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SLOW INWARD TAIL CURRENTS IN RABBIT CARDIAC CELLS

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SUMMARY

1. A whole-cell gigaseal suction microelectrode voltage-clamp technique has been used to study slow inward tail currents in single myocytes obtained by enzymatic dispersion of rabbit ventricle and atrium. A variety of stimulation protocols, Tyrode solutions and pharmacological agents have been used to test three hypotheses: (a) that the slow inward tail current is generated by an electrogenic Na^+–Ca^{2+} exchanger; (b) that a rise in $[Ca^{2+}]_i$, due to release from the sarcoplasmic reticulum can modulate the activity of this exchanger; and (c) that the uptake of calcium by the sarcoplasmic reticulum is a major determinant of the time course of the tail current.

2. As shown previously in amphibian atrium and guinea-pig ventricle, slow inward tail currents can be observed consistently under conditions in which action potentials and ionic currents are recorded using microelectrode constituents which only minimally disturb the intracellular milieu.

3. In ventricular cells, the envelope of these tail currents obtained by varying the duration of the preceding depolarizations shows that (a) the tail currents are activated by pulses as short as 10 ms, and reach a maximum for pulse durations of 100–200 ms, (b) the rate of decay of the tail current gradually increases as the activating depolarizations are prolonged, and (c) the tails cannot be due to deactivation of calcium currents, in agreement with other studies in frog heart.

4. When the mean level of $[Ca^{2+}]_i$ is raised following inhibition of the Na^+–K^+ pump by strophanthidin (10^{-5} M) or reductions in $[K^+]_o$ (0·5 mM), the slow inward tail grows in size prior to the onset of a contracture or other signs of calcium-induced toxicity.

5. In a number of different preparations, replacement of $[Ca^{2+}]_o$ with $BaCl_2$ markedly or completely inhibits the Na^+–Ca^{2+} exchanger, whereas Sr^{2+} replacement does not have this effect. In myocytes from rabbit ventricle the slow inward tails are reduced significantly and decay more slowly in 0·5–2·2 mM-$BaCl_2$ Tyrode solution, while in 2·2 mM $SrCl_2$ these tails are not altered.

6. The slow inward tail also shows a dependence on $[K^+]_o$, corresponding to previous data on Na^+–Ca^{2+} exchange in other tissues. Increasing $[K^+]_o$ in the Tyrode solution to a final concentration of 10–15 mM results in a marked inhibition of the

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slow tails. This effect cannot be accounted for by changes in the inwardly rectifying potassium current, I_{K1}.

7. The slow tail currents were changed significantly by increasing the temperature of the superfusing Tyrode solution. The major effect was a speeding up of the decay time. This was most apparent for tails following short depolarizations, which normally decay quite slowly at room temperature.

8. Caffeine (2–5 mM) produced a prolongation of the decay time of the slow tails, and a small reduction in slow tail amplitudes. The peak of the 'envelope' of the tails was shifted so that it occurred at longer depolarizations.

9. In atrial cells, somewhat similar tail currents could be recorded consistently. However, in atrium they decayed much faster and there was very little difference in decay time between short and long depolarizations. Caffeine also prolonged the decay time of these tail currents.

10. The slow tail currents in atrial cells are very sensitive to stimulus rate. They were large after short rest periods (30–60 s), and declined to a steady-state level within one to two pulses (at 1–3 Hz) after stimulation was resumed.

11. These results from rabbit ventricular and atrial cells support the hypothesis that the slow tail current reflects the electrogenic activity of the Na^+–Ca^{2+} exchanger. However, the observed changes in the slow tail currents caused by indirect manipulations of Ca^{2+} sequestration into the sarcoplasmic reticulum suggest that intracellular calcium homeostasis involves a complex interaction between Ca^{2+} sequestration into the sarcoplasmic reticulum and Ca^{2+} extrusion via the Na^+–Ca^{2+} exchanger.

INTRODUCTION

Recently, slow inwardly-directed tail currents have been identified in cardiac cells under experimental conditions which suggest that they are generated by an electrogenic Na^+–Ca^{2+} exchanger (Campbell, Robinson & Giles, 1984; Campbell, Shibata, Robinson & Giles, 1985; Hume & Uehara, 1986*a*, *b*; Kimura, Noma & Irisawa, 1986; London & Krueger, 1986; Mechmann & Pott, 1986; Fedida, Noble, Shimoni & Spindler, 1987; Kimura, Miyamae & Noma, 1987; Lipp & Pott, 1988*a*, *b*; Egan, Noble, Noble, Powell, Spindler & Twist, 1989). Further detailed study of the properties of a membrane current associated with the regulation of intracellular calcium (Mullins, 1979, 1981; Chapman, 1983; Noble, 1986; Sheu & Blaustein, 1986) is of importance for the understanding of both the electrical and the mechanical activity of the heart.

Quantitative studies of the Na^+–Ca^{2+} exchanger current, labelled I_{EX}, in cardiac cells have been hindered by the absence of a selective inhibitor (e.g. Mentrard, Vassort & Fischmeister, 1984; Bielefeld, Hadley, Vassilev & Hume, 1986). Nevertheless, several important features of I_{EX} in heart have been deduced, partly by analogy with a somewhat similar current that has been studied in other preparations (e.g. the retina), which do not exhibit transmembrane ionic currents which overlap and obscure I_{EX} (Yau & Nakatani, 1984; Schnetkamp & Bownds, 1987). In guinea-pig ventricular cells recent evidence strongly suggests that the rise in $[Ca^{2+}]_i$, associated with each contraction, triggers this current (Fedida *et al.* 1987). As indicated previously the lack of specific inhibitors for the Na^+–Ca^{2+} exchanger

prevents quantitative measurement of it under steady-state resting (diastolic) conditions. However, it is possible to record a transient current, presumably carried by the Na^+–Ca^{2+} exchanger when it is perturbed by a transient rise in intracellular calcium. I_{EX} increases very rapidly and then decays during diastole, in parallel to the removal of calcium from the cytosol (Barcenas-Ruiz & Wier, 1987; Barcenas-Ruiz, Beuckelmann & Wier, 1987).

The action potentials and K^+ currents in rabbit atrial and ventricular cells differ significantly (Giles & Imaizumi, 1988). In addition, there are important differences in the extent to which the atrium and ventricle exhibit Ca^{2+}-induced Ca^{2+} release. This issue has been examined in detail using skinned preparations (Fabiato, 1981) as well as pharmacological inhibitors of Ca^{2+} release (Bers, 1985; Sutko, Bers & Reeves, 1986). These results have suggested that in rabbit atrium a relatively large fraction of the Ca^{2+} needed for contraction is derived from the sarcoplasmic reticulum (SR), and that transsarcolemmal Ca^{2+} entry via I_{Ca} contributes relatively little of the Ca^{2+}. Since the current due to the Na^+–Ca^{2+} exchanger is activated and controlled by intracellular Ca^{2+}, $[Ca^{2+}]_i$, one would expect a different time course and/or amplitude of I_{EX} in rabbit atrium than in ventricle, if SR uptake and release are more pronounced in atrial cells.

The general aim of this study was to characterize the Na^+–Ca^{2+} exchanger current in single atrial and ventricular cells isolated from rabbit heart. The specific aims included: (1) establishing the size and time course of this current in rabbit ventricular cells, in comparison to guinea-pig ventricular myocytes; this information is of importance in view of a variety of reported interspecies differences (Bers, Philipson & Langer, 1981; Fabiato, 1982), and is needed to determine the role of this current in intracellular calcium homeostasis and in action potential configuration changes; (2) obtaining further evidence concerning whether the slow tail currents are due to the activity of the Na^+–Ca^{2+} exchanger; (3) testing whether I_{EX} is sensitive to calcium loading other than that associated with phasic contraction, given the close association between I_{EX} and cellular mechanical activity (Fedida *et al.* 1987); and (4) assessing the role of SR in the activation of I_{EX} and its decay, by pharmacological means (caffeine) and by comparing I_{EX} in ventricular and atrial cells.

METHODS

Single ventricular or atrial cells from the hearts of adult rabbits were used in these experiments. The details of the enzymatic dispersion procedure have been described previously (Giles & van Ginneken, 1985; Giles & Imaizumi, 1988). Briefly, the rabbit heart was excised rapidly and mounted on a Langendorff perfusion apparatus. After a short rinse in normal bicarbonate-buffered Tyrode solution the heart was perfused for 20 min in a nominally zero Ca^{2+} Tyrode solution. It was then perfused for 20 min in a collagenase-containing Tyrode solution. Thereafter, chunks of ventricle or the atria were removed and added to a HEPES-buffered, low $[Ca^{2+}]$ (50 μM) collagenase-containing Tyrode solution. Suspensions of cells were removed and washed with enzyme-free Tyrode solution at selected time intervals (10 min), to harvest viable single cells.

The isolated myocytes were placed in the experimental chamber and perfused with Tyrode solutions containing gradually increasing $[Ca^{2+}]_o$. Initially the isolated cells were placed in a HEPES-buffered Tyrode solution containing 50 μM $[Ca^{2+}]_o$. After 10 min external calcium, $[Ca^{2+}]_o$, was raised to 100 μM, and then 5 min later to 200 μM. If the cells showed no signs of contraction, $[Ca^{2+}]_o$ was raised to 500 μM for the following 10 min. After an additional 25–30 min, superfusion with control Tyrode solution ($[Ca^{2+}]_o$, 2·2 mM) was begun. This procedure was adopted since a

gradual increase in $[Ca^{2+}]_o$ yielded more myocytes which were tolerant to physiological $[Ca^{2+}]_o$ levels. The final control Tyrode solution contained (in mM): NaCl, 150; KCl, 5·4; $CaCl_2$, 2·2; $MgCl_2$, 1·0; HEPES, 5·0; and glucose, 5·5, and was titrated to a pH of 7·4 with NaOH, and then bubbled with 100% O_2. The microelectrodes were filled with a solution containing (in mM): potassium aspartate, 120; KCl, 30; Na_2ATP, 4; HEPES, 5; pH was titrated to 7·2 with KOH. Their DC

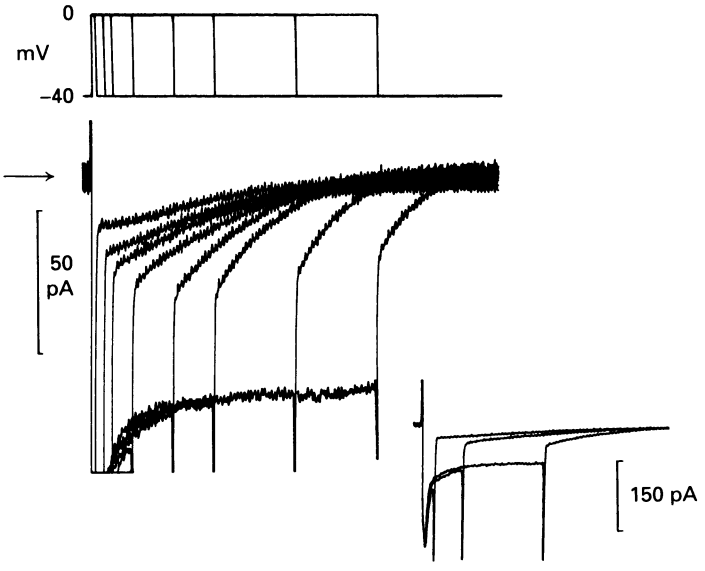

Fig. 1. Envelope of tail currents in a rabbit ventricular cell. The cell was depolarized from −40 to 0 mV at 0·1 Hz for durations varying between 10 ms (shortest) to 700 ms (longest pulse shown). The voltage-clamp steps are shown in the top panel. Following repolarization to −40 mV an inward tail current was observed. It increased in amplitude as the preceding activating depolarization was prolonged from 10 to 200 ms. For longer depolarizations, this tail current decreased in size. The rate of decay of the tail current was slow for short pulses, and much faster following long depolarizations. The current traces in the inset are at lower gain to show the calcium current and the tail currents elicited by 40 mV depolarizing pulses of 30, 100, and 300 ms duration. The arrow (beside the centre panel) indicates the baseline current level.

resistances varied from 2–5 MΩ. All experiments were carried out at room temperature (20–23 °C) except those in which temperature effects were studied. Measurements of transmembrane ionic currents were made using a whole-cell gigaseal voltage-clamp technique as described in detail in Giles & Shibata (1985). After forming a gigaseal the cells were ruptured by gentle suction. Careful measurement of the resting potential was made in order to obtain an accurate zero current level. No series resistance compensation was employed. Most of the experiments were carried out using a holding potential of −40 mV in order to inactivate the sodium current, I_{Na}. Under these experimental conditions, depolarizing pulses to between 0 and +20 mV consistently resulted in slow 'tail' currents immediately following repolarization to −40 mV.

RESULTS

Slow tails in rabbit ventricular cells

Our initial findings confirmed that in rabbit ventricular cells, depolarizing pulses from −40 mV to positive potentials (which are known to elicit contractions) are followed by slow inwardly directed tail currents. Similar phenomena have been

demonstrated previously in cells from guinea-pig ventricle and in bull-frog atrium. The initial amplitude of these tail currents was approximately 40–60 pA and they decayed back to the holding current level within 400–700 ms. Figure 1 illustrates typical slow inward tail currents following depolarizations from −40 to 0 mV.

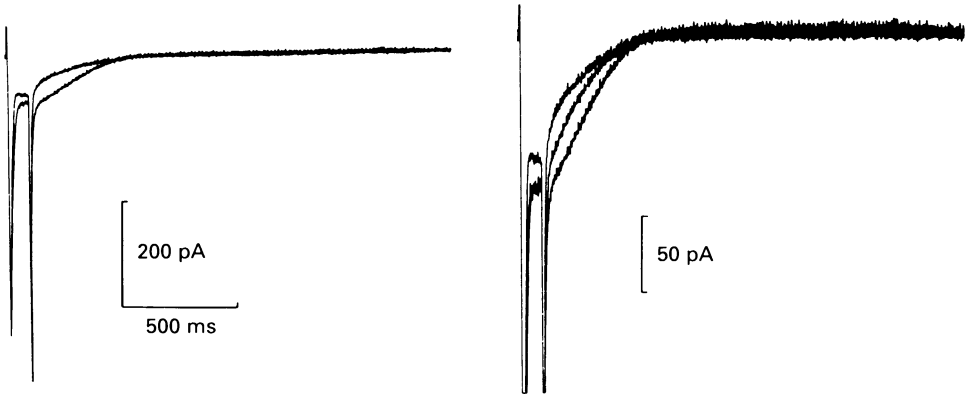

Fig. 2. Effects of blocking the Na^+–K^+ pump on the slow tail currents. The Na^+–K^+ pump was blocked by removing $[K^+]_o$. Voltage-clamp pulses of 100 ms were applied from −40 to +10 mV. The left panel shows superimposed current traces obtained in normal Tyrode solution and 7·5 min after changing to zero $[K^+]$ Tyrode solution. The slow tail was considerably larger, even though there was very little change in the calcium current, I_{Ca}. The right panel shows these current traces at higher gain, for control conditions (smallest tail current) and after 6·5 and 7·5 min in K^+-free Tyrode solution (largest tail current).

Fedida *et al.* (1987) have reported that in guinea-pig ventricle the amplitude of these tail currents is dependent on the duration of the preceding depolarization. Figure 1 confirms this and shows an important additional feature: the decay following short depolarizing pulses is considerably slower than the decay following longer pulses. These depolarizing pulses lasting 10–20 or 300–500 ms were followed by current tails with half-times of decay which differed by 2- to 4-fold. It is possible that these differences can be attributed to differences in the Ca^{2+}-sequestering capacity of the SR. This will be addressed later in this paper.

Previous work (Fedida *et al.* 1987) has shown that the activation of these current tails was closely linked to the phasic release of the calcium from the SR, such as the Ca^{2+} release which triggers contraction. We have examined whether a maintained increase in intracellular calcium ($[Ca^{2+}]_i$) could also augment the tail currents. $[Ca^{2+}]_i$ was elevated indirectly following inhibition of the Na^+–K^+ pump, by a reduction of extracellular potassium ($[K^+]_o$) or following exposure to 10^{-5} M-strophanthidin (Sheu & Fozzard, 1982). Both of these procedures ($[K^+]_o$ reduction, $n = 8$; strophanthidin, $n = 4$) consistently resulted in larger tail currents. Figure 2 illustrates the effects of $[K^+]_o$ reduction.

We next attempted to obtain additional evidence that in rabbit ventricle these tail currents are due to the electrogenic extrusion of Ca^{2+} by the Na^+–Ca^{2+} exchanger. The first experiments (results not shown) demonstrated that, as in guinea-pig cells, the tail current is reduced considerably by partial removal of extracellular sodium

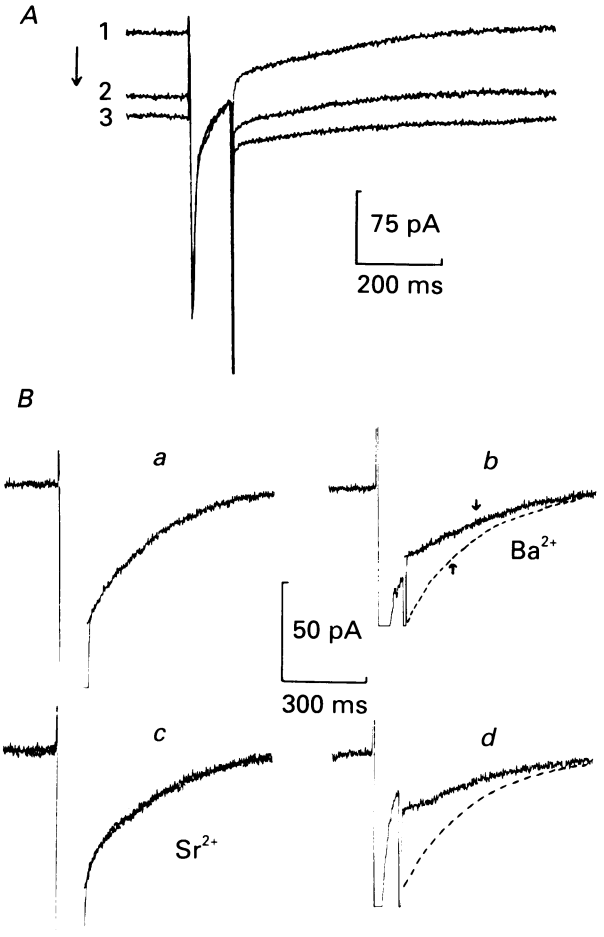

Fig. 3. Effects of Ba^{2+} and Sr^{2+} on the slow tail current. *A*, currents recorded following the addition of 2 mM-Ba^{2+} obtained at 1, 3 and 4·5 min (from top to bottom). In both *A* and *B*, 100 ms voltage-clamp pulses were applied from −40 to +10 mV. Following Ba^{2+} addition, at −40 mV there was a rapid inward shift of the holding current due to block of I_{K1} (see arrow). At the same time, the slow tail was reduced in amplitude and slower to decay. Note that even though the holding current shifted inward, the peak inward calcium current, I_{Ca}, was unchanged. *B*, current traces from another cell. *a*, in normal Tyrode solution. *b*, following addition of 0·5 mM-Ba^{2+}. The dashed line shows the contour of the tail in normal Tyrode solution. The arrows show the half-time of decay, illustrating that Ba^{2+} substantially slows the decay of the tail current. These effects of Ba^{2+} are reversible. *c*, following Ba^{2+} wash-out, 1 mM-Sr^{2+} was added. This panel shows two superimposed traces, one in normal Tyrode solution (after Ba^{2+} wash-out) and the other in 1 mM-Sr^{2+}. The slow tail currents are superimposable, indicating no effects of Sr^{2+}. *d*, Sr^{2+} was then washed off and 2 mM-Ba^{2+} re-added. The tail current was reduced even more than in *b*. The dashed line again indicates the contour of the tail current in normal Tyrode solution.

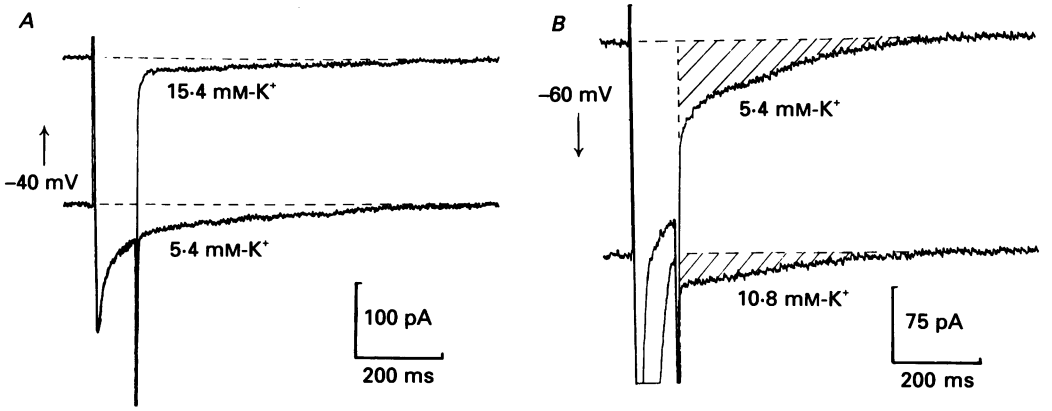

Fig. 4. Effects of increasing extracellular potassium, $[K^+]_o$, on the slow tail current. *A*, increasing $[K^+]_o$ from 5·4 to 15·4 mM, at a holding potential of −40 mV gave a large outward shift in the holding current (indicated by arrow) due to changes in I_{K1}. The peak inward current, I_{Ca} was unchanged, but the slow tail current following repolarization was significantly reduced. Clamp pulses were 100 ms in duration, from −40 to 0 mV. *B*, in another cell, held at −60 mV, increasing $[K^+]_o$ from 5·4 to 10·8 mM shifted the holding current in the inward direction (arrow). Once again, the elevated $[K^+]_o$ greatly decreased the slow tail current (shaded areas). In this cell, clamp pulses of 100 ms duration were applied from −60 to 0 mV.

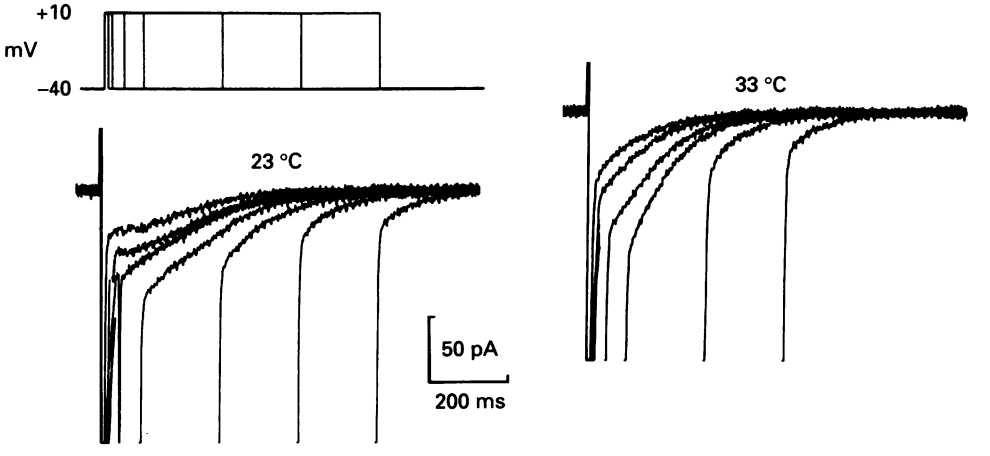

Fig. 5. Effects of increasing the temperature on the slow inward tail currents. Two 'envelopes' of slow tails are shown. Each was elicited in a ventricular cell held at −40 mV and depolarized to -10 mV for varying durations (10–700 ms). The slow tails were recorded following repolarization to −40 mV. The voltage-clamp steps are in the top panel. The middle panel shows currents recorded at 23 °C, and the bottom panel are the currents elicited after warming the Tyrode solution to 33 °C. At warmer temperatures there was a marked enhancement of the rate of decay. This was most notable following very short pulses.

($[Na^+]_o$). $[Na^+]_o$ was reduced to 25% of normal by replacing NaCl with LiCl. Following application of 25% $[Na^+]_o$. Tyrode solution a transient increase in the tail current was observed, perhaps due to the accompanying rise in $[Ca^{2+}]_i$.

In the absence of specific inhibitors of the Na^+–Ca^{2+} exchanger (Bielefeld *et al.* 1986; but see Lipp & Pott, 1988*a*, *b*), an important test for establishing whether this slow current is generated by the Na^+–Ca^{2+} exchanger consists of applying divalent ions other than Ca^{2+}. Previously, several investigators have shown that the Na^+–Ca^{2+} exchanger can carry Sr^{2+} but not Ba^{2+} (Tibbits & Philipson, 1982; Yau & Nakatani, 1984). In guinea-pig ventricle, Kimura *et al.* (1987) found that the Na^+–Ca^{2+} exchanger current also showed this pattern of selectivity for divalent cations. As shown in Fig. 3, the tail currents in rabbit ventricular myocytes were not changed when $SrCl_2$ was substituted for $CaCl_2$, but were inhibited by substitution of $CaCl_2$ with $BaCl_2$ ($n = 9$). Note that Ba^{2+} also slowed the decay of the tail current, consistent with an inhibition of the exchanger activity.

In rabbit ventricular cells the inwardly rectifying K^+ current, I_{K1}, generates a significant outward current at -40 mV. I_{K1} can be blocked by $BaCl_2$ (Giles & Imaizumi, 1988). However, this inhibition of I_{K1} is not responsible for the Ba^{2+}-induced block of the slow tail currents since in experiments using very low $[K^+]_o$ (0·54 mM) Tyrode solution, in which I_{K1} is very small at -40 mV, Ba^{2+} still blocked the slow tails (Shimoni & Giles, 1987; their Fig. 2). Since these experimental results supported the hypothesis that the slow tail current can be generated by the Na^+–Ca^{2+} exchanger, we labelled it I_{EX}.

I_{EX} is also very sensitive to increases in $[K^+]_o$. In the squid axon (Baker & McNaughton, 1976) and in amphibian retina (Yau & Nakatani, 1984; Schnetkamp & Bownds, 1987), fluxes mediated by the Na^+–Ca^{2+} exchanger and currents attributed to its activity can be inhibited by raising $[K^+]_o$. Consistent with this we have observed ($n = 8$) that the slow tail currents were reduced substantially by raising $[K^+]_o$ 2–3 times above its normal concentration (Fig. 4). Once again, it is important to ensure that the observed current changes do not arise from shifts in I_{K1} by measuring the changes in I_{EX} at different holding potentials. At -40 mV, increasing $[K^+]_o$ shifts the holding current inward, whereas at -60 mV the holding current is shifted outward, due to the 'cross-over' effect on I_{K1} (DiFrancesco & Noble, 1984). However, note that at both of these holding potentials I_{EX} is reduced significantly. Thus, in rabbit ventricle increases in $[K^+]_o$ appear to be able to block I_{EX}.

Additional experiments were done to test the temperature dependence of I_{EX}. Values of the temperature coefficient Q_{10} were obtained from meaurements of the time for decay of I_{EX} to 50% of the initial tail current. In eight cells the Q_{10} values (at 23–33 °C) averaged $2{\cdot}76 \pm 0{\cdot}13$ (mean ± S.E.M.). Figure 5 demonstrates the dramatic speeding up of the decay time of I_{EX} when the superfusing Tyrode solution was warmed to 33 °C. This effect was more pronounced in the slow tails elicited by short depolarizing pulses, where normally the decay is slowest.

These temperature-dependent effects on the kinetics of decay of the slow tails following depolarizing pulses of different durations raised questions which prompted a more detailed study of the change in the time course of decay of I_{EX} as a function of the duration of the preceding depolarization (Fig. 1). Our working hypothesis was that this effect may be due, in part, to altered Ca^{2+}-sequestering activity of the SR.

Since Ca^{2+}-induced Ca^{2+} release from the SR is regenerative, the Ca^{2+} release may continue after short depolarizing pulses have returned to −40 mV. Immediately following repolarization, Ca^{2+} extrusion may be achieved mainly by Na^+–Ca^{2+} exchanger activity. Short pulses may deplete the SR calcium stores relatively little,

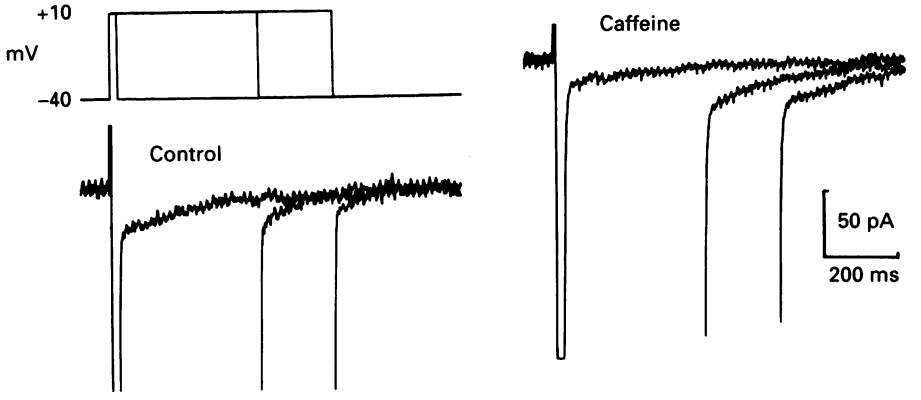

Fig. 6. Effects of 2 mM-caffeine on slow tails elicited by depolarizations of varying duration. This cell was held at −40 mV, and depolarized to +10 mV for 20, 400 and 500 ms. Voltage traces are shown at the top left. The currents in the control Tyrode solution (middle panel) decayed to half their initial amplitudes in 131, 74 and 30 ms, respectively. They decay faster following the longer pulses. In the right panel are traces obtained after adding 2 mM-caffeine. These currents decayed by 50% in 154, 137 and 120 ms, respectively. Thus, the difference between slow tails following short and long pulses was abolished. In addition, the activation of the slow tail shifted to later times, since the tail following the longer pulses actually increased, relative to the tail following the shorter pulse.

thus limiting SR re-uptake and leaving the Na^+–Ca^{2+} exchanger as the main mechanism for Ca^{2+} extrusion. In contrast, both during and following longer depolarizations, the SR (which has released more Ca^{2+}) can presumably sequester Ca^{2+}. Thus, the reduction of $[Ca^{2+}]_i$, and hence the decay of I_{EX} which was activated by the transient increase in $[Ca^{2+}]_i$ is achieved much faster (see Discussion). When the temperature is increased to 33 °C the rate of SR uptake is enhanced, and both release and uptake may commence earlier. Thus the peak of the I_{EX} envelope may shift to earlier times at warmer temperatures (Fig. 5). The temperature-induced enhancement in the rate of SR uptake of Ca^{2+} will result in a faster decay of I_{EX}, following both short and long depolarizing pulses.

This working hypothesis was tested by attempting to reduce the difference in I_{EX} decay times following short and long pulses by blocking SR calcium sequestration by the SR with caffeine. The data in Fig. 6 show that after ventricular cells were exposed to 2 mM-caffeine, the slow tails following short and long pulses decayed with very similar time courses.

Thus, it appears that the time course (and possibly the magnitude) of I_{EX} can be significantly modulated by the activity of the SR. It was therefore of interest to compare I_{EX} in ventricular cells and in atrial cells from the same species (rabbit) and under identical experimental conditions. In rabbit atrial cells the SR plays a much

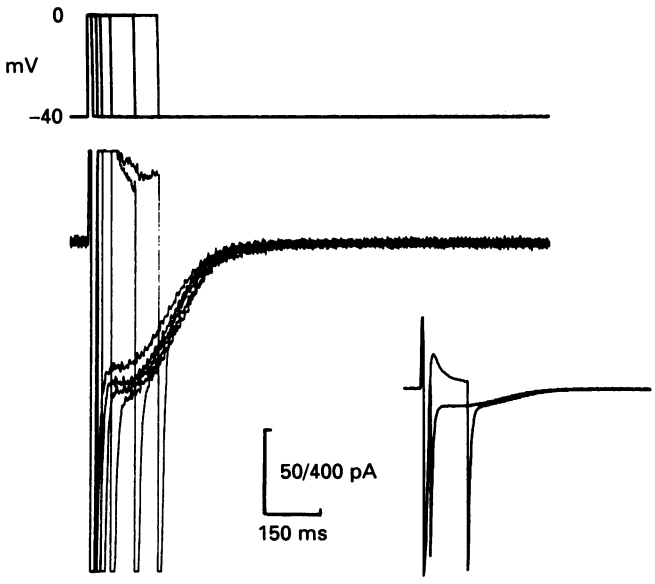

Fig. 7. An envelope of slow current tails in a rabbit atrial cell. These results were obtained under identical conditions to those used for the ventricular cell experiments. The durations of the depolarizing pulses shown are 10, 20, 30, 50, 100 and 150 ms. The bottom panel shows the tail currents which decayed considerably faster than the tails in ventricular cells. Furthermore, the rate of decay following short and long depolarizations is very similar. Note the 'sigmoid' shape of the tails, which was consistently observed following short pulses in atrial cells. The inset shows the complete current traces for two of the pulses (30 and 150 ms duration). The 400 pA, 150 ms calibration bars apply to the inset; 50 pA and 150 ms calibration apply to the middle panel.

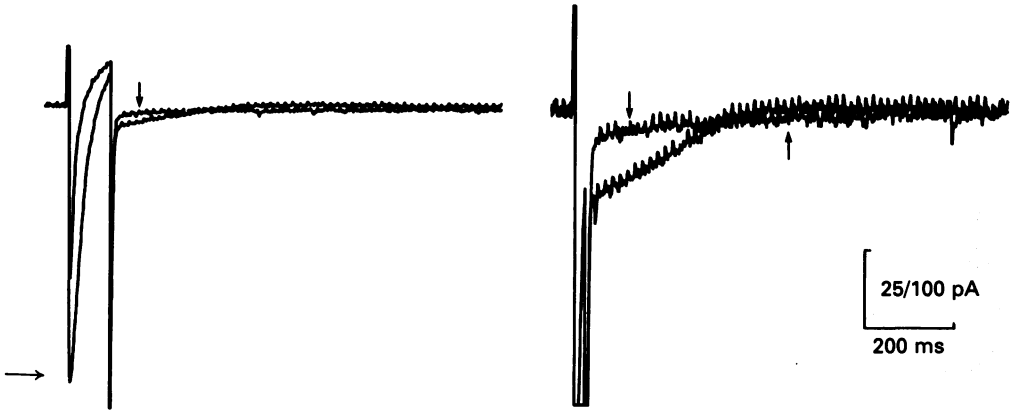

Fig. 8. Effects of caffeine on the slow tails in atrial cells. Two sets of superimposed current traces are shown. Current traces obtained in response to depolarizing the cell from −40 to +10 mV for 100 ms, at 0·2 Hz. In this cell no transient outward current was elicited under these conditions. Adding 4 mM-caffeine (arrows) augmented the peak inward current but the slow tail following repolarization was reduced and its decay was considerably slower. The right panel illustrates slow tails following 30 ms depolarizations (at a higher magnification). The vertical calibrations are 100 pA (left) and 25 pA (right).

more dominant role in Ca^{2+} homeostasis than it does in ventricle cells, as judged by force–frequency data and by studies of Ca^{2+}-induced Ca^{2+} release from 'skinned' preparations (cf. Bers, 1985; Sutko *et al.* 1986).

Slow tails in rabbit atrial cells

Measurements of slow tails in atrial cells ($n > 50$) showed a significant difference between atrium and ventricle; the decay of I_{EX} was considerably faster than that in

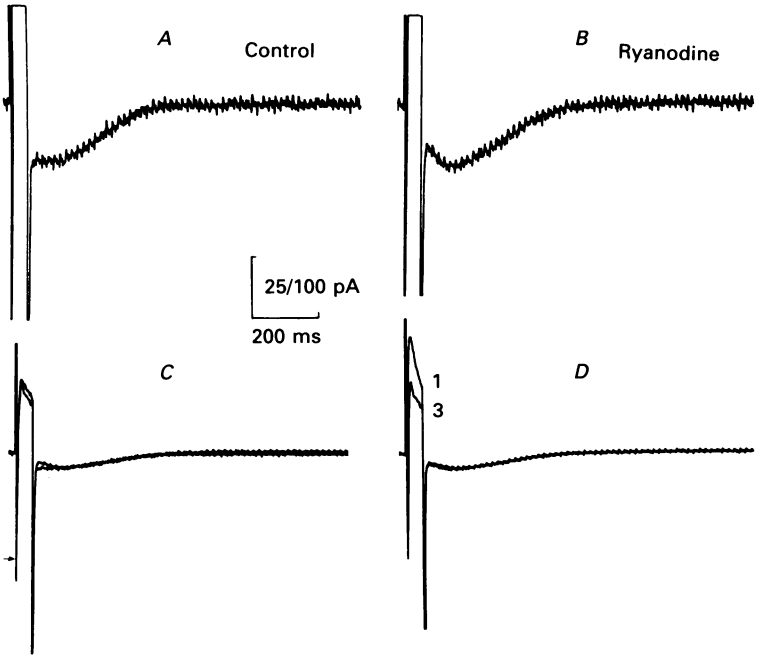

Fig. 9. Effects of ryanodine on the slow tails in atrial cells. This cell was held at −40 mV and depolarized to +10 mV for 50 ms at 0·1 Hz. The control tail current can be seen in *A*. Adding 5 μM-ryanodine delayed the onset of the tail (*B*). *C* shows that at the same time there was a slight reduction in both peak inward and transient outward currents. *D* illustrates that changes in peak transient outward current do not affect the amplitude or time course of the tail currents. After a 1 min rest, the cell was again clamped to +10 mV at 0·5 Hz. The first and third responses from the same cell are superimposed. Although the transient outward current showed its characteristic reduction related to higher rates, the slow tails were unchanged.

ventricular cells. In atrial myocytes at a holding potential of −40 mV, I_{EX} decayed completely within 200 ms following a 100 ms depolarization. Following longer depolarizations lasting longer than 200 ms no I_{EX} was observed, in marked contrast to ventricular cells, in which even 300–600 ms pulses were followed by prominent tail currents. The envelope of slow tails in atrial cells in Fig. 7 demonstrates this relatively fast decay of I_{EX} following depolarizing pulses.

Two other features of the slow tails in atrial myocytes are noteworthy. First, following very short pulses there is an apparent 'lag' period (50–70 ms) during which the initial size of I_{EX} changes very little before beginning to decay. This apparent 'lag' may be due to a decay tail of deactivating transient outward current, since this current is very large in rabbit atrial cells (Giles & Imaizumi, 1988). However, this

type of outward current tail cannot entirely explain the lag (see Discussion). Second, in atrial cells the time course of I_{EX} decay is very similar following short and long depolarizing pulses. This result and the relatively fast decay suggest that the rate of Ca^{2+} extrusion may be related to the active uptake of Ca^{2+} into the SR.

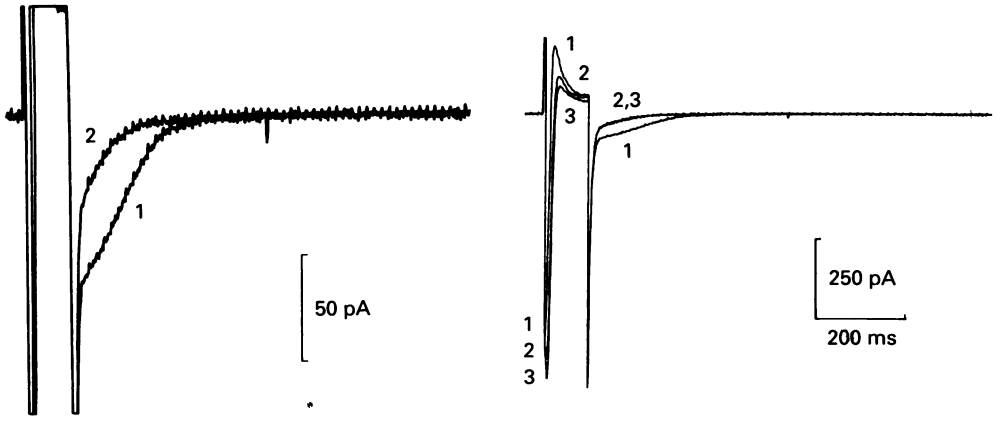

Fig. 10. Effects of a quiescent period on slow tail currents in atrial cells. This cell was held at −40 mV. After a rest of 1 min, 100 ms depolarizing pulses to 0 mV were given at 0·67 Hz. The left panel shows the first two current traces superimposed (at high gain). Note the marked reduction in the tail following pulse 2. The right panel shows the first three current traces. Although the peak transient inward current increased in size, and the transient outward current declined from the first to the third pulse, the slow tail was markedly reduced only between the first two pulses, i.e. there was no change between the second and third pulses. Thus, the changes in the slow tail apparently are not due to changes in the transient outward current.

To test whether the relatively rapid decay of the slow inward tail is mainly due to uptake of calcium by the SR, atrial cells were exposed to caffeine. In 1–4 mM-caffeine the amplitude of I_{EX} was decreased, and its time course of decay was slowed making the time for complete decay of I_{EX} in atrial myocytes similar to that in ventricular cells. An example of this caffeine-induced change is shown in Fig. 8.

Ryanodine ($1–5 \times 10^{-6}$ M), which prevents SR calcium uptake, produced some prolongation of I_{EX} decay ($n = 4$), although this effect was not as pronounced as that of caffeine. Ryanodine also decreased the calcium current, but did not change the peak size of I_{EX}. These results are similar to those obtained with guinea-pig ventricular cells (Fedida *et al.* 1987; their Fig. 12). Initially ryanodine induced a transient increase in the magnitude of I_{EX}, which may have resulted from an enhanced net calcium release or leakage from the SR (cf. Meissner, 1986). In some cells, exposure to 5 μM-ryanodine slowed the activation of I_{EX} sufficiently for its onset as well as its decay to be measured. Figure 9 shows an example of this. This result also indicates that the time 'lag' preceding the decay of I_{EX} in atrial cells is not due to an overlapping deactivation tail of the transient outward current.

Rabbit atrium exhibits prominent rate-dependent changes in action potential configuration, as well as very large post-rest contractions (Hilgemann, 1986). Consistent with this, very large I_{EX} currents were observed when rest periods of

30–60 s were followed by voltage-clamp depolarizations. This effect was transient; at rates of 0·67–1 Hz the size of the slow tails returned to its steady-state level within one to two pulses. Although qualitatively similar effects were observed in rabbit ventricular cells (as well as in guinea-pig ventricle), the magnitude of this effect was greater in rabbit atrial cells. An example is shown in Fig. 10. These records also show that I_{EX} does not change significantly between pulses 2 and 3, even when the transient outward current is still decreasing significantly. Thus, for 100 ms pulses, overlap of outward current does not seem to affect I_{EX}.

DISCUSSION

The sarcoplasmic reticulum can modulate the slow tail current

Our results demonstrate that in isolated cardiac cells an inwardly directed relatively slow transient inward tail current can be recorded consistently following depolarizing steps which are positive to the threshold for contraction. Several lines of evidence suggest that the 'tail' current in these cells are dependent on the increase in $[Ca^{2+}]_i$ which occurs during the preceding depolarization; and that most of this Ca^{2+} comes from the SR (cf. Fabiato, 1981, 1983; London & Krueger, 1986). Consistent with this hypothesis, periods of quiescence which are known to increase the Ca^{2+} load in the SR (cf. Bridge, 1986; Sutko *et al.* 1986), result in a significant post-rest potentiation of the tail current, even though I_{Ca} is not changed significantly (Fig. 10). Moderate increases in $[Ca^{2+}]_i$ following inhibition of the Na^+–K^+ pump can also enhance the slow inward tail currents (Fig. 2). Presumably, this effect can be explained by Ca^{2+}-induced release of Ca^{2+} from the SR being enhanced by moderate increases in $[Ca^{2+}]_i$ (Fabiato, 1981). The data obtained when the temperature of the superfusing Tyrode solution was varied also support the hypothesis that the slow inward tail is modulated significantly by Ca^{2+} release from the SR. Thus, the relatively high Q_{10} values obtained from measurements of the kinetic of decay of the slow tails are consistent with an active Ca^{2+}-pumping mechanism being importantly involved in the decay phase of the slow tails. However, these results need to be interpreted with caution since the Na^+–Ca^{2+} exchanger is also known to be temperature-sensitive. Previously, Kimura *et al.* (1987) have demonstrated that the Na^+–Ca^{2+} exchanger in guinea-pig ventricular myocytes shows a significant temperature-dependence and Blaustein & Oborn (1975) have also demonstrated that the Na^+–Ca^{2+} exchanger in vesicles obtained from mammalian nerve endings exhibits a Q_{10} of approximately 3.

Measurements of slow inward tail currents in cells obtained from rabbit atrium also suggest that Ca^{2+} release and re-uptake from the SR is an important regulator of the size and time course of the slow tails. As indicated previously, anatomical, electrophysiological, and mechanical experiments have shown that there is more SR in rabbit atrial tissue than in rabbit ventricle and that the SR plays a prominent role in the activation of contraction and in re-priming the mechanical activity (Fabiato, 1981; Bers, 1985; Capogrossi, Kort, Spurgeon & Lakatta, 1986; Hilgemann, 1986; Lewartowski & Pytkowski, 1987). Our measurements of I_{EX} in atrial cells consistently exhibited the following features: (i) an envelope with a much faster

time-to-peak than the corresponding one from rabbit ventricular cells; (ii) a substantial slowing effect of caffeine on the time course of decay; and (iii) a much faster time course of decay of the slow tail than in ventricular cells. Each of these findings is consistent with the hypothesis that an important determinant of the time course of the slow tail is the rate at which Ca^{2+} is sequestered by SR and hence $[Ca^{2+}]_i$ returns to normal diastolic levels.

Contribution of the Na^+–Ca^{2+} exchanger to the slow tail current

As indicated previously, no selective inhibitor of the Na^+–Ca^{2+} exchanger in intact cells is available at present. We were therefore forced to use a number of indirect tests of the hypothesis that the slow tail may be generated by the Na^+–Ca^{2+} exchanger as it is in bull-frog atrium (Campbell *et al.* 1985; Hume & Uehara, 1986*a*, *b*; Campbell, Giles, Robinson & Shibata, 1988), in myoballs from guinea-pig atrium (Lipp & Pott, 1988*a*, *b*) and guinea-pig ventricle (Fedida *et al.* 1987). Previously, it has been shown (Fedida *et al.* 1987; Shimoni & Giles, 1987) that the slow tail current is reduced when $[Na^+]_o$ is replaced by Li^+. Since in this experiment the electrochemical gradient for Na^+ is reduced and Li^+ is known to block the Na^+–Ca^{2+} exchanger, these findings strongly suggest that the slow tail in mammalian heart is also generated, at least partly, by the Na^+–Ca^{2+} exchanger. An independent test is based upon the observation that the Na^+–Ca^{2+} exchanger also exhibits selectivity for the divalent ions which are present, and in particular that Sr^{2+} will substitute for Ca^{2+}, but Ba^{2+} will not (see Yau & Nakatani, 1984; Campbell *et al.* 1985; Kimura *et al.* 1987; Campbell *et al.* 1988). Our results (Fig. 3) demonstrate that when $[Ca^{2+}]_o$ is substituted for Ba^{2+}, the slow tails are significantly reduced in magnitude and their decay time is slowed, consistent with a reduced capacity of the exchanger. As expected from previous work in the visual system and in heart (Tibbits & Philipson, 1982; Kimura *et al.* 1987), substitution of Ca^{2+} for Sr^{2+} did not significantly affect the slow tail currents. Previously, Fedida *et al.* (1987) observed a significant reduction in I_{EX} when Sr^{2+} was applied. The explanation for this difference is not obvious; particularly since Kimura *et al.* (1987), who also used guinea-pig ventricular myocytes, did not observe it.

A third test for the involvement of the Na^+–Ca^{2+} exchanger in the generation of the slow tail currents was made by increasing $[K^+]_o$ in the superfusing Tyrode solution. Previous results (Baker & McNaughton, 1976; Yau & Nakatani, 1984; Schnetkamp & Bownds, 1987) suggest that this manoeuvre should inhibit the Na^+–Ca^{2+} exchanger. Our data (Fig. 4) demonstrated this effect, suggesting further that the Na^+–Ca^{2+} exchanger is an important determinant in the slow tail.

'Envelope of tails' experiments

The 'envelope of the tails' experiments (Figs 1 and 7) demonstrate that this slow inward tail current is not due to a conventional time and voltage deactivation of I_{Ca}. Previous findings in a variety of other cardiac myocyte preparations are in agreement with this (bull-frog atrium: Campbell *et al.* 1985, 1988; Hume & Uehara, 1986*a*, *b*; guinea-pig ventricle: Barcenas-Ruiz *et al.* 1987; Fedida *et al.* 1987). The data obtained from these 'envelope' experiments also illustrate other important features of the slow tails. In rabbit ventricle, the tails decay significantly faster

following long depolarizations than they do after relatively short depolarizations (Fig. 1). In atrial myocytes this effect is much less prominent. Differences in the voltage-dependent behaviour of the Na^+–Ca^{2+} exchanger cannot contribute to this since the holding potential was −40 mV in both sets of experiments. One plausible explanation for this difference in kinetics is based upon known differences in the amount of SR in these two tissues. Since the atrial myocytes have more SR and the SR which is present appears to exhibit more prominent Ca^{2+}-induced release of Ca^{2+} than ventricle myocytes, the earlier time-to-peak and the more rapid decay of the inward tails in atrium could arise from faster, more substantial release of Ca^{2+} followed by relatively rapid sequestration into the more abundant SR. This explanation requires, however, that the Ca^{2+} pumps in the sarcolemma and in the SR of both cell types have approximately equal capacity and that the $[Ca^{2+}]_i$ buffering mechanisms are similar in both cell types (cf. Campbell *et al.* 1988). Previous studies have demonstrated conclusively that in rabbit heart there is a very complex interaction between the SR and the sarcolemmal mechanisms for Ca^{2+} homeostasis and that both Ca^{2+} pumps and the Na^+–Ca^{2+} exchanger are importantly involved (Bridge, 1986; Sutko *et al.* 1986).

Although these experiments were not designed to study in detail the time course of the envelope of tails, the sigmoid shape of the envelope in atrium was a consistent finding which may provide some insights into underlying mechanisms. One plausible explanation is that the sigmoidicity is due to an artifact resulting from the presence of a relatively large outward tail current due to the deactivation of the transient outward current in these preparations (Giles & Imaizumi, 1988). However, results in Figs 9*D* and 10 show that the slow inward tail can remain constant under conditions when the size of the transient outward current and, hence, of its tail would be expected to change significantly. Although the mechanism of this sigmoidicity has not been determined it seems likely that the time lag observed before the inward tail begins to grow is due to either a delay in the release of Ca^{2+} from the SR or significant, perhaps localized, Ca^{2+} buffering near the release sites so that the Na^+–Ca^{2+} exchanger which is located in the sarcolemma 'sees' the rise in $[Ca^{2+}]_i$ only after a measureable delay. It is apparent that experiments in which only electrophysiology is employed cannot answer important mechanistic questions of this kind. However, by using an approach involving simultaneous measurements of $[Ca^{2+}]_i$ transmembrane currents, and cell length (Barcenas-Ruiz *et al.* 1987; Cannell, Berlin & Lederer, 1987; Wier, Cannell, Berlin, Marban & Lederer, 1987) it may be possible to elucidate the main factors controlling Ca^{2+} efflux from the atrium and ventricle of the rabbit heart.

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