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CALCIUM-ACTIVATED INWARD CURRENT AND CONTRACTION IN RAT AND GUINEA-PIG VENTRICULAR MYOCYTES

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SUMMARY

1. Single ventricular cells from rat and guinea-pig hearts were voltage clamped, and contraction was monitored with an optical method.

2. In rat cells, short (2–10 ms) depolarizing pulses to 0 mV from a holding potential of −40 mV evoked current carried by calcium, and on repolarization to −40 mV there was a slow 'tail' current which decayed much more slowly than the expected deactivation of calcium current at this potential.

3. When rat cells were loaded with EGTA diffusing into the cytosol from an intracellular electrode, contraction and the tail current were both abolished, whereas the peak calcium current was not reduced.

4. Exposure of rat cells to ryanodine (1–2 μM) suppressed both contraction and the tail current, but not peak calcium current.

5. The tail current was unaffected by tetrodotoxin (10 μM), but was reduced by lowering extracellular sodium to 10% by replacement with lithium or choline.

6. In rat cells, exposure to nifedipine (1–5 μM) initially caused a marked reduction of calcium current while substantial contraction and tail current remained; longer exposure to nifedipine suppressed both contraction and the tail current. Isoprenaline (50–100 nM) caused a marked increase in peak calcium current, while under these conditions there was little or no increase in either contraction or tail current.

7. The amplitude of the tail current in rat cells varied with the duration of the depolarization at 0 mV; the tail current evoked by repolarization to −40 mV reached a peak just as contraction was beginning to develop and was back to undetectable levels just as relaxation became significant, as might be expected if the tail current were determined by the cytosolic calcium transient which triggered contraction.

8. In guinea-pig cells, a tail current was also recorded on repolarization to a holding potential of −40 mV, and, as in rat cells, the tail was suppressed by cytosolic EGTA and reduced by exposure of the cells to low-sodium solution.

9. It is concluded that the tail currents recorded in both rat and guinea-pig cells represent current activated by a rise in cytosolic calcium; in rat cells this is markedly

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dependent on ryanodine-sensitive release of calcium from internal stores. The origin of this current, and its possible role during the plateaux of action potentials are discussed.

INTRODUCTION

It has been recognized for some time that calcium ions play a crucial role in maintenance of the plateau of the cardiac action potential (Niedergerke & Orkand, 1966; Beeler & Reuter, 1970). In recent years, it has become apparent that a number of currents may contribute to the plateau and, hence, to the action potential time course (for details and computer model see DiFrancesco & Noble, 1985). Recordings of the calcium current in caesium-loaded Purkinje fibres (Marban & Tsien, 1982) and in isolated cardiac myocytes (see, e.g. Isenberg & Klockner, 1982; Lee & Tsien, 1982; Hume & Giles, 1983; Mitchell, Powell, Terrar & Twist, 1983; Matsuda & Noma, 1984) have indicated that this current is rapidly activated, the peak occurring a few milliseconds after stimulation, i.e. early in the plateau, rather than in tens of milliseconds as previously thought (see Noble, 1984). In addition to this fast calcium current, it has been suggested that a smaller, slowly inactivating calcium current is present during the plateau (Lee, Noble, Lee & Spindler, 1984). Action potential time course is modified by potassium currents, i.e. the transient outward current (Mitchell, Powell, Terrar & Twist, 1984*a*), the background rectifying current (Hume & Uehara, 1985) and the time-dependent late current (Kass & Wiegers, 1982; Mitchell, Powell, Terrar & Twist 1984*c*). In addition it has been suggested that a persistent sodium 'window' current is present throughout the plateau (Colatsky, 1982).

In rat ventricular cells, it appears that current carried by calcium plays a role in the early stages of the ventricular action potential; it has been suggested that the appearance of a distinct late component of the plateau at negative potentials is associated with an additional inward current activated by calcium, and that this current is secondarily dependent on current carried by calcium which in turn releases further calcium from internal stores (Mitchell, Powell, Terrar & Twist, 1984*b*). It has also been suggested that such an additional inward current activated by cytosolic calcium may play a role in action potentials from other species such as guinea-pig which do not show an obvious discontinuity in their plateaux phases (Mitchell *et al.* 1984*b*). The inward current was thought to be carried at least in part by sodium, and two possibilities (which are not mutually exclusive) are electrogenic sodium–calcium exchange (Kimura, Noma & Irisawa, 1986; Mechmann & Pott, 1986) and non-selective ion channels activated by cytosolic calcium (Colquhoun, Neher, Reuter & Stevens, 1981; Mechmann & Pott, 1986). Evidence for the role of such currents during the plateau of action potentials was provided by the observations that the late plateau in rat cells was suppressed by loading with cytosolic EGTA, by ryanodine which is thought to interfere with calcium release from internal stores, and by reducing extracellular sodium to low levels; in guinea-pig cells the plateau was shortened by injection of EGTA into the cytosol and by reducing extracellular sodium (Mitchell *et al.* 1984*b*).

The purpose of the experiments reported in the present paper was to investigate whether ionic currents suppressed by similar procedures could be recorded from rat and guinea-pig cells. Currents with these properties could be distinguished from

current carried by calcium by applying depolarizing pulses which were long enough to initiate contraction, and thus presumably induced a rise in cytosolic calcium, but which were sufficiently brief to show the presumably calcium-activated current as a slow inward tail on repolarization, whereas current carried by calcium would be expected to deactivate rapidly. Preliminary observations of these tail currents have been presented to the Physiological Society (Mitchell, Powell, Terrar & Twist, 1984*d*), and to IUPS (Mitchell, Powell, Terrar & Twist, 1986). The similarity of such currents with oscillating transient inward currents under conditions of 'calcium-overload' has been discussed by Mitchell, Powell, Terrar & Twist (1985).

METHODS

Methods were similar to those described in the accompanying paper (Mitchell, Powell, Terrar & Twist, 1987). Single ventricular cells were isolated from rat and guinea-pig hearts and superfused with warmed oxygenated solution. Electrical activity was recorded under voltage-clamp conditions, using a single-electrode voltage-clamp system (Dagan 8100 or Axoclamp 2). Contraction was monitored by an optical system, using a photodiode mounted in the eyepiece of the microscope. In all voltage-clamp records reported in this paper the holding current at −40 mV was outward and less than 0·5 nA. Ryanodine was a gift from Merck, Sharp & Dohme.

RESULTS

In rat ventricular cells, depolarization from a holding potential of −40 to 0 mV results in activation of the calcium current. This current has been shown to be maximal 2·5 ms after the step at 37 °C (Mitchell *et al.* 1983), and rapidly decays to a steady-state outward current within a 100 ms step depolarization. However, during the early plateau phase of the rat ventricular action potential, the membrane potential remains at 0 mV for only a few milliseconds (Watanabe, Delbridge, Bustamante & McDonald, 1983; Mitchell *et al.* 1984*a*), the longer later phase occurring at a more hyperpolarized potential. Thus, during voltage-clamp experiments, the effect of a short depolarization was investigated.

Figure 1*A* illustrates the currents evoked by a step depolarization from −40 to 0 mV and by repolarization after only 5 ms. During the depolarizing step, the calcium current was activated and partially decayed after reaching a peak. In addition, the short pulse was sufficient to initiate contraction of the cell. On repolarization to −40 mV, the fast capacity transient was observed to be followed by a slow tail of inward current. It is unlikely that this tail represents deactivation of the calcium current itself, as such a process would be expected to be rapid at this potential and to fuse with the capacity current. Indeed it will be illustrated later that the slow tail current can be abolished while the calcium current remains.

As the tail had a very slow time course in contrast to the rapid inactivation of the calcium current, it seemed possible that this tail might be related to the additional inward current postulated to underlie the late phase of the rat ventricular action potential and to be dependent on intracellular calcium and extracellular sodium (Mitchell *et al.* 1984*b*; Schouten & ter Keurs, 1985). Thus a series of experiments were performed to investigate the tail current and its ionic dependence.

Effect of cytosolic EGTA

The late phase of the action potential in rat cells is suppressed by cytosolic EGTA which has diffused from an intracellular electrode (Mitchell *et al.* 1984*b*). In voltage-clamped cells impaled with electrodes containing 0·5–1·0 M-EGTA, the contraction which normally accompanies step depolarizations was abolished. It can be seen from Fig. 1*B* that under these conditions the tail current following repolarization was also

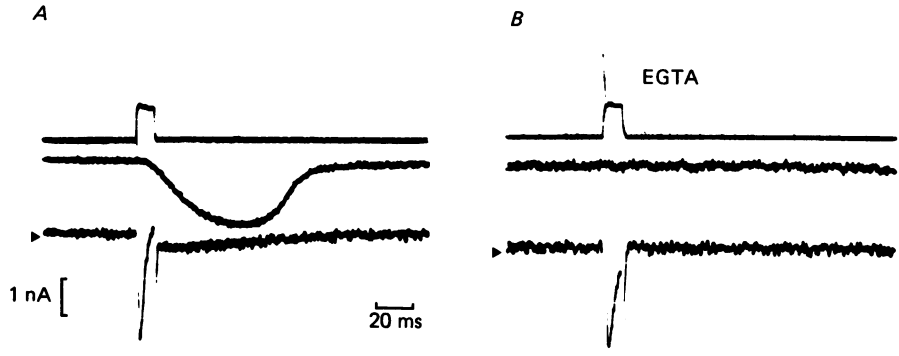

Fig. 1. The tail current and its suppression by internal EGTA. On depolarization from −40 to 0 mV (top trace) in *A*, current carried predominantly by calcium was activated (bottom trace) and this was accompanied by contraction (middle trace). On repolarization after 10 ms, there followed a long, slow tail of inward current. In *B* is illustrated the effect of impaling a cell with an electrode containing 1 M-EGTA. Here, the depolarization evoked calcium current as before but there was no contraction of the cell and no tail current was observed. 36 °C. Stimulation rate 0·3 Hz. The holding current at −40 mV was outward, and zero current is indicated by ▶.

abolished, whereas during the step depolarization the inward current carried by calcium persisted. In a few experiments, cells which had been impaled with EGTA-containing electrodes remained in place on the bottom of the superfusion chamber after withdrawal of the EGTA-containing electrode, and could be impaled again with a conventional electrode without EGTA in the filling solution; in these cases the suppression of both contraction and of tail currents associated with brief depolarizing pulses persisted, demonstrating that responses obtained with EGTA-containing electrodes do not result from any artifacts arising from abnormal filling solutions.

Effect of ryanodine

Figure 2 shows currents and contractions accompanying 5 ms depolarizations from −40 to 0 mV in the absence (Fig. 2*A*) and presence (Fig. 2*B*) of ryanodine (2 μM, applied for 3 min). Ryanodine suppressed both contraction and the tail current following repolarization without reducing peak inward current during the depolarization; indeed ryanodine (like injection of EGTA into the cytosol) slows inactivation of this current in rat cells (Mitchell, Powell, Terrar & Twist, 1984*e*, 1985). Thus, again the tail current shows a sensitivity to a procedure which suppresses the late plateau of action potentials in rat ventricular cells.

Effects of a reduction of extracellular sodium and of tetrodotoxin

Since the late plateau of rat cells is also suppressed by reduction of extracellular sodium, it was of interest to examine the effect of a similar reduction on the tail current following a step depolarization in a voltage-clamped cell. Figure 3 illustrates an experiment of this kind. In this case lithium ions were substituted for 90% of the

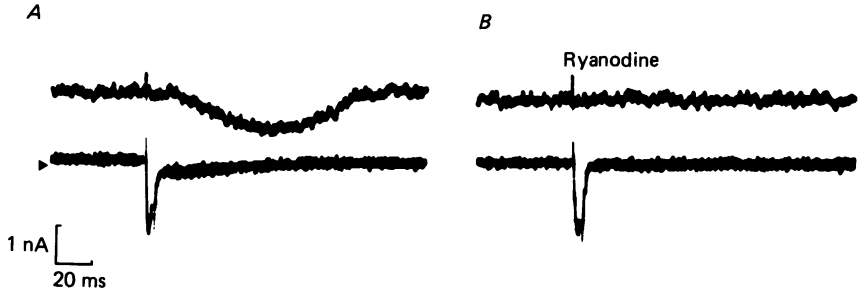

Fig. 2. Effect of ryanodine on the tail current. *A* illustrates the tail current after a 5 ms depolarization from −40 to 0 mv and the contraction activated by the step. After 3 min exposure to ryanodine (2 μM), the second inward current (I_{si}) was activated during the depolarization but the contraction and the tail current were suppressed (*B*). 36 °C. Stimulation rate 0·3 Hz. The holding current at −40 mV was outward, and zero current is indicated by ▶.

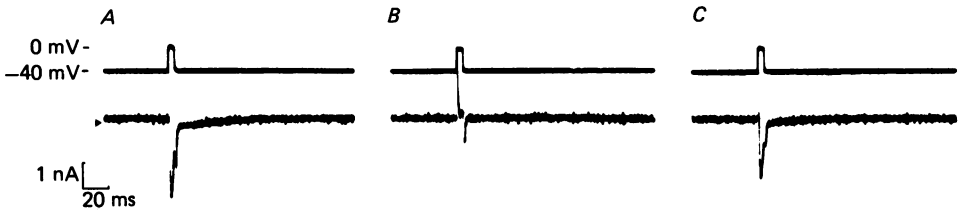

Fig. 3. Effects of reducing the extracellular sodium ion concentration on the tail current. Before (*A*), 45 s in low-sodium solution (*B*) and after 60 s recovery in normal solution (*C*). 90% of the sodium ion concentration in *B* was replaced by lithium ions. 36 °C. The holding current at −40 mV was outward, and zero current is indicated by ▶.

sodium. It can be seen that the tail current following repolarization was indeed suppressed by the reduction of extracellular sodium. However, the interpretation of this experiment is less straightforward than of the experiments described above, because the peak inward current was also reduced. (Note that calcium current is usually measured from the steady current at the end of a long (e.g. 100 ms) step at the depolarized level (see Mitchell *et al.* 1983) and the reduced current may still be inward relative to this although outward relative to the holding current at −40 mV.) Thus, the reduction in tail current may have been caused directly by the reduction in extracellular sodium, or might perhaps be a secondary consequence of the fall in amplitude of the peak inward current and modification of changes in the concentration of cytosolic calcium which normally accompany the calcium current. These points will be taken up again in the Discussion, but one explanation for the effect of

low extracellular sodium on current carried by calcium is that the resting level of cytosolic calcium is increased in low-sodium solution and this leads to calcium-induced inactivation of calcium currents. Thus, second inward currents in rat cells are not reduced by extracellular sodium when strontium is substituted for calcium in the external solution (Mitchell *et al.* 1983). Also in keeping with this interpretation, we have found that injection of EGTA to buffer cytosolic calcium at a low level reduces the effect of low extracellular sodium in causing a fall in amplitude of second inward current during a step depolarization, but since the tail current is also suppressed by cytosolic EGTA the influence of low sodium on tail currents cannot be examined under these conditions.

When tetrodotoxin (10 μM) was added to the solution bathing the cells, no change in the tail current was detected.

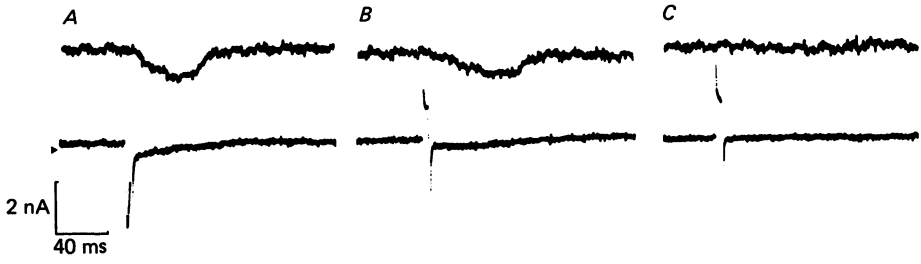

Fig. 4. Inhibition of the second inward current (I_{si}), contraction and tail current by nifedipine. Before (*A*), 90 s (*B*) and 3 min (*C*) exposure to 2 μM-nifedipine. 36 °C. Stimulation rate 0·3 Hz. The holding current at −40 mV was outward, and zero current is indicated by ▶.

Influence of nifedipine

Calcium influx blockers such as nifedipine are known to be selective in their effects in reducing currents carried by calcium (Lee & Tsien, 1983). In the case of the additional inward currents activated by cytosolic calcium described above, it would be expected that abolition of the current carried by calcium would lead to an abolition of the associated rise in cytosolic calcium and secondarily to a suppression of calcium-activated currents. However, the relationship between amplitude of inward current and contraction in rat cells is markedly non-linear for step depolarizations to 0 mV (see accompanying paper), and it is possible to block a substantial fraction of peak inward current during the depolarizations while still triggering a significant contraction. This point is illustrated in Fig. 4. In the absence of blocking drug (Fig. 4*A*), the step depolarization evoked the normal peak inward current during the pulse, together with contraction and a tail current following repolarization. During the onset of the effect of nifedipine (2 μM) applied in the solution flowing over the cell, there was a substantial reduction of peak inward current, but a large contraction was still recorded (interestingly with a slower rate of development) and this was associated with a significant tail current (Fig. 4*B*). After a longer exposure to nifedipine (3 min), contraction was suppressed, presumably as residual calcium current was suppressed, and at this time the tail on repolarization was no longer detectable (Fig. 4*C*). These observations are consistent with the suggestion that the tail current

reflects inward current activated by a rise in cytosolic calcium, and that provided sufficient calcium current remains unblocked by nifedipine to trigger release of calcium from internal stores there will be a significant rise in cytosolic calcium to activate both contraction and the tail current.

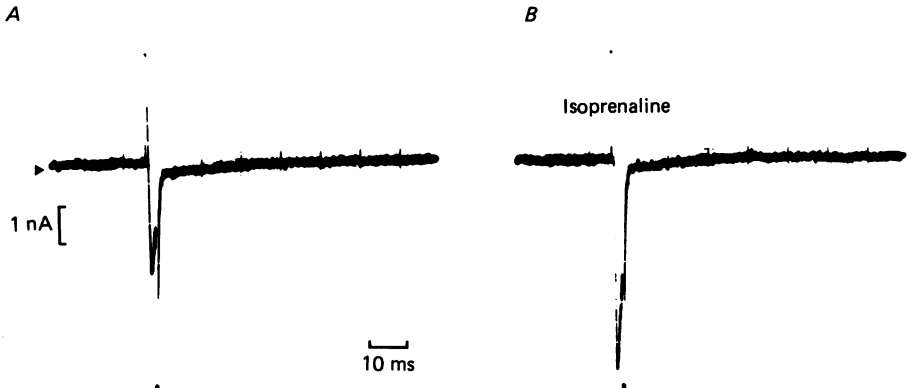

Fig. 5. Effects of isoprenaline (100 nM) on the tail current. A brief step depolarization from −40 to 0 mV was applied before (*A*) and in the presence of isoprenaline (*B*, 60 s exposure). The peak calcium current but not the tail current was increased by isoprenaline. 36 °C. Stimulation rate 0·3 Hz. The holding current at −40 mV was outward, and zero current is indicated by ▶.

The effect of isoprenaline and noradrenaline

A differential effect on the amplitudes of the calcium current and the tail current also occurred with isoprenaline. A substantial increase in peak calcium current was observed after 60 s exposure to 50–100 nM-isoprenaline (Fig. 5), with little or no change in the amplitude of the tail. Under these conditions (holding potential of −40 mV, 2·5 mM-extracellular calcium), there was little or no change in contraction amplitude in the presence of isoprenaline. Similar observations were made with 500 nM-noradrenaline.

The time course of calcium-activated current during a step depolarization to 0 mV

An indication of the time course of the presumed calcium-activated current at 0 mV can be obtained by applying a series of step depolarizations of different durations, and estimating the size of the calcium-activated current at the end of a depolarization to 0 mV from the size of the inward tail immediately following repolarization; capacity current and residual calcium current impose limitations on this procedure, although they are expected to decay much more rapidly than the calcium-activated current, which was estimated from the amplitude of the current at the discontinuity between rapidly inactivating and slow tail currents. An experiment of this kind is illustrated in Fig. 6. When the duration of the depolarizing pulse was increased from 2 to 5 ms, as in Fig. 6*A* and *B* the amplitude of the tail increased; note that the amplitude of the calcium current at the end of the depolarization had already decreased as a result of inactivation at this pulse duration, indicating once more a separation in behaviour of this current from that of the proposed calcium-activated current. As the pulse duration was progressively increased in Fig. 6*C–E*, the tail

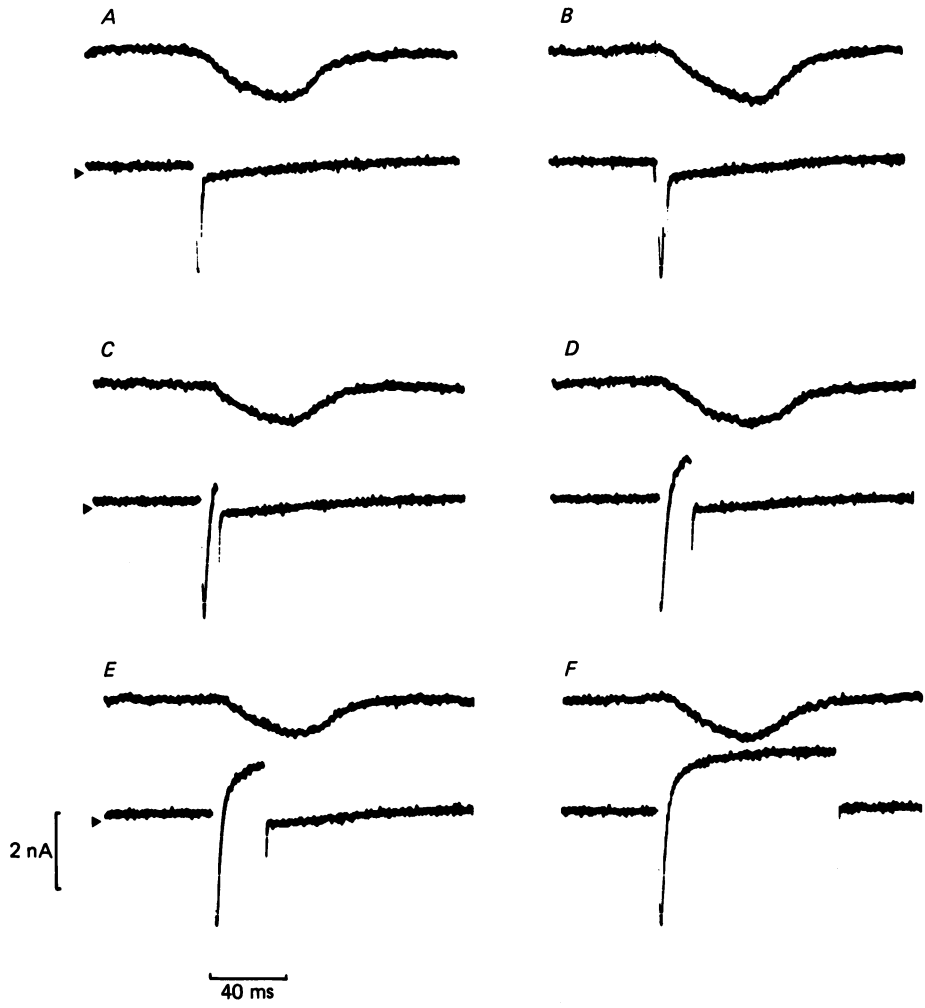

Fig. 6. Effect of depolarizing pulse duration on subsequent tail amplitude. In each of the six panels are shown the currents and contractions initiated by a step depolarization from −40 to 0 mV. The duration of the depolarization was increased from 2 (*A*) to 5 (*B*), 10 (*C*), 15 (*D*), 25 (*E*) and 95 ms (*F*). 36 °C. Stimulation rate 0·3 Hz. The holding current at −40 mV was outward, and zero current is indicated by ▶.

current was gradually reduced, until at 100 ms (Fig. 6*F*), when relaxation of contraction was complete, no slow tail current was detectable. Similar results from another cell are presented in a different form in Fig. 7. Estimated calcium-activated current at the end of the pulse is plotted as ordinate against duration of the pulse as abscissa; also shown (filled squares) is the time course of contraction in the same cell (maximum amplitude scaled to match peak tail current). As described above, it can be seen that the estimated calcium-activated current reached a peak just as contraction was beginning to develop, and the current was back to undetectable levels just as relaxation became significant.

How does the calcium-activated tail current vary with membrane potential?

It would be of great interest to investigate whether or not the tail current shows a significant variation with the membrane potential on repolarization after a brief step depolarization to 0 mV. However, such an investigation is hampered by additional currents both when the membrane is repolarized from 0 mV to a potential less negative than −40 mV (in which case residual calcium current appears) and when

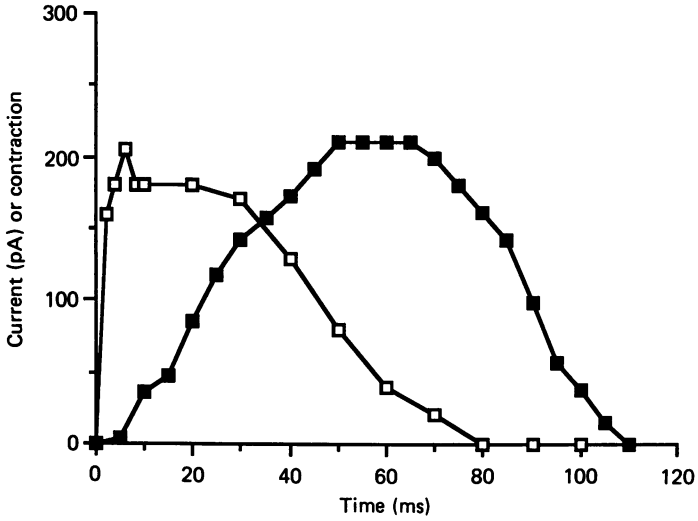

Fig. 7. Amplitude of tail current (open squares) and of contraction (filled squares) plotted as a function of time after the start of a step depolarization from −40 to 0 mV. Tail current amplitude was measured from a series of records with pulses of different duration as shown in Fig. 6 (see text).

repolarization is to a more negative potential than −40 mV. Thus, when the membrane potential was hyperpolarized from −40 to −80 mV, as in Fig. 8*A* (later part of trace at right), a 'tail' current was recorded; evidence will be presented that this 'tail' differs from the current which has been described up to this point and which is the main subject of this paper, but such an additional current would be expected to add to the complexity of the analysis at more hyperpolarized levels. It should be emphasized that in all previous records in this paper of the tail current which is thought to be activated by a rise in cytosolic calcium, repolarization was to −40 mV, and it will be recalled that a tail current was not recorded when repolarization from 0 to −40 mV was delayed until after contraction was complete (e.g. Fig. 6*F*). The 'tail' on hyperpolarization from −40 to −80 mV in Fig. 8*A*, unlike that following the previously described protocol of brief depolarization to 0 mV followed by repolarization to −40 mV, was not accompanied by a contraction observed by microscopic inspection. Following exposure to 3 μM-ryanodine for 3 min, both contraction accompanying a brief depolarization to 0 mV and the tail current on repolarization from 0 to −40 mV were suppressed to undetectable levels, but the 'tail' on hyperpolarization from −40 to −80 mV was not reduced (Fig. 8*B*); this was taken to support the hypothesis that the tail which in the absence of ryanodine was associated

with the contraction triggered by brief depolarization to 0 mV was activated by cytosolic calcium (as in Fig. 2), but that the 'tail' accompanying hyperpolarization from −40 to −80 mV arose from a different ryanodine-insensitive mechanism. Additional evidence that the 'tail' on hyperpolarization from −40 to −80 mV differed from the postulated calcium-activated tail current was provided by experiments in cells loaded with EGTA from an intracellular electrode, to buffer cytosolic calcium at a low level; in these cells contraction and the tail on repolarization to −40 mV after a brief depolarization to 0 mV were both suppressed, but the 'tail' on hyperpolarization from −40 to −80 mV was still recorded.

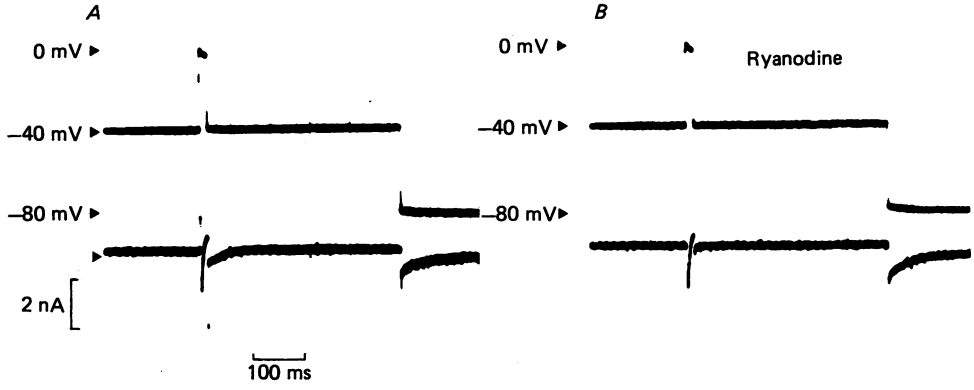

Fig. 8. Effect of ryanodine on the tail current, and on a 'tail' current on hyperpolarization. In *A*, the upper trace shows the membrane potential; a brief step depolarization from −40 to 0 and back to −40 mV was applied followed after a delay of approximately 500 ms by a hyperpolarization to −80 mV. The lower trace shows current: repolarization to −40 mV following the depolarization evoked the tail current which has been the subject of the paper up to this point; hyperpolarization to −80 mV also evoked a 'tail'. In *B*, recorded after more than 3 min exposure to 3 μM-ryanodine which suppressed contraction, it is seen that the tail current on repolarization to −40 mV was suppressed by ryanodine, but that the 'tail' on hyperpolarization was not reduced. 36 °C. Stimulation rate 0·3 Hz. The holding current at −40 mV was outward, and zero current is indicated by ▶.

If the 'tail' on hyperpolarization from −40 to −80 mV represents a separate, independent current mechanism, then subtraction of this component from the current on repolarization from 0 to −80 mV after a brief depolarization to 0 mV might be expected to leave the postulated calcium-activated current at −80 mV. An experiment of this kind is illustrated in Fig. 9. The currents on repolarization from 0 mV to a series of potentials (−40, −50, −60, −70 and −80 mV) are shown in Fig. 9*A*. The currents accompanying hyperpolarizations alone from −40 to −50, −60, −70 and −80 mV are shown in Fig. 9*B*. When the 'tail' accompanying hyperpolarization alone was subtracted from that at the same potential following the depolarizing pulse, the difference showed little or no increase with membrane potential (note that there are four records in Fig. 9*B* and five in 9*A*, since subtraction was not required for the first record from −40 to 0 and back to −40 mV).

The charge accumulation in the cell accompanying the inward tail current can be estimated from the area under the curve. This was done for the net current determined by subtraction described above for different potentials; in the experiment

shown in Fig. 9 the areas were (in arbitrary units): 24 at −40 mV; 22 at 50 mV; 23 at −60 mV; 23 at −70 mV; and 27 at −80 mV. Similar results were obtained in two other cells, with no substantial increase in the estimated charge accumulation associated with tail currents as the membrane potential following depolarization was made more negative in the range −40 to −80 mV.

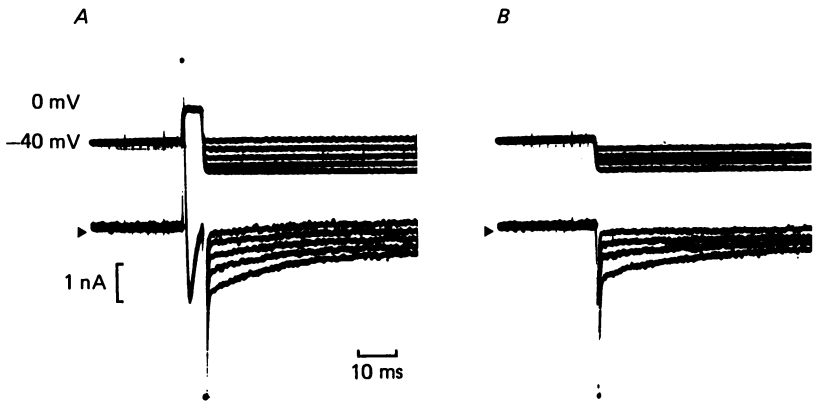

Fig. 9. Effect of membrane potential on the tail current. In *A*, the upper set of records shows superimposed traces of membrane potential for a sequence of step depolarizations from −40 to 0 mV, with repolarization to potentials between −40 and −80 mV; the corresponding currents are shown in the lower set of traces. Membrane potential (upper traces) and current (lower traces) are also shown (*B*) for hyperpolarizations alone to the potentials after the depolarizing steps in *A*. 36 °C. Stimulation rate 0·3 Hz. The holding current at −40 mV was outward, and zero current is indicated by ▶.

Calcium-activated currents recorded from guinea-pig cells

It was argued above and in an earlier paper (Mitchell *et al.* 1984*b*) that calcium-activated currents may play a role during the plateau of ventricular cells even when an obvious discontinuity in the plateau, such as that seen in rat cells, is absent. Such a possibility in guinea-pig cells may be examined by using the procedure of brief depolarizing pulses described above in relation to rat cells. An experiment of this kind is illustrated in Fig. 10. It can be seen that a 10 ms step depolarization from −40 to 0 mV evoked a submaximal but significant contraction which continued to develop after the end of the depolarization. On repolarization, as in rat cells, there was a tail of inward current which lasted much longer than the expected decays of capacity current and of residual current carried by calcium. When the pulse duration was increased to 60 ms, as in Fig. 10*B*, the contraction was slightly larger, and a significant tail current was recorded, but the tail was smaller in amplitude perhaps indicating that the cytosolic calcium transient was already beginning to fall at this time. At 200 ms, contraction was maximal for a step to 0 mV, and partial relaxation had occurred before the end of the pulse; a very small tail was recorded under these conditions. Thus, tail currents were recorded in guinea-pig cells which appeared to share some features of the tail currents described in rat cells. If the tail currents in guinea-pig cells reflected calcium-activated current, as was suggested for rat cells, they should be suppressed by loading with EGTA from a microelectrode. An ex-

periment to test this possibility is illustrated in Fig. 10 *D–E*, where it can be seen that cytosolic EGTA suppressed both contraction and tail currents. Another point illustrated in this Figure is that inactivation of current during the step depolarization appeared to be slower consistent with attenuation of calcium-induced inactivation when cytosolic calcium was buffered at low levels by cytosolic EGTA.

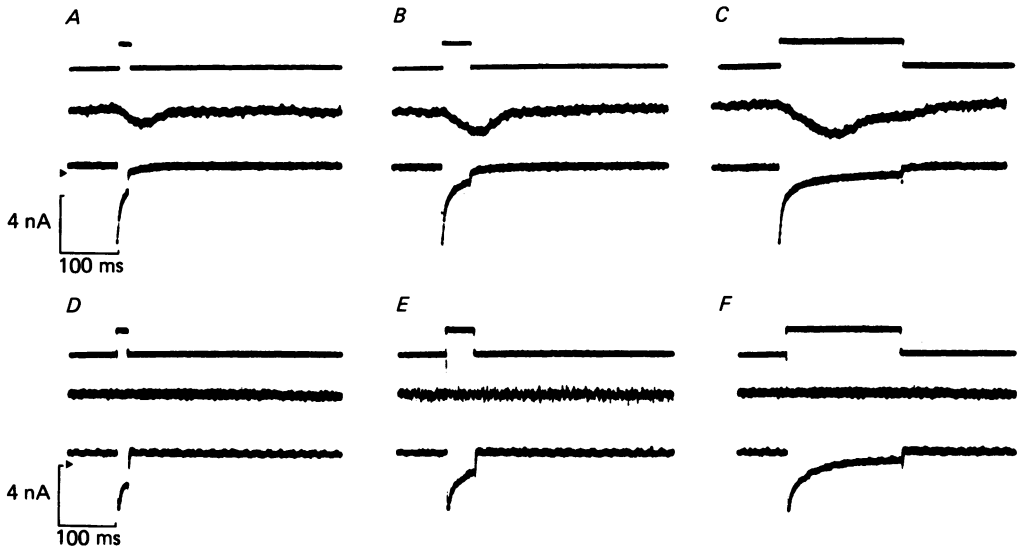

Fig. 10. The second inward current (I_{si}), contraction and tail current in guinea-pig cells and the effect of intracellular EGTA. Guinea-pig cells were voltage clamped at −40 mV and depolarizing steps to 0 mV applied. The top panel (*A*, *B* and *C*) shows the effect of different pulse durations on contractions (centre traces) and currents (lower traces). The lower panel (*D*, *E* and *F*) shows a similar voltage-clamp protocol applied to a cell impaled with an electrode containing 1 M-EGTA. 36 °C. Stimulation rate 0·3 Hz. The holding current at −40 mV was outward, and zero current is indicated by ▶.

As in rat cells, the tail current recorded from guinea-pig cells was reduced when extracellular sodium was reduced to 10% of normal by substitution of lithium or choline for sodium.

DISCUSSION

The main finding of this paper is that an inward current which is sensitive to cytosolic EGTA, ryanodine and reduced extracellular sodium, but not to tetrodotoxin, could be recorded from rat ventricular cells; these properties, together with its slow time course, are those expected of a current contributing to the late plateau of rat cells (Mitchell *et al.* 1984*b*; Schouten & ter Keurs, 1985). The current appeared as an inward tail on repolarization after a brief depolarization which evokes the calcium current and contraction. Its sensitivity to cytosolic EGTA (which buffers calcium at a low level) and to ryanodine (which interferes with release of calcium from the sarcoplasmic reticulum) is consistent with the hypothesis that this tail current is activated by a rise in cytosolic calcium.

It is clear from the experiments with cytosolic EGTA and with ryanodine that the tail current differed from the fast inward calcium current. Under these conditions, the calcium current was still present while the tail was abolished and it appeared that any tail current arising from inactivation of calcium current was very fast and indistinguishable from the capacity transient on repolarization. Further support for this is provided by the experiments with nifedipine and isoprenaline. In the case of nifedipine, an initial marked fall in calcium current was accompanied by a much smaller reduction in tail current (and contraction). With isoprenaline, a large increase of calcium current occurred with little or no increase in the amplitude of the tail current. The lack of increase of tail current may correspond with the observed lack of increase in contraction amplitude under these conditions, where contraction appeared to be already maximal, and Fabiato (1981) has found a similar lack of increase in contraction of rat cells exposed to catecholamines in 2·5 mM-extracellular calcium.

The sensitivity of the tail current to reduced extracellular sodium is difficult to interpret because current carried by calcium is also reduced in low-sodium solution in rat ventricular cells (see Mitchell *et al.* 1983), and thus the reduction in tail current could be a secondary consequence of the fall in calcium current. A reduction of the calcium current on exposure to low sodium may be interpreted in terms of a calcium-induced inactivation of calcium current, secondary to a rise in cytosolic calcium (Lee, Marban & Tsien, 1985). Since the tail current is thought to arise from different mechanisms from the calcium current, the sensitivity of the tail to reduced extracellular sodium could arise if the tail current were carried partly by sodium ions. Two possible currents which could account for this are (a) a calcium-activated current carried by electrogenic sodium–calcium exchange (Mullins, 1979, 1981; Kimura *et al.* 1986; Mechmann & Pott, 1986) and (b) current through non-selective cation channels (Colquhoun *et al.* 1981). Current components with these characteristics have been separated under patch-clamp conditions in atrial cell myoballs by Mechmann & Pott (1986), and it appears that the concentration of calcium to activate electrogenic sodium–calcium exchange is lower than that to activate non-selective channels (Colquhoun *et al.* 1981; Mechmann & Pott, 1986). In our experiments with reduced extracellular sodium, the fall in tail current when lithium ions are used as a sodium substitute might be taken as evidence in support of electrogenic sodium–calcium exchange as lithium might be expected to pass through ionic sodium channels, but the fall in calcium current mentioned above makes this conclusion less certain.

The tail current was observed under conditions where contraction occurred, but the time course of development of the tail current was a little different from that of contraction. This was pointed out in relation to Figs 6 and 7, where the development of tail current was assessed from the amplitude of tails following depolarizing pulses of progressively increasing duration. It appeared that the calcium-activated current reached a peak just as contraction was beginning to develop, and the current was back to undetectable levels while relaxation was occurring. Such observations would be expected if the tail current were determined by the cytosolic calcium transient which triggered contraction; indeed the time course of the tail current and its relation to contraction are approximately similar to those of aequorin transients in cardiac muscle (Allen & Blinks, 1978; Marban & Wier, 1985).

If the tail current is activated by a rise in cytosolic calcium, the source of the

calcium might be influx through the surface membrane carried by calcium current, or release of calcium from intracellular stores. In the steady state calcium entry must be balanced by calcium extrusion, but slow processes of calcium entry and extrusion may occur so that calcium entry during the calcium current need not necessarily match any calcium extruded during the tail current.

Whatever the immediate source of calcium to activate the tail current, the relation of this current with membrane potential is of interest. When the membrane potential during the tail was made more negative, the amplitude of the tail appeared to increase, as described by Mitchell *et al.* (1984*d*), but hyperpolarizing pulses alone to similar potentials also evoked a 'tail' current and evidence was presented that this 'tail' on hyperpolarization from −40 mV to more negative values arose from different mechanisms from the postulated calcium-activated tail which, unlike the 'tail' on hyperpolarization alone, was suppressed when contraction was attenuated by ryanodine or cytosolic EGTA. When the 'tail' on hyperpolarization alone was subtracted from that following a step depolarization (as in Fig. 9), there was little change in the calculated calcium-activated current over the range −40 to −80 mV; this might seem surprising if, for example, the current were electrogenic sodium–calcium exchange, but such behaviour over certain potential ranges can be accounted for by some models of sodium–calcium exchange (Eisner & Lederer, 1985).

If the charge associated with a hyperpolarization alone was subtracted from that associated with a hyperpolarization following a brief depolarization to evoke contraction, the net charge associated with the tail current was approximately constant over the range −40 to −80 mV. This observation is consistent with the hypothesis that the amount of calcium extruded remained approximately constant over this range of potentials, perhaps because the calcium transient which activates both contraction and the tail current remained approximately constant. If calcium-activated non-selective ion channels were to make a major contribution to the tail current, then for a fixed number of open channels the inward current, and consequently the accumulated charge, would be expected to increase as the membrane potential was hyperpolarized away from the reversal potential for the current. The mechanism underlying the 'tail' which appeared with hyperpolarizations alone remains for further investigation.

It has been argued that a calcium-activated current may occur in guinea-pig as well as rat cells (Mitchell *et al.* 1984*b*). Evidence for such a current was provided by experiments of the kind illustrated in Fig. 10. A tail current following brief depolarizing pulses could be observed, and as in rat cells, this current was abolished by cytosolic EGTA (Fig. 10*B*). Also as in rat cells, the tail current in guinea-pig cells was reduced by replacing 90% of the external sodium with lithium or choline, though once again the reduction of current carried by calcium leads to difficulties in interpretation of this experiment. Ryanodine does not suppress peak contraction in guinea-pig cells, and its effects were not investigated.

Thus, in both rat and guinea-pig cells a tail current appears to be activated by a rise in cytosolic calcium. In rat cells, this is likely to be associated with the late plateau. In guinea-pig cells, rectification of outward current during the plateau of the action potential is probably such that a delicate balance between inward and outward

currents maintains the plateau; several components of inward current carried by calcium, as well as residual sodium current and calcium-activated current may play a role.

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