ca} (Einwachter et al., 1972; Leoty and Raymond, 1972; Vassort and Rougier, 1972), whereas tonic tension appears to continue to increase at very positive potentials where i_{Ca} becomes small, and has been postulated to be importantly controlled by an Na/Ca exchange mechanism (Horackova and Vassort, 1979a; Chapman and Tunstall, 1980).

Fig. 9 shows representative results obtained from five experiments in which the voltage dependence of phasic contractions was monitored optically, under control conditions and after exposure to monensin. Fig. 9A shows superimposed current traces elicited by identical 200-ms voltage pulses from a holding potential of −90 to +10 mV under control conditions and after 10 min exposure to the Na ionophore monensin (5 × 10^{-6} M). Typical changes in membrane current
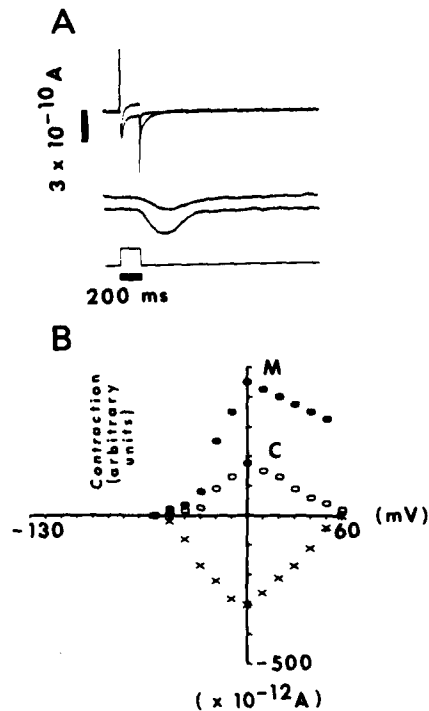
A

B

FIGURE 9.   Effect of Na loading on membrane currents and mechanical activity monitored optically. (A) The top traces are superimposed membrane currents elicited by 200-ms voltage-clamp steps from −90 to +10 mV before and after exposure to monensin (5 × 10^{-6} M); the middle traces are optical signals accompanying mechanical activity (top: control; bottom: after exposure to monensin); the bottom trace is membrane voltage. (B) Relative changes in light intensity plotted as a function of membrane potential under control conditions (C) and after exposure to monensin (M). Also plotted is the voltage dependence of the peak amplitude of i_{Ca} recorded under control conditions (×'s).

were produced by monensin: an increase in outward current during the depolarizing pulse, followed by an inward creep current upon repolarization back to the holding potential. Below the current traces are the changes in light intensity that accompanied the contractions elicited by these voltage-clamp steps. After monensin exposure, there appeared to be a greater change in light intensity compared with control. Fig. 9B is a plot of the magnitude of the change in light intensity (in arbitrary units) as a function of the voltage of the clamp steps under

control conditions (open circles) and after exposure to monensin (solid circles). Before monensin exposure, the voltage dependence of the change in the magnitude of light intensity had the same voltage dependence as that of the peak amplitude of i_{Ca} (X's). This is similar to the known relationship between phasic tension and voltage. This result provides evidence that this technique for optically monitoring contractions in single myocytes can provide reliable information with regard to the voltage dependence of phasic tension, although quantitative changes in the force of contraction cannot be assessed accurately. Interestingly, after Na loading there appears to be an increase in the recorded magnitude of light intensity over the entire range of potentials examined. The greatest change in light intensity after Na loading seems to be at very positive potentials, where the peak magnitude of i_{Ca} has become very small. Overall, the changes produced by Na loading are strikingly similar to the changes in the peak tension-voltage relationship (generated with 300-ms pulses) previously observed in intact frog atrial trabeculae after exposure to ouabain (Vassort, 1973). These changes were attributed to an increase in the tonic component of tension in response to an elevation of $[Na]_i$. A similar mechanism may also explain the data in Fig. 9.

To examine in more detail the possible relationships between the creep currents induced by Na loading of atrial cells and mechanical activity, changes in light intensity were monitored during longer-duration voltage-clamp pulses, which would be expected to elicit strong tonic contractions. The data in Fig. 10 were recorded from an atrial cell that had been Na-loaded by 15 min exposure to monensin (5×10^{-6} M). In this experiment, 1-s voltage-clamp pulses were applied in 10-mV increments to potentials ranging from −80 to +50 mV. Since these long-duration pulses inevitably produce significant activation of the delayed K current (Hume and Giles, 1983), the holding potential was set to −100 mV (near E_K) to prevent contamination of inward creep currents by outward tail currents associated with i_K. The top traces in Fig. 10A are superimposed currents that were recorded in response to 1-s voltage-clamp steps to −10, +10, +30, and +50 mV. The middle traces are the accompanying changes in light intensity that were monitored optically; the bottom traces are superimposed voltage records. Initially, a transient inward i_{Ca} was activated; this was followed by the slower activation of the delayed outward K current by each of the depolarizing voltage-clamp pulses. As the pulses were applied to progressively more positive potentials, the peak amplitude of i_{Ca} diminished and the amplitude of i_K increased (outward creep currents, although present, were not so apparent since the currents had not been subtracted from the controls). Note that after the depolarizing pulses, inward creep currents can be observed, and their amplitude increases as the prepulse potential is made more positive. The accompanying changes in light intensity during these voltage-clamp pulses become larger as the pulse potential is made more positive. Upon the return to the holding potential, there is a rapid decay of the optical signal that occurs shortly after the inward creep currents. The decay of the optical signal can be attributed to a relaxation of the preceding contraction.

Fig. 10B is a plot of the peak amplitude of the change in light intensity (in arbitrary units) as a function of membrane potential. In the presence of monensin,

the relationship between the peak amplitude of the change in light intensity and the membrane potential is very similar to the known voltage dependence of tonic tension in frog atrium (Vassort and Rougier, 1972; Morad and Goldman, 1973). Furthermore, the voltage dependence of the tonic contraction, as monitored optically, is nearly identical to the voltage dependence of the outward creep currents induced by Na loading (see Fig. 1 B). It can probably be concluded that during the time course of the outward creep currents, there is a significant
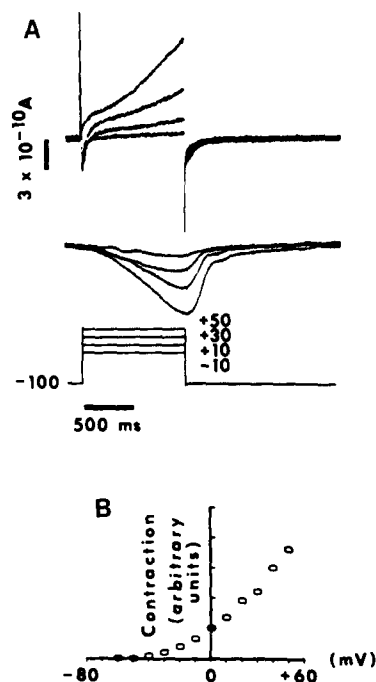

FIGURE 10.   Membrane currents and associated mechanical activity monitored optically from an Na-loaded atrial cell. In this experiment, a series of 1.0-s voltage-clamp steps was applied from −100 mV (holding potential) to potentials ranging from −80 to +50 mV in 10-mV increments. (A) The top traces are superimposed membrane currents elicited in response to depolarizing pulses to −10, +10, +30, and +50 mV; the middle traces are the superimposed optical signals that accompanied the changes in membrane current; the bottom traces are superimposed membrane voltages. (B) Relative changes in light intensity (in arbitrary units) plotted as a function of the voltage-clamp pulse potential.

increase in [Ca]ᵢ, and that as the potential is made more positive, coincident with a progressively increasing magnitude of outward creep current, there is a progressively elevated [Ca]ᵢ. During the time course of the inward creep current, there may be a significant reduction of [Ca]ᵢ. These experiments raise an important question: what is the relationship between the relaxation of mechanical activity and the inward creep current?

We have compared the rate of decay of the inward creep current with the rate of mechanical relaxation (rate of decay of the optical signal) at the holding potential after depolarizing voltage-clamp pulses. Fig. 11 A shows a plot of the decay of the inward creep current (left) and a semilogarithmic plot of the decay of the optical signal (mechanical relaxation; right), both recorded after the 1-s voltage-clamp step to +50 mV, shown previously in Fig. 10. The inward creep current decay is well fit by a single exponential with a time constant of 98 ms. Two exponential components are evident in the decay of the optical signal. The slow component, with a time constant of 610 ms, was subtracted from the total
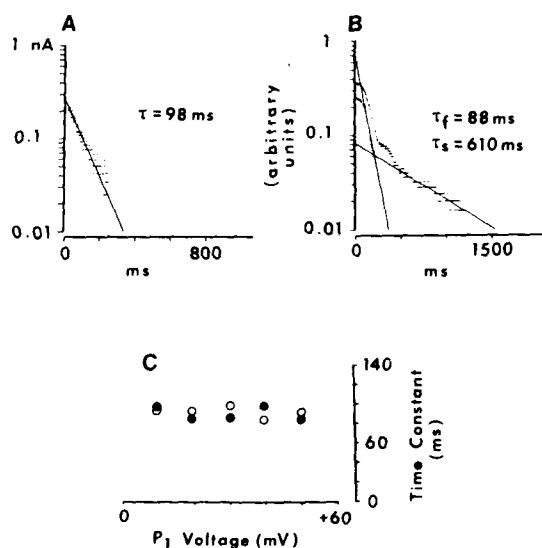
FIGURE 11.   Comparison of the time course of decay of the inward creep current with the time course of decay of the optical signal. (A) Semilogarithmic plot of the time course of decay of the inward creep current recorded after a voltage-clamp step to +50 mV (same experiments as in Fig. 10). (B) Semilogarithmic plot of the time course of decay of the optical signal (arbitrary units) recorded after the voltage-clamp step to +50 mV. (C) Comparison of the time constant of decay of the inward creep current (open circles) with the fast time constant of decay of the optical signal (solid circles) after voltage-clamp steps to +10, +20, +30, +40, and +50 mV (both decay rates measured at the holding potential, −100 mV). See text for further details.

current record to yield the faster component, with a time constant of 88 ms. In this example, the time constant of the fast component of decay of the optical signal is similar to the time constant of decay of the inward creep current. The direct measurement of tension in bullfrog atrial muscle has provided evidence that mechanical relaxation after depolarization is a biexponential process (Goto et al., 1972). A comparison of the time constant of decay of the inward creep currents and the time constant of the fast component of decay of the optical signal after voltage-clamp pulses to a range of potentials is plotted in Fig. 11 B. These data show that after voltage-clamp pulses to potentials ranging from +10

to +60 mV, the time course of the decay of the inward creep currents is remarkably similar to the time course of the fast component of decay of the optical signal, which reflects the rate of mechanical relaxation. Such a similarity between the time courses of the electrical and mechanical events suggests either that the events are mediated by some common underlying event, or that the electrical event is the direct cause of the mechanical event.

DISCUSSION

Flux experiments in squid axon have established that the Na/Ca exchange mechanism is electrogenic (Baker et al., 1969; Blaustein, 1977). Although an Na/Ca exchange has been implicated in heart as a primary mechanism for the regulation of $[Ca]_i$ (Reuter and Seitz, 1968; Jundt et al., 1975), until recently it was not possible to decide whether such a mechanism was electrogenic or electroneutral. Convincing evidence recently obtained from flux studies in isolated cardiac sarcolemmal vesicles indicates that more than two Na ions are transported for each Ca and that the exchanger is voltage dependent (Pitts, 1979; Bers et al., 1980; Caroni et al., 1980; Reeves and Sutko, 1980). Studies using measurements of intracellular ion activities also have provided evidence that the Na/Ca exchange mechanism in the heart is electrogenic (Bers and Ellis, 1982; Sheu and Fozzard, 1982; Chapman et al., 1983a; Eisner et al., 1983).

In recent years, several attempts have been made to measure a transmembrane ionic current associated with the operation of the Na/Ca exchange mechanism in the heart. Attempts in frog atrium have relied upon examination of ionic currents before and after changes in $[Na]_o$ or $[Ca]_o$. Benninger et al. (1976) failed to detect any measurable change in current in response to a reduction in $[Na]_o$, but the recent data of Mentrard et al. (1984), using a similar experimental procedure, revealed small changes in membrane current that were considered to be consistent with the thermodynamic model of electrogenic Na/Ca exchange proposed by Mullins (1979). However, any change in the Na/Ca exchange current under these conditions must be quite transient in nature, and would have to be measured quickly, before subsequent changes in $[Na]_i$ could occur (Ellis and Deitmer, 1978). In mammalian heart, the ability to measure ionic currents associated with the operation of an electrogenic Na/Ca exchanger may be more difficult since other mechanisms may also be important for the regulation of $[Ca]_i$ (Chapman, 1979).

A variety of experimental manipulations and agents that produce an increase in $[Na]_i$ in mammalian heart inevitably result in the appearance of transient inward currents (TI's) and aftercontractions. Although creep currents were observed under similar experimental conditions (Eisner and Lederer, 1979), most subsequent electrophysiological studies have focused on the properties and ionic basis of the TI's, and little attention has been devoted to the study of creep currents. In recent years, some controversy has developed over the ionic basis of TI's. Both a Ca-activated channel selective to Na and K and an electrogenic Na/Ca exchange mechanism have been proposed (Kass et al., 1978b; Brown et al., 1984; Arlock and Katzung, 1985) as possible mechanisms. However, the magnitude and time course of both the outward and inward creep currents as

originally shown in Eisner and Lederer (1979, see Fig. 10), as well as those studied in the present experiments in single frog atrial cells, bear a striking resemblance to the changes in membrane current that have been predicted to result from the operation of an electrogenic Na/Ca exchanger (see Fig. 7.3 in Mullins, 1981). In fact, in a recent review, Chapman (1983) has speculated that outward currents associated with an increase in tonic tension and inward currents associated with a relaxation of tonic tension in earlier studies (Leoty and Raymond, 1972; Eisner and Lederer, 1979) were due to the operation of an electrogenic exchange mechanism. It is also noteworthy that recent studies of caffeine actions in mammalian heart (Clusin et al., 1983; Vassalle and Gennaro, 1985) have described a transient inward current (tail current) induced by caffeine that may be related to the inward creep current induced by Na loading. If creep currents are mediated by electrogenic Na/Ca exchange, then they may be expected to be induced by a variety of conditions and agents that act to elevate $[Ca]_i$.

In the preceding article (Hume and Uehara, 1986b), evidence was presented which indicated that creep currents in frog atrial cells are not likely to be directly mediated by voltage-gated Na, Ca, or K channels or by an electrogenic Na,K pump, but are induced by conditions that elevate $[Ca]_i$. The sensitivity of inward creep currents to Ca channel antagonists was found to depend upon the route of Ca entry: elevation of $[Ca]_i$ via the Ca channel produced inward creep currents that were sensitive to Ca channel antagonists, whereas elevation of $[Ca]_i$ via other routes of Ca entry induced inward creep currents that were insensitive to Ca channel antagonists. It was concluded that creep currents could be attributed either to an electrogenic Na/Ca exchange mechanism or to a nonselective channel activated by $[Ca]_i$. In this article, additional properties of creep currents have been examined to distinguish between these two alternative mechanisms.

An examination of the voltage and time dependence of the changes in membrane current induced by Na loading of atrial cells reveals that depolarizing voltage-clamp pulses away from the holding potential (-90 mV) produce outward creep currents, followed by inward creep currents upon the return to the holding potential. Hyperpolarizing voltage-clamp pulses away from the holding potential produce inward creep currents followed by outward creep currents upon the return to the holding potential. The symmetry in terms of the direction of current change and the magnitude and time course of current change (Fig. 1) strongly suggest that any voltage displacement away from the holding potential represents a shift away from one set of equilibrium conditions existing at the holding potential to some new equilibrium that is dependent upon the membrane voltage. A new equilibrium can only be achieved after time-dependent changes in current subside. This interpretation is supported by experiments in which the stability of the inward creep current reversal potential after depolarizing voltage-clamp pulses was examined. Current-voltage relations of creep currents were found to be extremely labile and dependent on both the time and voltage of the preceding clamp steps. Similarly, the magnitude of the outward creep currents during depolarizing clamp steps is also a unique function of time and voltage, becoming larger at more positive potentials, but decaying in amplitude as the

duration of the pulse to any given potential is prolonged. These observations suggest that the reversal potential of inward creep currents is modified by the magnitude of the outward creep currents that occur during the preceding voltage-clamp steps. Furthermore, the observations that exactly reciprocal current changes are observed in response to hyperpolarizing pulses away from the holding potential, and that the current-voltage relations of both inward and outward creep currents during both depolarizing and hyperpolarizing voltage-clamp steps intersect the voltage axis at the same voltage, indicate that both inward and outward creep currents may be generated by the same mechanism. This mechanism appears to have the unique capability of regulating its equilibrium potential as a function of its own activity. A likely candidate for such a mechanism is the Na/Ca exchanger (Mullins, 1979; Noble, 1986).

Tonic tension in both amphibian and mammalian myocardial preparations has been postulated to result from the operation of an Na/Ca exchange mechanism (Horackova and Vassort, 1979*a*, *b*; Chapman and Tunstall, 1980; Coraboeuf et al., 1981; Eisner et al., 1983). In the preceding article (Hume and Uehara, 1986*b*), it was shown that inward creep currents are induced by conditions that elevate either $[Na]_i$ or $[Ca]_i$ (isoproterenol). It was hypothesized, based upon the sensitivity of the inward creep currents to a variety of Ca channel antagonists and to a reduction in $[Ca]_o$, that the inward creep current in Na-loaded cells was triggered by an elevation of $[Ca]_i$. The possibility that the outward creep current is involved in mediating this increase in $[Ca]_i$ is suggested by the similarity between the voltage dependence of the outward creep current (Fig. 1) and the voltage dependence of tonic tension (Morad and Goldman, 1973; Horackova and Vassort, 1979; Chapman and Tunstall, 1980). Our experiments, using an optical monitor of contraction, verify that Na loading increases tonic contractions in single frog atrial cells. Moreover, the voltage dependence of this tonic component of contraction is similar to the known voltage dependence of tonic tension in frog myocardium and to the voltage dependence of the outward creep current induced by Na loading (compare Figs. 1 and 10). These results, along with the observation that outward creep currents are also abolished when $[Ca]_o$ is removed, strongly suggest that the outward creep current is involved in transport of Ca across the sarcolemma.

Relaxation of tension in cardiac muscle may involve a reduction of $[Ca]_i$ by three potentially important processes: (*a*) extrusion of Ca into the extracellular space by an Na/Ca exchanger operating in reverse (Langer, 1982), (*b*) an ATP-dependent Ca pump (Caroni et al., 1980), and (*c*) sequestration of Ca into the sarcoplasmic reticulum (SR). It has been postulated that, in amphibian heart, Na/Ca exchange alone controls the relaxation of tonic tension (Goto et al., 1972; Chapman, 1979; Roulet et al., 1979), since the sarcoplasmic reticulum is much less extensive in this tissue. The recent data of Chapman and Rodrigo (1985) also strongly suggest that Na/Ca exchange mediates the relaxation of tonic tension in frog atrial trabeculae. In our experiments, an involvement of the inward creep current in Ca extrusion from the cell is implied by the temporal relationship between the visually observed relaxation of tension after depolarizing voltage-clamp pulses and the occurrence of the inward creep current. We

attempted to examine the relationship between tension relaxation and inward creep currents by comparing the kinetics of the decay of the inward creep current with the decay of an optical signal that monitors contraction. Our results indicate that the kinetics of both processes are very similar, which suggests that the inward creep current may be important in the relaxation of tonic tension in amphibian heart. A second, slower phase of decay of the optical signal was observed in our experiments, which might indicate that tension relaxation is also mediated by a second process that is independent of the inward creep currents. This process may be the electrically neutral ATP-dependent Ca pump (Caroni et al., 1980), which has recently been histochemically identified in frog atrial myocardium (Meyer et al., 1982). The actual relationship between inward creep currents and mechanical relaxation needs to be assessed carefully under conditions in which the time course of the inward creep current can be compared with the time course of mechanical relaxation as assessed by direct tension measurements.

Mathematical Simulation of Creep Currents

If the creep currents produced by Na loading or Ca loading of frog atrial cells are mediated by an Na/Ca exchange mechanism, then an important question must be considered. In the Mullins (1979, 1981) model of Na/Ca exchange, an increase in $[Na]_i$ should produce a parallel shift of the equilibrium potential (E_{ex}) and the current-voltage relationship for the exchanger in the hyperpolarizing direction similar to that which would occur in response to a decrease in $[Na]_o$. Both an elevation of $[Na]_i$ and a reduction of $[Na]_o$ should yield a difference curve that is always positive and is a U-shaped function of membrane potential (e.g., Mentrard et al., 1984). Why does an elevation of $[Na]_i$ in single atrial cells yield a difference curve that intersects the voltage axis? To address this question, a mathematical simulation of Mullins' (1976, 1977) model as recently modified by Noble (1986) has been used to predict changes in electrogenic current that might be expected in response to an elevation of $[Na]_i$.

According to Mullins (1981), the sinh expression for the exchange current may only be valid for very small voltage displacements, and Noble (1986) has derived an alternative expression for the exchange current that may be more consistent with the rather large variations of $[Ca]_i$ that may occur in response to larger voltage displacements:

$$I_{ex} = K\{(a^i_{Na})^n\, (a^o_{Ca})\, \exp(E_m \cdot F \cdot \gamma / RT)$$
$$- (a^o_{Na})^n\, (a^i_{Ca})\, \exp[-E_m \cdot F(1 - \gamma)/RT]\}. \tag{1}$$

In this expression, I_{ex} is the current generated by the Na/Ca exchanger, K is a scaling factor, γ is the partition parameter (0.5 assumed), n is the coupling coefficient (3), and R, T, and F have their usual meanings. If estimated ion activities are substituted into this expression, current-voltage relations for the exchange current like those shown in Fig. 12A are obtained. The curve labeled a corresponds to equilibrium conditions at rest with $a^i_{Na} = 15$ mM (Chapman, 1979) and $a^i_{Ca} = 0.3\ \mu M$ (Fabiato and Fabiato, 1975; Marban et al., 1980). The equilibrium potential for the exchanger (E_{ex}) occurs at approximately -50 mV. The curve labeled b is obtained after an elevation of a^i_{Na} to 23 mM, which can

be reasonably assumed to occur after exposure to monensin or intracellular Na dialysis. An elevation of a^i_{Na} does not merely produce a parallel shift of the current-voltage relation for I_{ex}, but produces a larger shift in the positive portion of the curve. As a result, the difference current obtained (dotted lines) is a
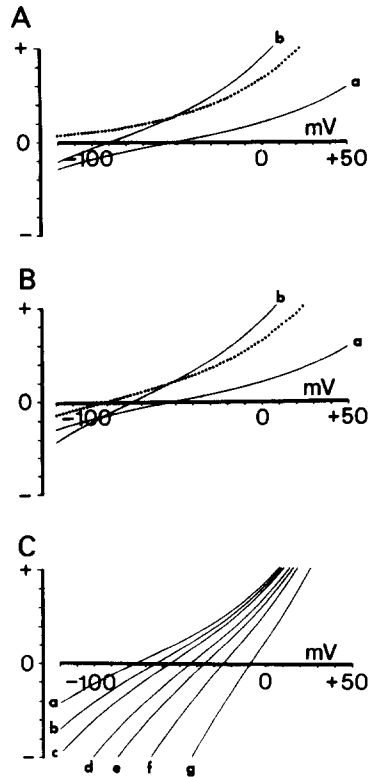

FIGURE 12.   Simulated current-voltage relationships for Na/Ca exchange calculated from Eq. 1. Current is plotted in arbitrary units. (A) The current-voltage relationship for Na/Ca exchange under control conditions is labeled a (ion activities: 110 mM Na$_o$, 15 mM Na$_i$, 750 μM Ca$_o$, 0.3 μM Ca$_i$); that after exposure to monensin is labeled b (110 mM Na$_o$, 23 mM Na$_i$, 750 μM Ca$_o$, 0.3 μM Ca$_i$). The dotted line is the difference current-voltage relationship obtained from b − a. (B) Monensin exposure is assumed to produce an elevation of both Na$_i$ and Ca$_i$. The current-voltage relationship for control conditions is labeled a (ion activities: 110 mM Na$_o$, 15 mM Na$_i$, 750 μM Ca$_o$, 0.3 μM Ca$_o$); that after monensin exposure is labeled b (110 mM Na$_o$, 23 mM Na$_i$, 750 μM Ca$_o$, 0.5 μM Ca$_i$). The dotted line is the difference current-voltage relationship obtained from b − a. (C) The influence of elevated Ca$_i$ on simulated current-voltage relationships for Na/Ca exchange. The curve labeled a corresponds to Na/Ca exchange current-voltage relationship after monensin exposure (ion activities: 110 mM Na$_o$, 23 mM Na$_i$, 750 μM Ca$_o$, 0.5 μM Ca$_i$). Curves b–g were calculated based upon progressive elevation of Ca$_i$ to 0.75 (b), 1.0 (c), 1.5 (d), 2.0 (e), 3.0 (f), and 5.0 (g) μM. For these calculations, γ, the partition parameter, was assumed to be 0.5, $n = 3$, $T = 25°C$; F and R have their usual values.

monotonically increasing function of membrane voltage. Inspection of curve a reveals that, at rest, I_{ex} may produce net inward current near the resting membrane potential of the cell (-90 mV). This inward current, which presumably represents electrogenic extrusion of Ca, may be an important factor in Ca homeostasis at rest, since it may act to balance any inward leak of Ca (Niedergerke, 1963). In response to an elevation of a^i_{Na}, the ability of the exchanger to balance this resting leak of Ca will be compromised because of the negative shift of E_{ex}. This may be especially true in a voltage-clamp experiment; the imposed holding potential may be expected to significantly influence equilibrium conditions. Overall, the shift from curve a to curve b in response to an elevation of a^i_{Na} is probably accompanied by a small elevation of a^i_{Ca}. In Fig. 12B, it is assumed that the same elevation of a^i_{Na} is accompanied by a small elevation of a^i_{Ca} to 0.5 μM (curve b). In this case, curves a and b intersect, with the resulting difference curve itself intersecting the voltage axis near -90 mV. This difference current-voltage relationship is very similar to that observed experimentally after Na loading of atrial cells (Fig. 1). The supposition that an elevation of [Na]$_i$ is accompanied by a small increase in [Ca]$_i$ at rest is consistent with results obtained from ion-sensitive electrode measurements (Sheu and Fozzard, 1982; Lee, 1981; Bers and Ellis, 1982).

Finally, we examined the sensitivity of the simulated I_{ex} to larger changes in a^i_{Ca} (Fig. 12C). The response of the simulated I_{ex} to an elevation of a^i_{Ca} is very similar to the experimentally observed positive shift of the voltage dependence of the inward creep currents in response to alterations in the voltage and duration of the preceding voltage-clamp depolarizations (Fig. 4–6). These curves are also consistent with the experimental observation that agents that elevate a^i_{Ca} via enhancement of i_{Ca} will lead to the appearance of an inward creep current (Fig. 9, Hume and Uehara, 1986b). These simulations are consistent, therefore, with the hypothesis that outward creep currents may be associated with Ca influx during depolarizing clamp pulses; the Ca influx produces an elevation of a^i_{Ca}, which in turn results in a positive shift of E_{ex}. Inward creep currents may be associated with Ca efflux, a subsequent decline in a^i_{Ca}, and a negative shift of E_{ex}. Our selection of this particular mathematical model of Na/Ca exchange (Noble, 1986) is, however, somewhat arbitrary, and our data certainly do not prove the validity of this particular model or exclude the validity of other models (e.g., Eisner and Lederer, 1985).

Our experimental results and simulations describe the behavior of creep currents under conditions in which [Na]$_i$ has been elevated to levels considerably in excess of that expected under normal physiological conditions. What role, if any, might creep currents play under conditions in which [Na]$_i$ levels are more physiological? The control current-voltage relationship for the exchanger shown in Fig. 12 assumes $a^i_{Na} = 15$ mM and $a^i_{Ca} = 0.3$ μM. Under these conditions, the exchanger would be involved in Ca homeostasis at rest and would balance any inward leak of Ca. If a^i_{Ca} is higher than estimated or if a^i_{Na} is lower than estimated, then the current-voltage relationship for the exchanger may be displaced in the positive direction on the voltage axis under normal conditions. The recent finding that the tonic tension-voltage relationship in single frog atrial cells under

normal conditions may occur at significantly more depolarized potentials than indicated by previous determinations in multicellular preparations may be consistent with this (Tarr et al., 1985). We might speculate that under these conditions, inward creep currents may contribute more significantly to the electrical activity of the cell during membrane depolarizations up to 0 mV or even more positive. Fig. 1 illustrates the occurrence of an inward creep current during hyperpolarizing voltage-clamp pulses in Na-loaded cells that closely resembles both i_{Ca} and the persistent inward current previously described in single frog atrial cells during depolarizing voltage-clamp pulses (Hume and Giles, 1983). The possibility exists that, under normal conditions, inward creep currents, because of operation of the exchanger, may overlap with and contribute significantly to waveforms that have been assumed solely to represent the second inward current, i_{Ca} (Noble, 1984).

Creep Currents: Mediation by Na/Ca Exchange or [Ca]ᵢ-activated Channels?

The difficulty in distinguishing experimentally between the transmembrane current generated by an electrogenic Na/Ca exchange mechanism or by a nonselective cation channel activated by $[Ca]_i$ is exemplified by the recent controversy surrounding the ionic basis of transient inward currents (see Noble, 1984; Eisner and Lederer, 1985). As originally pointed by Kass et al. (1978b), reversal potential measurements may provide information that allows a definitive distinction between these two mechanisms to be made. A genuine reversal of current would be expected in the case of a $[Ca]_i$-activated, nonspecific channel, whereas in the case of an electrogenic Na/Ca exchanger, increases in $[Ca]_i$ may produce only inward currents and not a genuine reversal of the current direction. However, the demonstration of a genuine reversal potential may not be a valid criterion for distinguishing between the two possible mechanisms, since it has recently been pointed out that reversal potential predictions for an electrogenic Na/Ca exchange critically depend upon specific models (Eisner and Lederer, 1985).

Reversal potential measurements of creep currents in Na-loaded frog atrial cells provide a number of results that seem more consistent with properties expected of an electrogenic Na/Ca exchange mechanism than with a nonspecific channel activated by $[Ca]_i$. Current-voltage relationships for inward creep currents are extremely labile and shift toward more positive voltages, as preceding voltage-clamp pulses are either prolonged in duration or applied to more positive potentials (both conditions are expected to result in increases in $[Ca]_i$). In the case of either a $[Ca]_i$-activated, nonspecific channel or a $[Ca]_i$-activated K channel, large variations in $[Ca]_i$ would be expected to produce corresponding changes in current amplitude without producing changes in the equilibrium potential (Eisner and Lederer, 1985). The observed dependence of the creep current equilibrium potential on the holding potential (e.g., Fig. 2) is also more consistent with an exchange system than with a $[Ca]_i$-activated channel. Changes in holding potential can be expected to result in changes in $[Ca]_i$, but not to produce changes in the equilibrium potential for a $[Ca]_i$-activated membrane channel.

When voltage-clamp pulses are applied to potentials negative to the creep

current equilibrium potential (e.g., holding potential), a genuine reversal of creep currents is observed (Fig. 1). This might be predicted for an electrogenic Na/Ca exchange current: negative displacements away from equilibrium would increase electrogenic Ca efflux during the voltage pulse, followed by an increased electrogenic Ca influx as the original equilibrium is re-established when the voltage is returned to the holding potential. Moreover, it is difficult to explain why an inward creep current, if mediated by a nonspecific channel and activated by $[Ca]_i$, would be observed during voltage-clamp pulses to -150 mV, which would be expected to decrease $[Ca]_i$. A nonspecific channel activated by $[Ca]_i$ might be observed at such negative membrane potentials only if it were preceded by a positive voltage-clamp depolarization, which would significantly elevate $[Ca]_i$ (cf. Kass et al., 1978b).

Finally, the insensitivity of creep currents induced by Na loading of atrial cells to Ca channel antagonists, which was demonstrated in the preceding article (Hume and Uehara, 1986b), is in marked contrast to the sensitivity of transient inward currents to these agents (Ferrier and Moe, 1973; Kass et al., 1978a). If creep currents are generated by a nonselective channel activated by $[Ca]_i$, then they should be greatly attenuated by Ca channel antagonists because of their ability to eventually deplete intracellular Ca stores (cf. Kass et al., 1978a). Creep currents induced by Na loading of atrial cells, however, are quite insensitive to Ca channel antagonists and remain robust even after long periods of exposure.

In summary, we believe that many of the properties of creep currents in single frog atrial cells are more consistent with an electrogenic Na/Ca exchange mechanism compared with $[Ca]_i$-activated membrane cation channels. Recent experiments that have examined the pharmacological properties of creep currents also support this conclusion: several different compounds that block Na/Ca exchange flux activity in isolated cardiac vesicle preparations are also potent inhibitors of creep currents in single frog atrial cells (Hadley et al., 1985); however, the specificity of these compounds needs to be examined carefully. In future studies, it may be useful to directly compare the properties of creep currents with the properties of transient inward currents in the same preparation under similar experimental conditions.

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