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Strontium, nifedipine and 4-aminopyridine modify the time course of the action potential in cells from rat ventricular muscle

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1 Action potentials, initiated by brief depolarizing pulses, were recorded from single cells isolated from rat ventricular muscle. These action potentials showed a rapid upstroke to about +30 mV, followed by two phases of repolarization referred to as the early and late phases of the action potential.

2 Nifedipine (1 μM), which blocks the second inward current (I_{si}) carried by Ca in these cells, shortened the early phase.

3 Substitution of strontium for calcium in the solution bathing the cells, a procedure which prolongs I_{si}, prolonged the early phase.

4 4-Aminopyridine (1 mM), which inhibits transient outward current, prolonged the early phase with either calcium or strontium in the external solution.

5 It is concluded that both I_{si} and transient outward current contribute to the early phase of the action potential in rat ventricular muscle. It is also suggested that I_{si} does not directly contribute to the late phase, since the characteristics of the late phase are not compatible with such a role, and the possibility of additional inward current is investigated in the accompanying paper (Mitchell *et al.*, 1984).

Introduction

The action potentials of most mammalian ventricular muscle have a long plateau at positive potentials and the second inward current (I_{si}), which is mainly carried by calcium ions, is thought to play an important role in the maintenance of this plateau (cf. Reuter, 1973). However, in rat multicellular ventricular preparations (Aronson, 1980) and single ventricular cells (Powell *et al.*, 1980), the plateau shows two phases, the first being very brief and followed, at more negative potentials, by a prolonged phase. The separation of these components of repolarization in rat cells might be fortunate for the study of the underlying mechanisms, if this separation were to reflect a difference in the currents that contribute to the two phases. Recent investigations have shown that the kinetics of I_{si} are more rapid than previously reported (Mitchell *et al.*, 1983b). The present study on the early plateau phase of the action potential of isolated ventricular cells of the rat suggests that I_{si} contributes to this phase because pharmacologically induced changes in I_{si} produce corresponding changes in action potential time course. In addition, outward currents flowing during repolarization would be expected to modify the time course of the action potential. 4-Aminopyridine (4-AP), which inhibits a transient outward current in these cells (Mitchell *et al.*, 1983b), is shown to modify this phase of the action potential. Some of these results have been communicated to the Physiological Society (Mitchell *et al.*, 1983a)

Methods

Cells were isolated from ventricular muscle of adult Sprague-Dawley rats using collagenase as previously described (Powell *et al.*, 1980). The cells were held on the surface of agar gel in a perspex chamber to allow superfusion (8 ml min^{-1}) of solution (pH 7.4, 36–37°C), containing (mM): NaCl 118.5, $NaHCO_3$ 14.5, KCl 2.6, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgSO_4$ 1.2, glucose 11.1 and bovine serum albumin 5 mg ml^{-1} (Pentex Fraction V, Miles Laboratories). In experiments with strontium, $CaCl_2$ was replaced

by $SrCl_2$ (2.5 mM). Nifedipine and 4-aminopyridine (4-AP) were obtained from Sigma. Membrane potentials were recorded with glass microelectrodes (40–60 MΩ), filled with 3M KCl and linked to a preamplifier incorporating a bridge circuit (Model 8100, Dagan Corporation, Minneapolis). Changes in the composition of the superfusing solution sometimes caused slight changes in the membrane potential, and these were offset by applying a constant current through the recording electrode. This allowed comparison of action potentials elicited from the same initial potential and avoided difficulties in interpretation of results arising from differences in the time course of action potentials which have been stimulated from different resting levels. The cells were initially impaled in solution containing 5 mM calcium, which improved sealing in of the electrodes and allowed attainment of stable membrane potentials (−75 to −80 mV) within 90 s. These potentials remained stable on decreasing the external calcium concentration to 2.5 mM (Powell *et al.*, 1980). The cells were stimulated by applying short (0.5–1 ms) depolarizing pulses (1 Hz) through the recording electrode. Data was recorded on an FM tape recorder (Racal Store 4 at 15 in s^{-1}, 1 db down at 5 kHz) and displayed on a Gould Digital Storage Oscilloscope (OS4020).

Results

Time course of the rat action potential

The action potential shown in Figure 1 illustrates the time course generally seen in rat ventricular cells. The time course of the first 20 ms of this action potential is shown more clearly on the right at a faster sweep speed. On stimulation from a resting potential of −80 mV, there is a rapid upstroke to +25 mV,

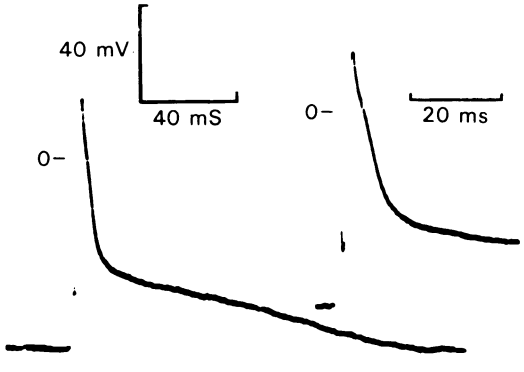

Figure 1 Time course of the rat ventricular action potential. Inset (right) shows the early time course more clearly at a faster sweep speed.

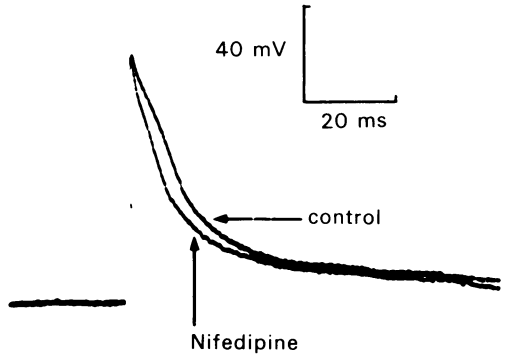

Figure 2 Reduction of the duration of the early plateau phase of the rat ventricular action potential by nifedipine. The record in nifedipine (10^{-6} M) was taken 60 s after exposure of the cells to the drug.

followed by a rapid repolarization to approximately −50 mV and a long slow repolarization to the resting potential. The latter two phases will be referred to as the early and late phases of the rat ventricular action potential.

Effect of nifedipine on the action potential

An inward current carried largely by calcium ions, and referred to as the second inward current or I_{si}, has been recorded from rat ventricular cells (Powell *et al.*, 1981; Mitchell *et al.*, 1983b). This current is activated at potentials more positive than −40 mV, and has a current-voltage curve and rapid kinetics appropriate for a role during the early phase of the action potential in these cells (Mitchell *et al.*, 1983b). The possible contribution of I_{si} during the early phase was examined by exposing the cells to 1 μM nifedipine, a calcium channel blocker which abolishes I_{si} in rat ventricular cells (Mitchell *et al.*, 1983b). Figure 2 shows that when nifedipine was applied in the external solution there was a shortening of the early phase of the action potential.

Effect of strontium on the action potential

Strontium is known to be able to substitute for calcium as a charge carrier in Purkinje fibres (Vereecke & Carmeliet, 1971) and ventricular muscle (Kohlhardt *et al.*, 1973). In rat ventricular cells strontium prolongs the second inward current (Mitchell *et al.*, 1983a,b) and, as the experiment with nifedipine above indicated that I_{si} might be important in the early phase of the action potential, it was of interest to discover what changes in action potential time course would be caused by strontium.

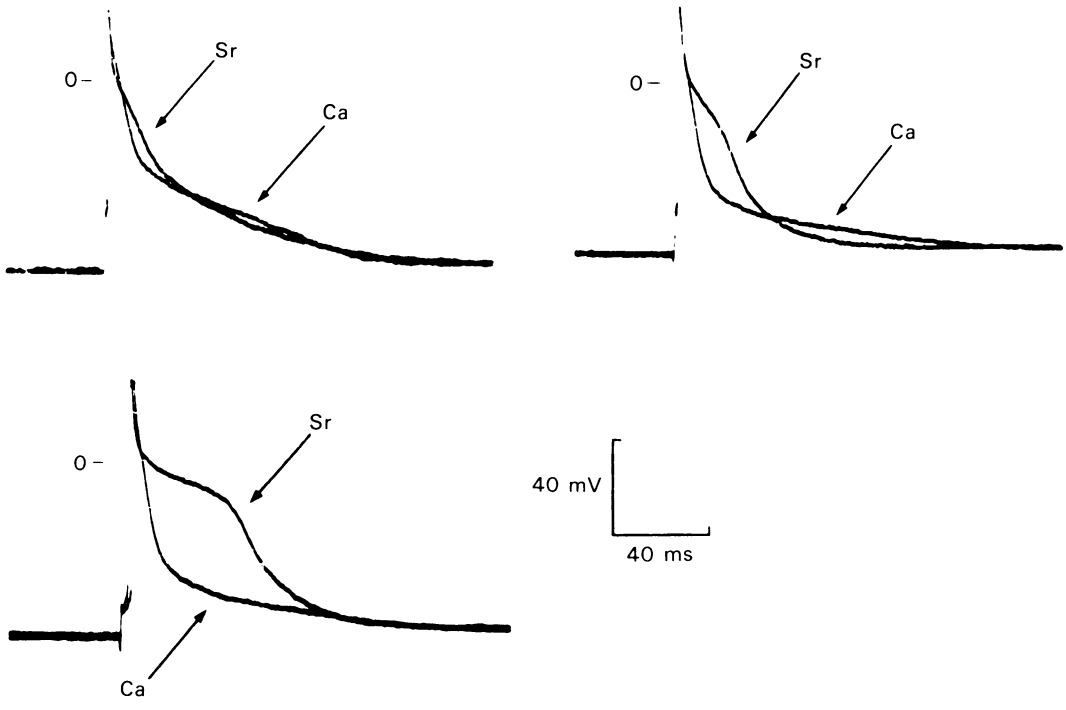

Figure 3 Effect of strontium substitution for calcium on the time course of the rat ventricular action potential. Records are from three different cells and the traces of the action potentials in strontium were taken 60 s after substitution of Ca^{2+} by Sr^{2+}.

The effect of strontium ions on the action potential is illustrated in Figure 3 where records from three different cells are shown. In each case the action potential obtained in the presence of strontium has been superimposed on that obtained in calcium for ease of comparison. It can be seen that strontium has a consistent effect in that it prolongs the early phase of the plateau. The effect of strontium on the late phase was variable and it was not possible to ascertain whether strontium had a specific effect on the late phase as the time course of this phase may itself be affected by the changes in the early phase.

The effect of strontium was also investigated in experiments where the external potassium ion concentration in the superfusing solutions was either higher or lower than the normal concentration of 3.8 mM. It will be shown in the following paper that a decrease in external potassium (to 1.2 mM) enhances the late phase of the plateau, while an increase of external potassium (to 12 mM) diminishes the late plateau. In cells bathed in low potassium, strontium had little effect on the late phase but increased the duration of the early phase (Figure 4a). In the presence of 12 mM potassium there was not an obvious late phase and the early phase was again enhanced by strontium (Figure 4b).

Action potential time course and 4-aminopyridine

The substitution of strontium for calcium, in addition to prolonging I_{si}, causes a reduction of transient outward current in Purkinje fibres (Siegelbaum & Tsien, 1980). Such an effect might contribute to the prolongation of the action potential by strontium in Figures 3 and 4. In order to investigate the possible contribution of transient outward current to the time course of repolarization, both in normal calcium and in strontium containing solution, the effects of 4-AP were examined; this compound inhibits transient outward current both in Purkinje fibres (Kenyon & Gibbons, 1978) and in rat ventricular cells (Mitchell *et al.*, 1983b).

4-AP prolongs the early phase of the action potential in rat cells when the divalent cation in the external solution is calcium (Figure 5a). Prolongation of the early phase by 4-AP also occurred when strontium was substituted for calcium (Figure 5b). The effect of 4-AP is very rapid with either cation, appearing within 5 s of exposure to the drug. Although longer exposures (1–5 min) of the cells to strontium sometimes prolong the first phase further, this is a slow effect compared to that of 4-AP. Thus, even though strontium is known to inhibit outward currents

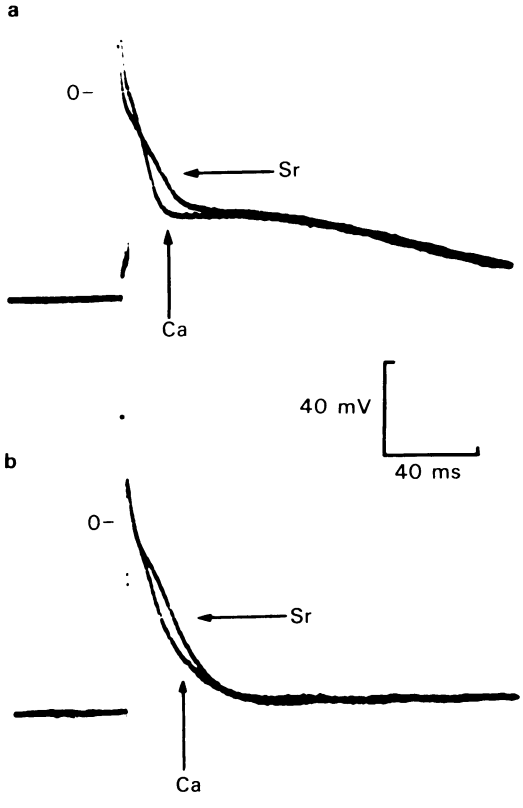

Figure 4 Effect of strontium on the action potentials in low and high external K concentrations. In the upper records the $[K^+]_o$ was 1.2 mM and in the lower records $[K^+]_o$ was 12 mM. The action potentials in Sr^{2+} were taken 60 s after substitution of Ca^{2+} by Sr^{2+}.

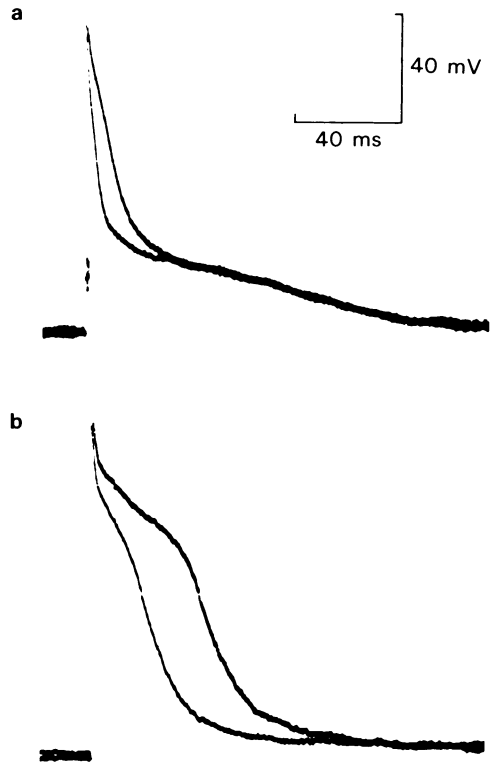

Figure 5 Effect of 4-aminopyridine (4-AP) on action potential time course. In both Ca^{2+} (a) and Sr^{2+} (b) 4-AP (1 mM) prolonged the early plateau phase. The action potentials in 4-AP were photographed at 60 s (a) and 30 s (b) after exposure to this drug. The cell in (b) was exposed to Sr for 60 s before addition of 4-AP.

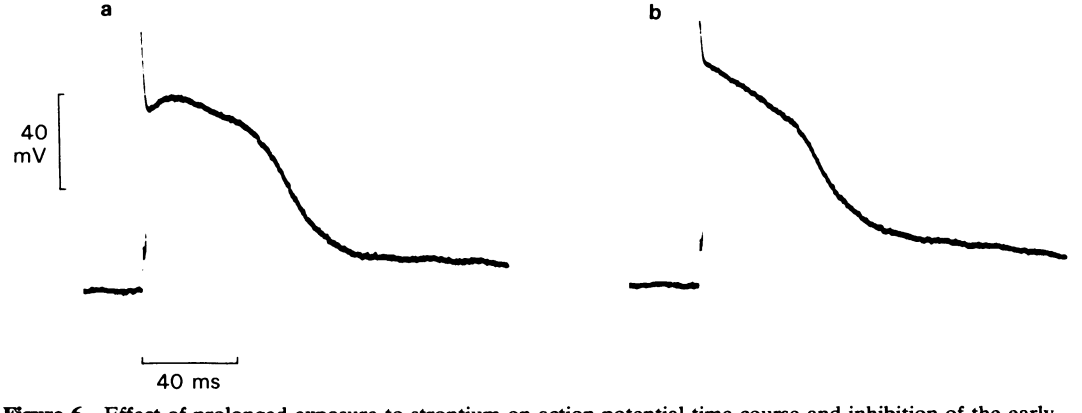

Figure 6 Effect of prolonged exposure to strontium on action potential time course and inhibition of the early repolarization by 4-aminopyridine (4-AP): (a) shows an action potential recorded after 3 min exposure of the cell to Sr^{2+}. The early 'notch' is rapidly abolished in 4-AP (1 mM) as shown by the action potential recorded after 30 s in 4-AP (b).

(Siegelbaum & Tsien, 1980; Coraboeuf & Carmeliet, 1982), the prolongation by 4-AP suggests that substantial outward current remains in the presence of strontium.

In some cells after 3–5 min exposure to strontium, a secondary depolarization appeared producing a 'notch', and the action potential was greatly prolonged as illustrated in Figure 6a. In these cells, 4-AP was found to reduce the extent of the initial fast repolarization (Figure 6b), thus abolishing the notch. This suggests that there is an outward current which is sensitive to 4-AP, and which continues to influence repolarization even following prolonged exposure to strontium.

Discussion

The observation that nifedipine, which blocks I_{si}, shortened the early phase of the action potential in rat ventricular muscle is consistent with a contribution of I_{si} to the time course of this phase. Such a contribution would be expected in view of the fact that I_{si} is activated at potentials positive to -40 mV and decays rapidly (Mitchell *et al.*, 1983b). However, it seems unlikely that a current with these characteristics could contribute to the late plateau phase which appears at potentials negative to -40 mV, and the possibility of additional currents which could contribute to this late phase will be discussed in the following paper.

The prolonging effect of strontium on the early phase of the action potential is consistent with a prolongation by strontium of I_{si}. Nevertheless, it remains possible that an additional factor contributing to the effect on the early phase is a reduction of a transient outward current by strontium. Such an inhibitory effect of strontium on outward currents has been seen in Purkinje fibres (Siegelbaum & Tsien, 1980; Coraboeuf & Carmeliet, 1982). The contribution of an outward current to early repolarization in calcium-containing solution is shown by the effect of 4-AP, which prolongs the early phase of the action potential. The rapid prolongation by 4-AP was also observed when strontium was present, suggesting that 4-AP inhibited an outward current, which was still contributing to early repolarization in the presence of strontium. Transient outward current would be expected to exert its major influence during the first 10–20 ms of repolarization (Siegelbaum & Tsien, 1980; Mitchell *et al.*, 1983b). In some cells, extended exposure (3–5 min) to strontium-containing solution resulted in very prolonged action potentials in which there was a notch and subsequent plateau close to 0 mV. It seems unlikely that suppression of transient outward current alone could have resulted in these prolonged action potentials, and in such cases the effect of inhibition of transient outward current by 4-AP was to abolish the notch.

In ventricular cells of other species, the action potential shows a prolonged plateau at positive potentials (Powell *et al.*, 1981; Isenberg & Klockner, 1982; Trautwein *et al.*, 1982). It is possible that I_{si} in these cells also contributes to the initiation of the action potential plateau and that, although two phases of the action potential plateau are not visible, another inward current is present to maintain the plateau at a depolarized level. This possibility is considered in detail in the accompanying paper (Mitchell *et al.*, 1984). As seen above, in the presence of strontium, rat action potentials themselves may show only one long plateau, thus obscuring two underlying phases (cf. Figure 6).

It should be mentioned that while it seems clear that both I_{si} and transient outward current contribute to the early phase, the relative contributions of these two currents to the time course of the action potential in rat ventricular muscle may vary with experimental conditions. It has been pointed out (Powell *et al.*, 1980) that the precise time course of the action potential depends, among other factors, on the resting membrane potential and rate of stimulation. These factors may account for some small differences between the action potentials reported by Powell *et al.* (1980; and the present study) and those of Watanabe *et al.* (1983), in addition to any differences between cells of different origin in rat ventricular muscle (Watanabe *et al.*, 1983).

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