Br. J. Pharmac. (1984), **81**, 543–550

The effects of ryanodine, EGTA and low-sodium on action potentials in rat and guinea-pig ventricular myocytes: evidence for two inward currents during the plateau

Mary R. Mitchell, T. Powell*, D.A. Terrar & V.W. Twist*

Departments of Pharmacology and Therapeutics, and of * Medical Physics and Institute of Nuclear Medicine, Middlesex Hospital Medical School, Cleveland Street, London W1P 6DB

1 Action potentials were recorded from single cells isolated from rat and guinea-pig ventricular muscle. In rat cells the repolarization showed two distinct phases, referred to as the early and late phases. In guinea-pig cells there was a maintained plateau.

2 Reducing external sodium by replacement with lithium or choline suppressed the late phase of the action potential in rat cells, and shortened the plateau of the action potential in guinea-pig cells.

3 Intracellular EGTA abolished contraction while suppressing the late phase of the action potential in rat cells, and shortening the plateau in guinea-pig cells.

4 Ryanodine (1 μM), which is thought to inhibit the release of calcium from internal stores, suppressed contraction and the late phase of the action potential in rat cells. In guinea-pig cells, there was no substantial effect of ryanodine (1 μM) on either contraction or the time course of the action potential.

5 The late phase of the action potential in rat cells was suppressed by increasing the external potassium concentration to 12 mM, and enhanced by reducing external potassium to 1.2 mM.

6 It is concluded that an inward current activated by internal calcium contributes to the late phase of the action potential in rat cells, and to the plateau in guinea-pig cells. Two possibilities are a current arising from electrogenic sodium-calcium exchange, and a current through ion channels activated by calcium. The effects of reducing external sodium would be consistent with either mechanism. The contribution of such an inward current would be expected to be modified by outward currents through a rectifying potassium conductance which varies with external potassium concentration. In the rat, but not the guinea-pig, the rise in internal calcium which activates the inward current seems to be largely dependent on ryanodine-sensitive release of calcium from internal stores.

Introduction

In the accompanying paper (Mitchell *et al.*, 1984a) it was shown that the plateau of the rat ventricular action potential has a complex time course consisting of a brief early phase and a long late phase and that the second inward current (I_{si}) and a 4-aminopyridine (4-AP)-sensitive outward current determine the time course of the early phase. It was suggested that the separation of two components of repolarization in rat cells may be fortunate for the study of the underlying mechanism if the separation were to reflect a difference in the currents contributing to the two phases. The underlying cause of the late phase in these cells still remains to be determined. If there were additional current(s) contributing to the late phase of the action potential in rat cells, it seems possible that a similar current might contribute to the action potentials of other mammalian cardiac cells which do not show any obvious sign of discontinuity in the plateau. The kinetics of I_{si} are rapid in guinea-pig (Lee & Tsien, 1982) and human (Mitchell *et al.*, 1982) ventricular muscle, and both these types of muscle cell show a prolonged plateau (Powell *et al.*, 1981; Isenberg & Klockner, 1982; Mitchell *et al.*, 1982). Our experiments on ventricu-

lar cells from both rat and guinea-pig suggest that an inward current activated by a rise in intracellular calcium is involved in the generation of the late plateau phase in the rat and in the maintenance of the plateau in the guinea-pig.

It has been proposed that in rat cells I_{si} induces the sarcoplasmic reticulum to release the calcium required to activate contraction (Fabiato & Fabiato, 1975). Ryanodine is an alkaloid which has long been known to reduce the force of contraction in heart muscle (Jenden & Fairhurst, 1969) and is believed to inhibit release of calcium from internal stores (Sutko *et al.*, 1979). The uncoupling by ryanodine of the electrical and mechanical events in mammalian cardiac muscle (Penefsky & Kahn, 1969) has been linked with a dissociation of the T-tubules from the sarcoplasmic reticulum (Penefsky, 1974). The extent of the inhibition of contraction varies between species, being more obvious in those cardiac tissues with a more highly developed sarcoplasmic reticulum e.g. in the rat (Sutko & Willerson, 1980). Ryanodine has also been found to have little effect on tension in the guinea-pig compared to the rat (Hajdu, 1969) and this is confirmed in the present experiments, where ryanodine is found to abolish contraction in rat but not in guinea-pig cells. Ryanodine also decreases the late phase of the action potential in rat cells but has no effect on the time course of the action potential in guinea-pig cells, suggesting that the sarcoplasmic reticulum is more important in the rat as a source of calcium for activation of the late inward current.

Methods

Cells were isolated from rat and guinea-pig ventricles by collagenase digestion (Powell *et al.*, 1980; Lee & Tsien, 1982). The cells were mounted on the surface of agar in a perspex chamber. The superfusing solution (pH 7.4, 36–37°C) for the rat cells contained (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}. In most experiments on guinea-pig cells, the superfusing solution was as above except for an increase in $NaHCO_3$ (15.4 mM) and KCl (4.2 mM). In some experiments e.g. low sodium exposure (Figure 2b), guinea-pig cells were superfused with solution containing (mM): NaCl 113.1, KCl 4.6, $MgCl_2$ 1.2, NaH_2PO_4 3.5, $NaHCO_3$ 21.9, glucose 5.5 (cf. Bustamante *et al.*, 1981) and bovine serum albumin 5 mg ml^{-1}. To avoid precipitation, the calcium concentration was reduced to 1 mM in this solution. In experiments where the sodium ion concentration was reduced, NaCl was replaced by LiCl or iso-osmotically by choline chloride. In the latter solution atropine (1 μM) was added to antagonize any effects of choline on muscarinic receptors. Atropine sulphate was obtained from BDH Laboratories, tetrodotoxin from Sigma, and ryanodine was a gift from Merck, Sharp and Dohme.

Membrane potentials were recorded as in the previous paper and records of contractions were obtained by monitoring the change in intensity of light passing through the cell to a photodiode mounted in the eyepiece of the microscope (Purves *et al.*, 1974; Kass, 1981). The amplitude of contraction in the figures is in arbitrary units. Contraction records were low-pass filtered at 700 Hz, or in the case of Figure 5 at 100 Hz. In some experiments, electrodes were filled with 400 mM EGTA, titrated to pH 7.4 with 1M KOH. Rat cells were initially impaled in 5 mM calcium to allow rapid sealing of the electrodes, and the resulting resting potentials remained stable at −75 to −80 mV on reduction of the external calcium concentration to 2.5 mM (Powell *et al.*, 1980). Stable membrane potentials (−80 to −90 mV) were obtained in guinea-pig cells in 2.5 mM calcium. Action potentials in both rat and guinea-pig cells were initiated by brief depolarizing current pulses (0.5–1 ms at 1 Hz).

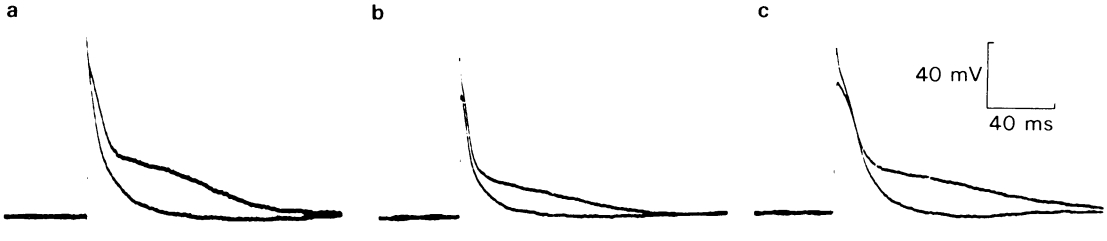

Figure 1 Superimposed action potentials recorded from three rat ventricular cells in solution containing normal and 11% sodium (shortened action potentials). In (a) NaCl was replaced by LiCl and in (b) and (c) by choline chloride. The divalent action in (a) and (b) was Ca^{2+} (2.5 mM) and in (c) was Sr^{2+} (2.5 mM). Records in low sodium were taken 1 min after reduction of the external Na^+ concentration.

Results

Effect of low sodium on rat and guinea-pig ventricular action potentials

Action potentials initiated by a brief depolarizing current pulse in rat ventricular cells show a rapid upstroke followed by two phases of repolarization, referred to as the early and late phases, as described in the previous paper. In rat cells, lowering the sodium ion concentration to 11% by substituting LiCl for NaCl caused a reduction in action potential duration with a decrease in both early and late phases (Figure 1a). In some experiments NaCl was isoosmotically replaced by choline chloride, and atropine (1 μM) was added to counteract any effects of the choline on muscarinic receptors. In solutions containing either calcium (Figure 1b) or strontium (Figure 1c), the late phase of the plateau was again diminished by reducing sodium, but unlike experiments in which Li substituted for Na, the overshoot of the action potential was reduced.

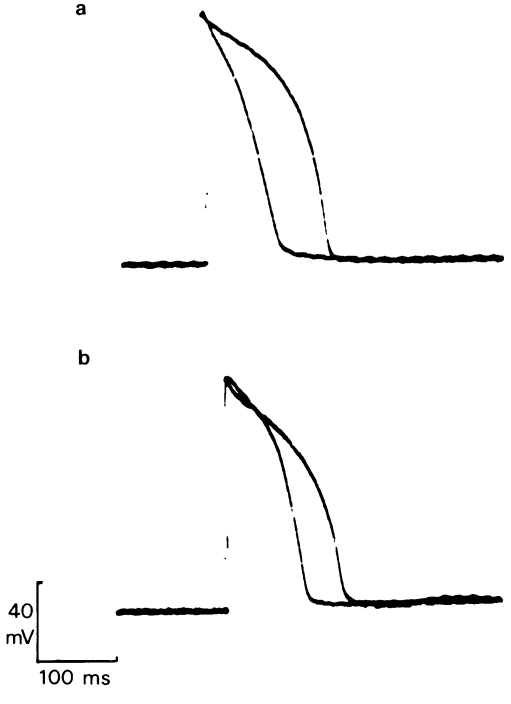

Figure 2 Superimposed records of action potentials from guinea-pig ventricular cells in normal and after 60 s in low sodium (shortened action potentials in (a) and (b)). In (a) the Na^+ concentration was reduced to 16% by substitution of NaCl by LiCl and the Ca^{2+} concentration was 1 mM (see Methods). In (b) Na^+ was reduced to 11% by choline substitution and the Ca^{2+} concentration was 2.5 mM.

The action potential of guinea-pig ventricular cells has one long plateau at positive potentials and the duration of this plateau is found to be reduced when the external sodium ion concentration is decreased. In three cells in 2.5 mM calcium, where the sodium concentration was reduced to 11% by substitution of NaCl by LiCl (Figure 2a), there was an average reduction in action potential duration of 44%. In separate experiments where the calcium concentration was 1 mM (see Methods), the sodium concentration was reduced to 16% by choline substitution (Figure 2b). The average reduction of action potential duration in three cells was 37%.

In both rat and guinea-pig cells, reduction of external sodium by the above methods in calcium-containing solution resulted in low frequency (approximately 3–5 Hz) oscillations in tension with associated small fluctuations in membrane potential (see Discussion).

The effects of reducing external sodium, in reducing the late phase of the action potential in the rat, and in shortening of the action potential in the guinea-pig, are consistent with the contribution of an inward current carried at least in part by sodium in these cells. Since Li is a good substitute for sodium in the rapid sodium current responsible for the upstroke of the action potential, and yet substitution of Li for Na suppressed the late plateau, it seems unlikely that a non-inactivating component of such a current could contribute markedly to the late plateau. As a further test of this possibility, tetrodotoxin (TTX) was used at a concentration of 10 μM, which reduces the fast inward sodium current in isolated rat cells by 80% (Brown *et al.*, 1982). TTX markedly reduced the overshoot of the rat action potential and only slightly reduced the late phase. In guinea-pig cells, TTX was seen to reduce action potential duration by an average of 10% in three cells. It is therefore unlikely that a TTX-sensitive steady sodium current (Colatsky, 1982) can adequately account for the maintenance of rat and guinea-pig action potential plateaux.

Effect of intracellular EGTA

Cells of both rat and guinea-pig were impaled with electrodes (resistance 60–80 MΩ) containing 400 mM EGTA. Contraction of the cells accompanying action potentials was rapidly abolished under these conditions, consistent with leakage of EGTA from the electrode and consequent buffering of the intracellular calcium at a low level. Such a conclusion is consistent with a simple calculation of the possible intracellular concentration of EGTA under these conditions. Salt leakage from a microelectrode filled with 1 M KCl (resistance 50–75 MΩ) into *Neurospora* cells has been estimated from direct measurements of intracellular ions to be ap-

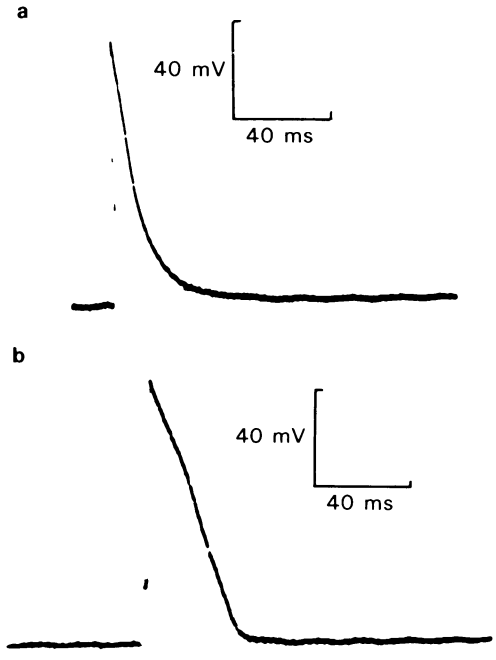

Figure 3 Action potentials recorded from rat (a) and guinea-pig (b) ventricular cells with electrodes containing 400 mM EGTA (pH 7.4).

proximately 4–5 fmol s^{-1} (Blatt & Slayman, 1983). If the leakage of EGTA from a microelectrode filled with 400 mM EGTA were to occur at approximately 1 fmol s^{-1}, then in 1 min the intracellular concentration of EGTA in a cell with internal volume of 20 pl would rise to 2.5 mM. This value is in the order of magnitude of concentrations of EGTA required to keep internal calcium below 1 nM.

When intracellular calcium was buffered by internal EGTA in this way the action potentials recorded both from rat and guinea-pig were shorter in duration (Figure 3a and b) than those recorded with conventional KCl electrodes (cf. Figures 1 and 2). No obvious differences were seen in input resistances measured at the resting potential with EGTA-containing electrodes compared to those recorded with more conventional electrodes. The attenuation cannot therefore be due to gross changes in membrane resistance arising from impalement by EGTA-containing electrodes.

Effects of ryanodine

Further experiments were carried out with ryanodine, which interferes with calcium release from internal stores (Sutko & Willerson, 1980). Figure 4 shows the action potential and accompanying contraction in a rat cell before (a), and at 90 s (b) and 3 min (c) after exposure to 1 μM ryanodine. The abolition of contraction is consistent with the proposal that calcium from internal stores (the sarcoplasmic reticulum) plays the major role in activation of contraction in the rat (Fabiato & Fabiato, 1979). In addition to inhibiting contraction in these cells, ryanodine produces a gradual decrease in the late plateau phase (Figure 4) suggesting that intracellular calcium released from the sarcoplasmic reticulum is important in producing this phase. In contrast to these observations in rat cells, ryanodine does not inhibit contraction nor does it markedly affect the time course of the action potential in guinea-pig cells (Figure 5).

Inward rectifying properties of ventricular cells

In the mammalian heart there is known to be a background potassium conductance which is inward rectifying (see Noble, 1979), and which influences repolarization. Figure 6 shows that in rat ventricular cells a decrease in the external potassium ion concentration slows repolarization, presumably because membrane resistance was increased, and the postulated additional inward current was opposed by less

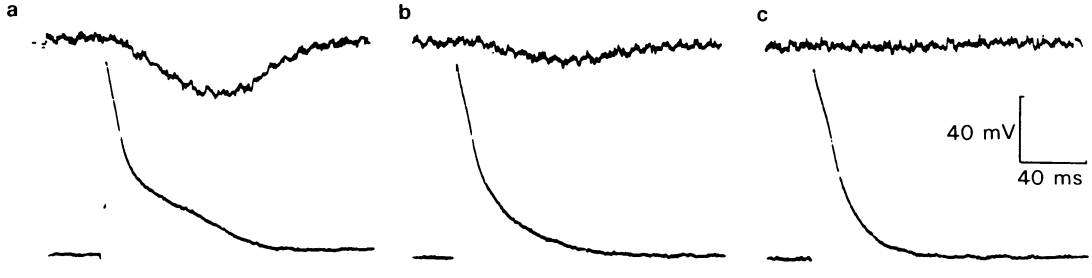

Figure 4 Effect of ryanodine on the rat ventricular action potential and contraction. Upper traces show the time course of contraction monitored by a photodiode in the microscope eyepiece. Lower traces show action potentials recorded simultaneously with the contraction traces before (a) and at 90 s (b) and 3 min (c) after exposure to ryanodine 1 μM.

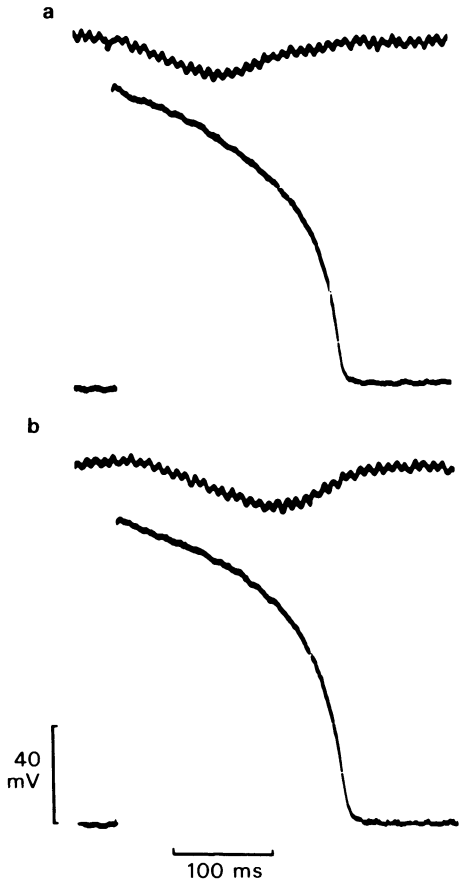

Figure 5 Ryanodine and the guinea-pig ventricular action potential and contraction: upper traces are of contractions and lower traces are of the action potentials recorded from a guinea-pig ventricular cell before (a) and 3 min (b) after exposure to ryanodine 1 μM.

outward current. Conversely, when external potassium was increased, the late plateau was suppressed because the membrane resistance was reduced and the additional inward current was balanced by outward current under these conditions.

In guinea-pig cells input resistance was monitored by applying hyperpolarizing pulses (duration 100–200 ms, amplitude 100–200 pA). On depolarization with a steady current, while giving the hyperpolarizing pulses, there was a rapid transition from a stable membrane potential of approximately −70 mV with a low resistance to a new stable potential at approximately +20 mV with a high resistance (cf. Gadsby & Cranefield, 1977). This rapid switching between stable levels of membrane potential with gradual changes in injected current was reproducible over many cycles of depolarization and repolarization. The rapid switching suggests the presence of a high resistance between these two potentials. A rectifying potassium conductance which is presumably responsible for these effects has been described for guinea-pig ventricular muscle by Daut (1982).

Discussion

The major findings of the present study in relation to the mechanisms underlying the action potential are that: (1) reduction of external sodium reduces the late phase of the action potential in rat cells, and shortens the plateau of the action potential in guinea-pig cells; (2) intracellular EGTA also reduces the late phase in rat cells and shortens the plateau in guinea-pig cells; and (3) ryanodine suppresses the late phase and contraction in rat cells, whereas the guinea-pig cells seem to be insensitive to ryanodine in that neither the action potential nor contraction show any substantial changes in the presence of ryanodine. These observations are consistent with the existence of an inward current during the late phase in the rat and the plateau in the guinea-pig, which is initiated by a rise in internal calcium. This rise in calcium

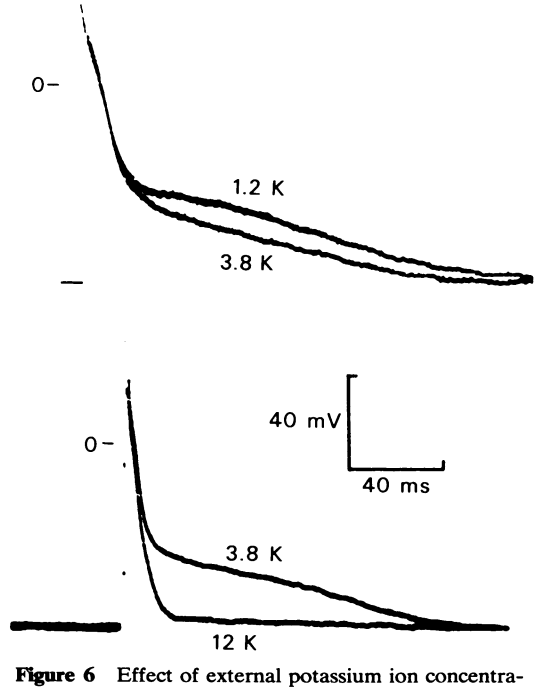

Figure 6 Effect of external potassium ion concentration on rat ventricular action potential time course. The upper records show the effect of reducing the external K^+ concentration and the lower records the effect of increasing the external K^+ concentration on action potentials recorded from rat ventricular cells.

would be dependent on a ryanodine-sensitive release of calcium from internal stores in the case of the rat, whereas in the guinea-pig the rise in calcium would result from mechanisms which do not depend on release from such internal stores. One current which may account for these observations is an electrogenic current arising from sodium-calcium exchange, which would allow accumulated calcium to be removed from the cell (cf. Mullins, 1981). Another possibility is that the additional inward current flows through the ion channels activated by internal calcium described by Colquhoun *et al.* (1981). The dependence of the additional inward current on external sodium is consistent with either of these two suggestions since both electrogenic sodium-calcium exchange and a current through the ion channels described by Colquhoun *et al.* would be carried at least in part by sodium.

In the rat, I_{si} does not have appropriate characteristics to make a direct contribution to the late phase, since it is activated at potentials positive to -40 mV and inactivates rapidly, with little steady component at 0 mV (Mitchell *et al.*, 1983b). Nevertheless, I_{si} would be expected to contribute to the rise in internal calcium both by influx of calcium through the surface membrane, and by triggering release of calcium from internal stores, and thus I_{si} should exert a profound indirect influence on the postulated additional inward current. In the guinea-pig, I_{si} is activated over a similar potential range to the rat, and shows rapid kinetics, but there appears to be a maintained component which could contribute to the plateau (Lee & Tsien, 1982). The sensitivity of this plateau to intracellular EGTA, which does not block I_{si} in these cells (Lee & Tsien, 1982) indicates that I_{si} cannot be the only inward current during the plateau.

It also seems unlikely that a non-inactivating component of rapid sodium current could alone be responsible for the late phase in the rat, or the plateau in the guinea-pig, since rapid sodium current would not be expected to be greatly influenced by replacing some of the external sodium with Li, or by internal EGTA, procedures which cause marked suppression of the late phase or plateau. A similar argument applies to ryanodine in the rat. Further evidence against a major contribution by a component of rapid sodium current is the observation that 10 μM TTX caused only small changes in the late phase and the plateau, while the rate of rise and the overshoot were greatly reduced. The small changes which were observed in the late phase and the plateau might perhaps be secondary consequences of the reduced rate of rise and overshoot.

Whether or not the postulated inward current activated by internal calcium makes a noticeable contribution to the action potential duration depends on the underlying potassium conductances. The results here show that a background rectifying potassium current affects the time course of the rat action potentials; the higher the membrane resistance and the lower the outward potassium current, the more pronounced will be the effect of an inward current on the action potential plateau. In the guinea-pig, rectification may be more pronounced than in the rat, so that any inward current will have a more marked effect and allow the plateau to be held at a more positive potential. In addition, rat ventricular cells have an early outward current which is important in the fast initial repolarization of the action potential, as shown in the previous paper.

The marked reduction of contraction and of the late phase of the action potential plateau in rat cells on exposure to ryanodine suggests that internal stores are an important source of calcium in rat ventricle for activation of contraction and for providing calcium which activates the additional inward current. In contrast, the lack of effect of ryanodine on contraction and action potential duration in guinea-pig cells suggests either that guinea-pig cells are less sensitive to the ryanodine or that the sarcoplasmic reticulum is not such an important source of calcium ions in these cells. Differences between rat and guinea-pig cells in their sensitivity to ryanodine may reflect differences in the source of calcium for contraction in these two species (Mitchell *et al.*, 1984b). The contraction in guinea-pig cells was lengthened on exposure to ryanodine (Figure 5). This might arise if, in the guinea-pig, relaxation is promoted by calcium uptake into the sarcoplasmic reticulum and this is inhibited by ryanodine.

Lithium is able to substitute for sodium in the initial fast increase in conductance thus maintaining the overshoot of the action potential. A similar effect of lithium has been observed on the action potential of skeletal muscle (Keynes & Swan, 1959). However, in other circumstances, lithium is unable to substitute for sodium. For example, it has been found that, unlike sodium, lithium cannot promote calcium efflux from squid axons (Baker *et al.*, 1967). In addition, replacement of external sodium by lithium, but not by choline, promotes sodium efflux and concomitant calcium influx in these axons (Baker *et al.*, 1969). In guinea-pig auricles, calcium efflux is decreased when sodium is replaced either by lithium (Reuter & Seitz, 1968) or by choline (Glitsch *et al.*, 1970), and in rat ventricular cells exposure to low sodium, choline solution causes an increase in spontaneous activity presumably due to inhibition of sodium-calcium exchange and resulting increase in intracellular calcium (Mitchell *et al.*, 1983b). The more marked effect of lithium than choline substitution in the attenuation of both rat and guinea-pig action potentials may result from a greater increase in

internal calcium in lithium, if lithium promotes calcium influx in addition to inhibiting efflux as seen in squid axons: the increased intracellular calcium could then cause greater inactivation of I_{si} (Mitchell *et al.*, 1983b) and thus decrease the early phase of the action potential to which I_{si} contributes (see preceding paper). Although separate phases are not seen in guinea-pig action potentials, a similar mechanism may also exist here to account for the more marked reduction in action potential duration on substitution of sodium by lithium. It should also be mentioned in this context that inhibition of I_{si} does not occur in low sodium, choline-containing, solution when strontium replaces calcium in the external solution (Mitchell *et al.*, 1983b). Thus, suppression of the late phase under these conditions cannot be attributed to a secondary effect of low sodium on I_{si}, which might in turn influence the additional inward current.

In the guinea-pig the functional significance of the additional inward current may be to help keep the cell at a depolarized level at which calcium may continue to enter to activate contraction (Mitchell *et al.*, 1984b). In the rat, where contraction may be controlled by calcium released from internal stores following I_{si} during the early part of the action potential, the functional significance of the additional inward current is less clear. If it were to reflect electrogenic Na-Ca exchange, it may simply be the consequence of removal of calcium following the activation of contraction.

A very slow inward current in guinea-pig cells has recently been reported by Lee *et al.* (1983). Whether or not this is related to the additional inward current in our experiments deserves further study.

For simplicity, the additional inward currents activated by internal calcium have been discussed together above as if they were similar in both rat and guinea-pig ventricular muscle. While it is clear that such currents can make an important contribution to the time course of action potentials in both rat and guinea-pig cells and that they share many features discussed above, it remains to be determined to what extent they share a common ionic mechanism. Experiments under voltage-clamp conditions may be required to approach this problem. It also remains for further study to investigate whether such currents are a feature of other mammalian cardiac muscle.

This work was supported by grants from the B.H.F. and M.R.C.

References

BAKER, P.F., BLAUSTEIN, M.P., HODGKIN, A.L. & STEINHARDT, R.A. (1967). The effect of sodium concentration on calcium movements in giant axons of *Loligo forbesi*. *J. Physiol.*, **192,** 43–44P.

BAKER, P.F., BLAUSTEIN, M.P., HODGKIN, A.L. & STEINHARDT, R.A. (1969). The influence of calcium on sodium efflux in squid axons. *J. Physiol.*, **200,** 431–458.

BLATT, M.R. & SLAYMAN, C.L. (1983). KCl leakage from microelectrodes and its impact on the membrane parameters of a nonexcitable cell. *J. membrane Biol.*, **72,** 223–234.

BROWN, A.M., LEE, K.S. & POWELL, T. (1981). Sodium current in single rat heart muscle cells. *J. Physiol.*, **318,** 479–500.

BUSTAMANTE, J.C., WATANABE, T. & McDONALD, T.F. (1981). Single cells from adult mammalian heart: isolation procedure and preliminary electrophysiological studies. *Can. J. Physiol. Pharmac.*, **59,** 907–910.

COLATSKY, T.J. (1982). Mechanisms of action of lidocaine and quinidine on action potential duration in rabbit cardiac Purkinje fibers. An effect on steady state sodium currents. *Circulation Res.*, **50,** 17–27.

COLQUHOUN, D., NEHER, E., REUTER, H. & STEVENS, C.F. (1981). Inward current channels activated by intracellular Ca in cultured cardiac cells. *Nature, Lond.*, **294,** 752–754.

DAUT, J. (1982). The passive electrical properties of guinea-pig ventricular muscle as examined with a voltage-clamp technique. *J. Physiol.*, **330,** 221–242.

FABIATO, A. & FABIATO, N. (1975). Contractions induced by a calcium-triggered release of calcium from the sarcoplasmic reticulum of single skinned cardiac cells. *J. Physiol.*, **249,** 469–495.

FABIATO, A. & FABIATO, N. (1979). Calcium and cardiac excitation-contraction coupling. *A. Rev. Physiol.*, **41,** 473–484.

GADSBY, D.C. & CRANEFIELD, P.F. (1977). Two levels of resting potential in cardiac Purkinje fibres. *J. gen. Physiol.*, **70,** 725–746.

GLITSCH, H.G., REUTER, H. & SCHOLZ, H. (1970). The effect of the internal sodium concentration on calcium fluxes in isolated guinea-pig auricles. *J. Physiol.*, **195,** 451–470.

HAJDU, S. (1969). Mechanism of the Woodworth staircase phenomenon in heart and skeletal muscle. *Am. J. Physiol.*, **216,** 206–214.

ISENBERG, G. & KLOCKNER, U. (1982). Calcium tolerant ventricular myocytes prepared by preincubation in a "KB medium". *Pflugers Arch.*, **395,** 6–18.

JENDEN, D.J. & FAIRHURST, A.S. (1969). The pharmacology of ryanodine. *Pharmac. Rev.*, **21,** 1–25.

KASS, R.S. (1981). An optical monitor of tension for small cardiac preparations. *Biophys. J.*, **34,** 165–170.

KEYNES, R.D. & SWAN, R.C. (1959). The permeability of frog muscle fibres to lithium ions. *J. Physiol.*, **147,** 626.

LEE, K.S., LEE, E.W., NOBLE, D. & SPINDLER, A.J. (1983). A very slow inward current in single ventricular cells. *J. Physiol.*, (in press).

LEE, K.S. & TSIEN, R.W. (1982). Reversal of current through calcium channels in dialysed single heart cells. *Nature. Lond.*, **297,** 498–501.

MITCHELL, M.R., POWELL, T., TERRAR, D.A. & TWIST, V.W. (1982). Action potentials and second inward current recorded from individual human ventricular muscle cells. *J. Physiol.*, **332,** 51–52P.

MITCHELL, M.R., POWELL, T., TERRAR, D.A. & TWIST, V.W. (1983a). Contribution of the second inward current to action potential time course in isolated rat ventricular mycotes. *J. Physiol.*, **334,** 57–58P.

MITCHELL, M.R., POWELL, T., TERRAR, D.A. & TWIST, V.W. (1983b). Characteristics of the second inward current in cells isolated from rat ventricular muscle. *Proc. R. Soc. B.*, **219,** 447–469.

MITCHELL, M.R., POWELL, T., TERRAR, D.A. & TWIST, V.W. (1984a). Strontium, nifedipine and 4-aminopyridine modify the time course of the action potential in cells from rat ventricular muscle. *Br. J. Pharmac.*, **81,** 551–556.

MITCHELL, M.R., POWELL, T., TERRAR, D.A. & TWIST, V.W. (1984b). Membrane potential and contraction in voltage-clamped cells from rat and guinea pig ventricular muscle. *J. Physiol.*, (in press).

MULLINS, L.J. (1981). The generation of electric currents in cardiac fibers by Na/Ca exchange. *Am. J. Physiol.*, **236,** C103–C110.

NOBLE, D. (1979). *The Initiation of the Heartbeat.* Chapter 9. Oxford: University Press.

PENEFSKY, Z.J. (1974). Ultrastructural studies of the site of action of ryanodine on heart muscle. *Pflugers Arch.*, **347,** 185–198.

PENEFSKY, Z.J. & KAHN, M. (1969). Mechanical and electrical effects of ryanodine on mammalian heart muscle. *Am. J. Physiol.*, **218,** 1682–1686.

POWELL, T., STURRIDGE, M.F., SUVARNA, S.K., TERRAR, D.A. & TWIST, V.W. (1981). Intact individual heart cells isolated from human ventricular tissue. *Br. med. J.*, **283,** 1013–1015.

POWELL, T., TERRAR, D.A. & TWIST, V.W. (1980). Electrical properties of individual cells isolated from adult rat ventricular myocardium. *J. Physiol.*, **302,** 131–153.

PURVES, R.D., HILL, C.E., CHAMLEY, J.H., MARK, C.E., FRY, D.M. & BURNSTOCK, C. (1974). Functional autonomic neuromuscular junctions in tissue culture. *Pflugers Arch.*, **350,** 1–7.

REUTER, H. & SEITZ, N. (1968). The dependence of calcium efflux from cardiac muscle on temperature and external ion composition. *J. Physiol.*, **195,** 451–470.

SUTKO, J.L. & WILLERSON, J.T. (1980). Ryanodine alteration of the contractile state of rat ventricular myocardium. Comparison with dog, cat, and rabbit ventricular tissues. *Circulation Res.*, **46,** 332–343.

SUTKO, J.L., WILLERSON, J.T., TEMPLETON, C.H., JONES, L.R. & BESCH, H.R., Jr. (1979). Ryanodine: Its alterations of cat papillary muscle contractile state and responsiveness to inotropic interventions and a suggested mechanism of action. *J. Pharmac. exp. Ther.*, **209,** 37–47.

(*Received September 8, 1983.*
Revised October 24, 1983.)