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THE SLOW REPOLARIZATION PHASE OF THE ACTION POTENTIAL IN RAT HEART

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SUMMARY

1. Intracellular action potentials and isometric force were measured from thin trabeculae of the right ventricle of rat heart. Characteristic for the action potential of rat myocardium is a short plateau and a slow final repolarization phase. We have studied the influence of ionic composition of the medium and of stimulation frequency on the slow phase of repolarization and its relation to peak force.

2. The results confirmed a positive correlation between peak force and the duration of the slow phase of repolarization, as has been reported for other species.

3. An increase of $[Ca^{2+}]_o$ caused a shortening of the slow phase of repolarization when peak force was kept constant.

4. In low $[Na^+]_o$ peak force was increased and the slow phase of repolarization was shortened. Reperfusion with normal medium after a period in low $[Na^+]_o$ induced a transient prolongation of the slow phase of repolarization and reduction of peak force. The transient lasted about 20 min.

5. In the presence of the Ca^{2+} entry blocker nifedipine the action potential duration and peak force were reduced. Low $[Na^+]_o$ caused less shortening of the slow phase of repolarization and a greater increase of peak force. The slow phase of repolarization was prolonged transiently following reperfusion at normal $[Na^+]_o$, but only during a few beats.

6. These results are in agreement with the hypothesis that the slow phase of repolarization is due to an inward current generated by Na^+–Ca^{2+} exchange, as the latter mechanism is known to be sensitive to the intracellular and extracellular concentrations of both Na^+ and Ca^{2+}.

INTRODUCTION

The nature and kinetics of many transmembrane currents that occur during the cardiac action potential have been studied in great detail (Reuter, 1973; Trautwein, 1973; Boyett & Jewell, 1980). The contribution, particularly of the second inward current, to excitation–contraction coupling has been well established (Morad & Goldman, 1973; Reuter, 1974; Wohlfart & Noble, 1982).

The second inward current, i_{si}, is important for the genesis of the plateau of the action potential. In atrial and ventricular myocardium of various species a slow final

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phase of repolarization has been observed (Vaughan Williams, 1959; Sleator, Furchgott, de Gubareff & Krespi, 1964; Coraboeuf & Vassort, 1968; Ravens, 1983). The slow phase of repolarization is more pronounced during forceful contractions and is due to an inward current (Simurda, Simurdova, Braveny & Sumbera, 1981). In isolated myocytes from rat heart it is much reduced in low $[Na^+]_o$, which suggests that Na^+ acts as a charge carrier for the current involved (Mitchell, Powell, Terrar & Twist, 1984).

The action potential of rat myocardium has a short plateau phase with an intense i_{si} (Isenberg & Klöckner, 1980; Payet, Schanne & Ruiz-Ceretti, 1981), whereas the slow phase of repolarization is pronounced. This renders rat myocardium a suitable preparation to study the properties of the latter. Our results confirm a positive relationship between the duration of the slow phase of repolarization and peak force. Furthermore, we found evidence that the slow phase of repolarization depends on both intracellular and extracellular Na^+ and Ca^{2+} concentrations. The results can be explained if the current underlying the slow phase of repolarization is generated by a Na^+–Ca^{2+} exchange mechanism.

METHODS

Preparation

The heart was rapidly excised from adult rats of either sex (180–550 g) under ether anaesthesia. Long (1·5–4 mm) and thin (0·06–0·25 mm) free-running trabeculae were dissected from the right ventricle at the tricuspid valve. The preparation was suspended in a muscle bath (volume 1·5 ml) between the clamps of a force transducer and a length adjustment device. The muscle was stretched to a length where passive force was 2–5 % of active force. Before starting the experiments the muscle was stimulated at 1 Hz for at least 1 h and after this period length was adjusted again. Stimulus frequency during experiments was 0·2 Hz unless mentioned otherwise.

Solutions

Standard solution had the following composition (mM): NaCl, 120; KCl, 5; $MgCl_2$, 1·2; $CaCl_2$, 1·0–2·5; NaH_2PO_4, 2; Na_2SO_4, 1·2; $NaHCO_3$, 27; glucose, 10. The solution was recirculated via a 0·5 l reservoir which was in equilibration with 95 % O_2 and 5 % CO_2, p_{O_2} was 590–650 mmHg; p_{CO_2} 39–45 mmHg, pH 7·35–7·45. $[Ca^{2+}]$ was varied by adding the appropriate amounts of 1 M-$CaCl_2$ stock solution to Ca^{2+}-free medium. Low $[Na^+]$ was obtained by substituting 120 mM-LiCl or choline chloride or 240 mM-sucrose for 120 mM-NaCl. Flow through the muscle bath was 7–10 ml/min. Temperature in the bath was maintained at 26 ± 0·3 °C.

Experimental procedure

The intracellular potential was measured by means of glass micro-electrodes with a 3 mm long flexible shaft which was bent 70–150 deg. The resistance of the micro-electrodes (filled with 3 M-KCl) was 60–140 MΩ measured in the muscle bath. Frequently single cell impalements lasted up to 8 h although the preparation contracted vigorously and the site of impalement moved over a distance of 0·1 mm during the contractions. The signal of membrane potential was amplified and was fed into an action potential analyser which produced at its outputs the value of resting membrane potential (V_{rest}), sampled 5 ms prior to the stimulus and the duration of the action potential (a.p.d.) at 20 % (a.p.d.$_{20}$) or 50 % (a.p.d.$_{50}$) of the amplitude. Force, V_{rest} and a.p.d. were recorded on a chart recorder (Gould Brush 440). Action potentials were photographed on Polaroid paper from the screen of an oscilloscope.

Stimulation protocols

In order to obtain variation in force of contraction two different stimulation protocols were used.

(*1*) *Post-extrasystolic potentiation.* The priming period (0·2 Hz) was terminated by a varied number of extrasystoles at 0·20 s intervals. Subsequently the preparation was paced at 0·1 or 0·2 Hz (see inset Fig. 1). The first test beats following the extrasystoles were measured.

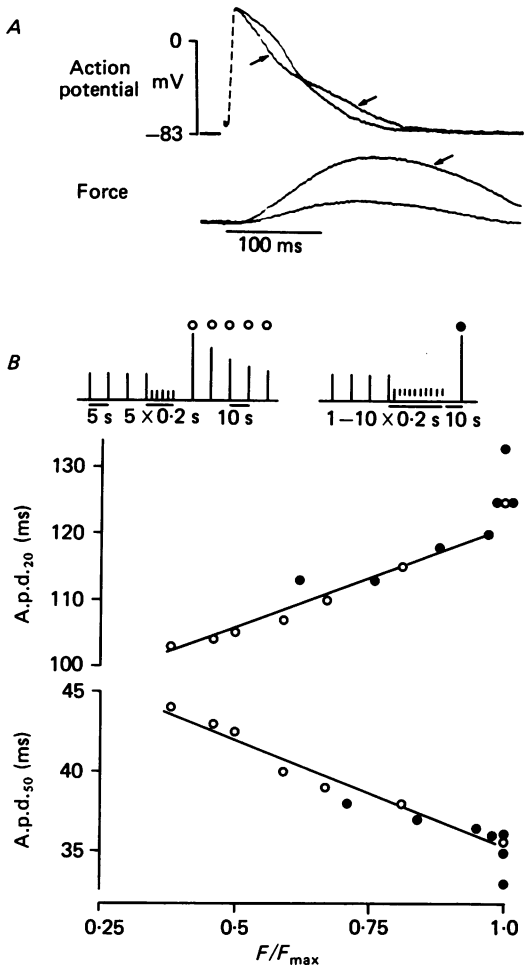

Fig. 1. A.p.d.–F relationships during post-extrasystolic potentiation. A, traces show action potentials and contractions of the first (arrows) and the tenth beat during the negative force staircase following ten extrasystoles. B, the negative a.p.d.$_{50}$–F relationship and the positive a.p.d.$_{20}$–F relationship measured from potentiated beats. ○, data obtained from the negative staircase following five extrasystoles (see left part of the inset). ●, data of the first potentiated beat only following different numbers of extrasystoles (see right part of the inset). Results in A and B were from different trabeculae. $[Ca^{2+}]_o$ was 0·8 mM.

(2) *Mechanical and electrical restitution.* The preparation was paced at a constant priming frequency of 0·2 Hz. When it was in the steady state a varied test interval was interposed (see inset of Fig. 2). From the test beats peak force and a.p.d. were measured.

RESULTS

The correlation between a.p.d. and peak force (F) was investigated by means of two different stimulation protocols. A typical result of the potentiation protocol (see Methods) is shown in Fig. 1. The first test beat following the extrasystoles was

potentiated roughly in proportion to the number of extrasystoles. More than ten to fifteen extrasystoles, however, did not cause a further increase of potentiation and the maximal force (F_{max}) probably reflects saturation of one of the steps in excitation–contraction coupling. The force of the next beats decayed, creating a negative

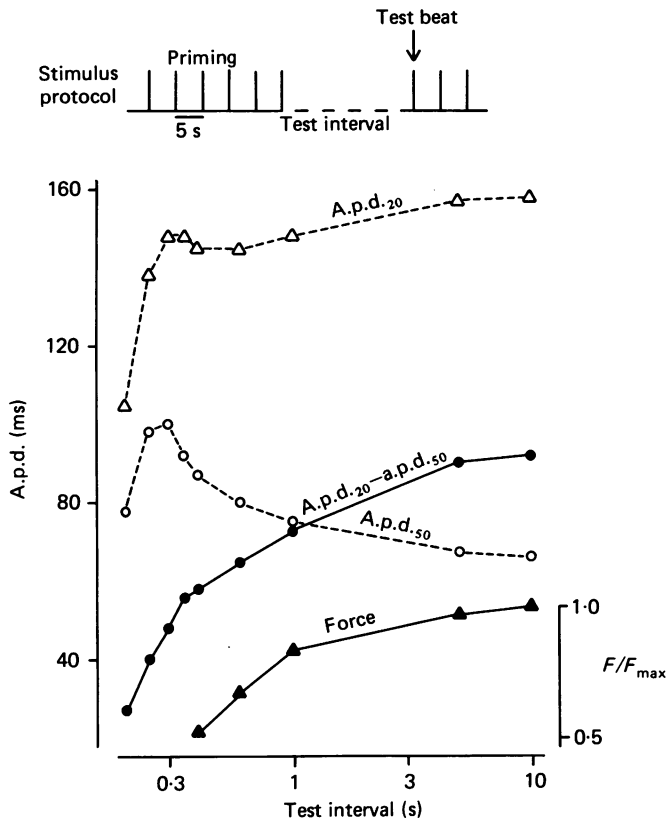

Fig. 2. Mechanical and electrical restitution curves from rat cardiac trabeculae. A.p.d.$_{50}$ refers to action potential duration at 50% of its amplitude and a.p.d.$_{20}$ at 20% of the amplitude; force refers to peak force of the twitches. Top: stimulation protocol; a varied test interval was interposed in a train of 5 s intervals when the preparation was in the steady state.

staircase. The relation between a.p.d.$_{20}$ and F during the negative staircase was positive as opposed to a.p.d.$_{50}$, which showed a negative relationship to F (○, Fig. 1*B*). Similar relationships were obtained if a.p.d.$_{20}$ or a.p.d.$_{50}$ was correlated with F using the first potentiated beat following a varied number of extrasystoles (●, Fig. 1*B*).

Fig. 2 shows the influence of variation of test interval on a.p.d. and F. The curves relating F and a.p.d. to test interval are conventionally called the mechanical and electrical restitution curves (Bass, 1975). The curve of a.p.d.$_{50}$ shows a maximum at a test interval of 0·25 s similar to that found in rabbit heart (Wohlfart, 1979). The

curve of a.p.d.$_{20}$ shows a maximum at 0·25 s and at 10 s. Peak force and the difference a.p.d.$_{20}$ − a.p.d.$_{50}$ increased monotonically with test interval. The values of F for test intervals shorter than 0·35 s were omitted from Fig. 2 because twitch summation occurred with the last control contraction. Fig. 2 shows that there is a negative correlation between a.p.d.$_{50}$ and F and a positive correlation between a.p.d.$_{20}$ and F.

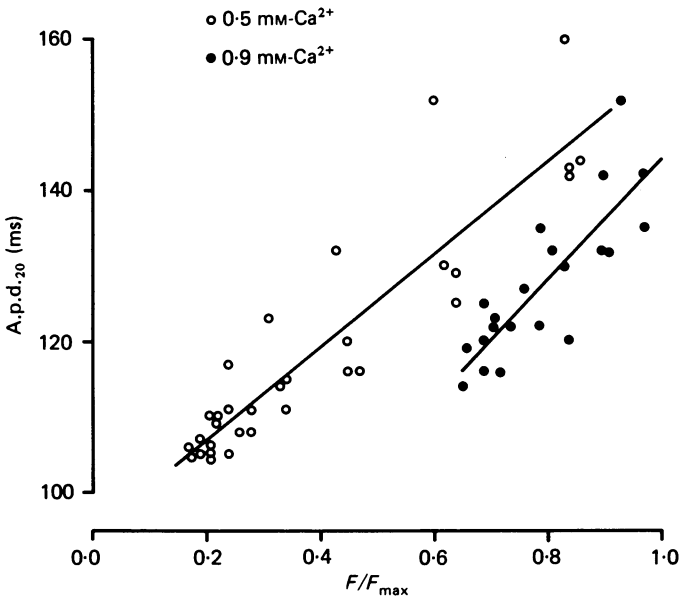

Fig. 3. A.p.d.$_{20}$–F relationship in two different $[Ca^{2+}]_o$. ○, data from 0·5 mM-Ca^{2+}; ●, data from 0·9 mM-Ca^{2+}. The data were obtained from the negative force staircase following extrasystoles as in Fig. 2. The sequence was: two series in 0·5 mM-Ca^{2+}, three series in 0·9 mM-Ca^{2+}, two series in 0·5 mM-Ca^{2+}. The scatter of data in the individual series was comparable to that in Fig. 2. Lines represent least-squares linear fit to the two sets of data. The a.p.d.$_{20}$ measurements were obtained from one cell.

It should be noted, however, that between preparations a.p.d.$_{50}$ varied from 15 to 65 ms (n = 66) and a.p.d.$_{20}$ from 35 to 160 ms (n = 45). In the preparations with a short slow phase of repolarization (a.p.d.$_{20}$ − a.p.d.$_{50}$ smaller than about 50 ms) the positive a.p.d.$_{20}$–F relationship was not apparent with the two protocols. Both stimulation protocols always resulted in a negative a.p.d.$_{50}$–F relationship.

Ionic composition of the medium

The most likely charge carriers for the inward current that underlies the slow phase of repolarization (Simurda *et al.* 1981) are Na^+, Ca^{2+} and possibly Cl^-. We tested therefore the effects of these ions on the preparations.

Influence of $[Ca^{2+}]_o$

In Ca^{2+}-free medium contractile activity of the preparation was abolished and the action potential was shortened. With increasing $[Ca^{2+}]_o$ peak force increased to reach

a maximum in about 3 mM-Ca^{2+}. The action potential prolonged, but beyond 0·5 mM-Ca^{2+} the duration of the plateau and of the slow phase of repolarization remained almost constant. In the previous section a positive a.p.d.$_{20}$–F correlation was shown. Therefore the influence of $[Ca^{2+}]_o$ on the slow repolarization phase has to be corrected for the simultaneous changes in F. We used the potentiation protocol to vary F in two different extracellular Ca^{2+} concentrations. An example of the two a.p.d.$_{20}$ relationships obtained in this way is given in Fig. 3. The a.p.d.$_{20}$–F relationship was shifted to shorter a.p.d.$_{20}$ when $[Ca^{2+}]_o$ was increased. Apparently extracellular Ca^{2+} tends to shorten the slow phase of repolarization.

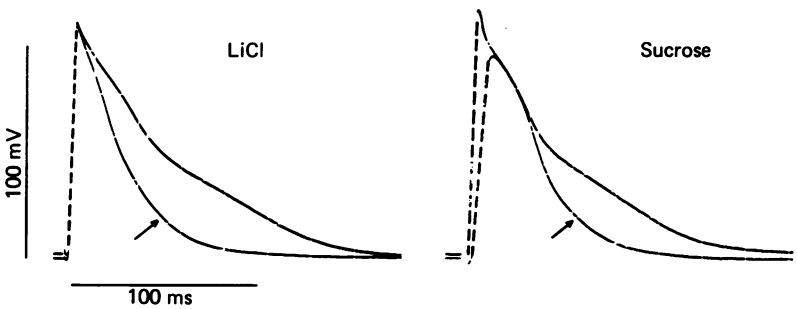

Fig. 4. Influence of NaCl substitutes on the action potential of rat ventricle. 120 mM-NaCl was substituted by 120 mM-LiCl (left panel) or by 240 mM-sucrose (right panel). All action potentials shown here were recorded from the same cell. The upstroke of the action potentials is retouched.

Reduction of the Na^+ and Cl^- concentrations

Three different substitutes were used to reduce the Na^+ and/or Cl^- concentrations to 30 mM: LiCl, sucrose or choline chloride. Their common effect was to reduce a.p.d.$_{20}$, to increase F, to induce after-contractions and a contracture during the first 3 min after substitution. The effect of the different substitutes also revealed differences: Li^+ substitution caused shortening of the plateau phase and of the slow phase; sucrose had the same effects but also abolished the spike (Fig. 4). Experiments with left atrial strips suggested that choline had a muscarinic effect as well and caused very short action potentials with a reduced amplitude. The choline effects in atrium could be mimicked by methacholine (1 μg/l). The same amount of atropine necessary to block the metacholine effects only partially reversed the choline chloride effects, suggesting a residual effect of Na^+ substitution. Because of the possible muscarinic effects of choline its use was abandoned for further experiments. The observed reduction of the slow phase in LiCl or sucrose solutions indicates that Na^+ is the responsible charge carrier, whilst Cl^- is probably rather unimportant.

Time course and reversibility of low $[Na^+]_o$ effects

Upon reduction of $[Na^+]_o$ the action potential shortened within 30 s. Reperfusion with normal solution caused a transient prolongation that lasted 10–30 min. Fig. 5 shows a rather outspoken example. In this preparation a.p.d.$_{20}$ prolonged to 540 ms

upon reperfusion with normal medium, the control duration of 115 ms was established again after 30 min. A more typical result is given in Fig. 6*A* which shows that the changes in *F* followed a more complex course. In low $[Na^+]_o$ *F* increased in two phases before reaching a steady state after about 4 min. During this initial period a transient

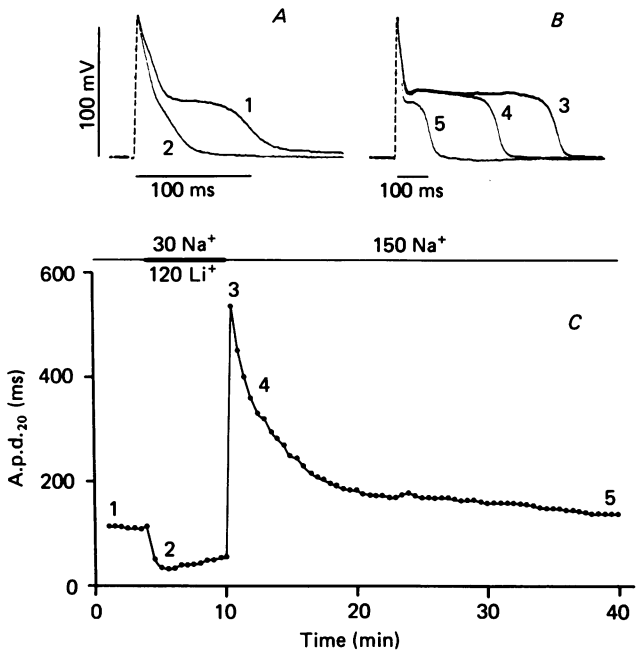

Fig. 5. Time course and reversibility of the effects of low $[Na^+]_o$ perfusion on a trabecula from the right ventricle of rat. *A*, action potentials recorded before (1) and during (2) perfusion with medium in which 120 mM-NaCl was replaced by 120 mM-LiCl. *B*, action potentials recorded 20 s (3), 120 s (4) and 30 min (5) after reperfusion with normal solution. *C*, redrawing of the continuous recording of $a.p.d._{20}$ during perfusion with Li^+-containing medium and reperfusion with normal solution. The bar above *C* indicates the period of perfusion with Li^+ medium. All data in this Figure were obtained during a continuous impalement of one cell. The upstroke of the action potentials is retouched.

contracture and after-contractions were generally observed. Reperfusion with normal solution first caused a small increase of *F*. Subsequently *F* fell rapidly to a low level, generally much lower than in the example of Fig. 6*A*. Finally it gradually increased to the steady state within 10–30 min. Thus the changes in *F* were opposite to the changes in a.p.d. but the time courses were comparable. It should be noted, however, that upon reperfusion with normal solution the prolongation of $a.p.d._{50}$ and the decrease of peak force were delayed by a few beats with respect to the prolongation of $a.p.d._{20}$. To illustrate this the arrows in Fig. 6*A* indicate the a.p.d. and *F* values of one of the significant beats. It is also evident from the action potentials numbered 3–5 in Fig. 6*A*.

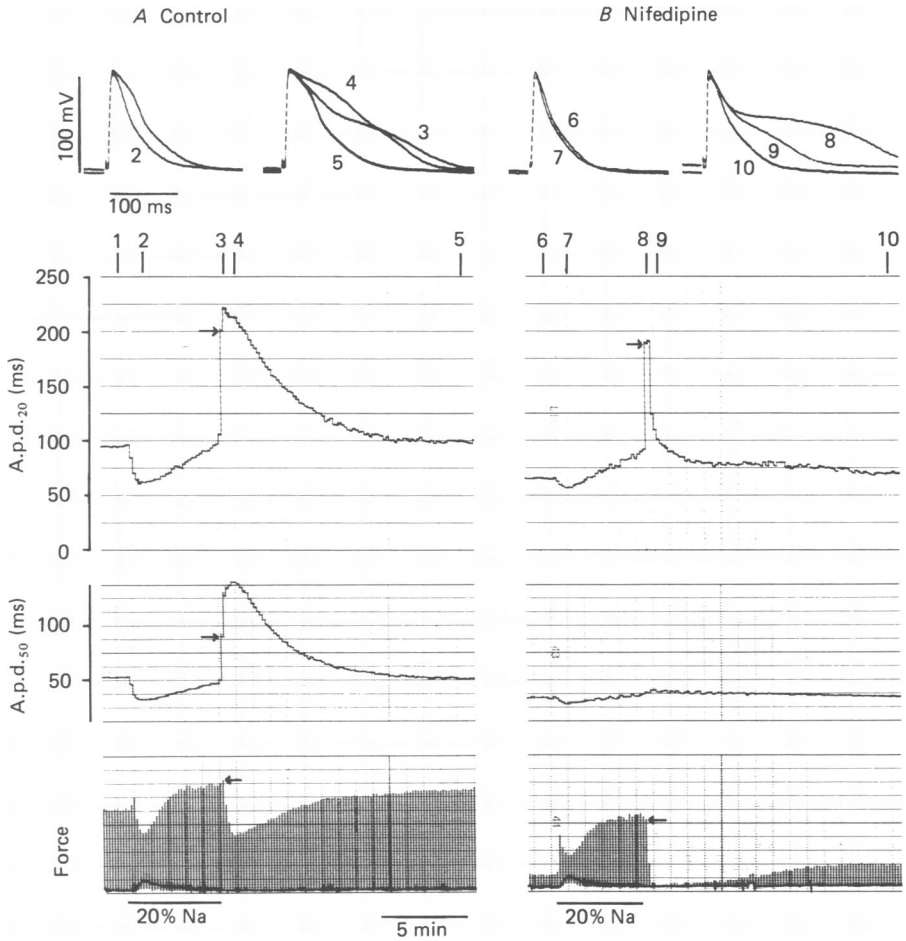

Fig. 6. The effect of low $[Na^+]_o$ on $a.p.d._{20}$, $a.p.d._{50}$ and contractile force in the absence (*A*) and in the presence of nifedipine (0·5 mg/l) (*B*). 120 mM-NaCl was substituted by 120 mM-LiCl for 5 min as indicated by the bars below the force traces. The numbers near each photographed action potential (upper panel) correspond to the numbers above the $a.p.d._{20}$ traces. Arrows in *A* and *B* indicate the values of $a.p.d._{20}$, $a.p.d._{50}$ and peak force of one of the first beats following reperfusion with normal $[Na^+]_o$.

Influence of nifedipine

The transient prolongation of the plateau ($a.p.d._{50}$) following reperfusion with normal $[Na^+]_o$ may indicate a greater influx of Ca^{2+}. This may have influenced the transient prolongation of the slow phase of repolarization ($a.p.d._{20}$). Therefore we repeated the low $[Na^+]_o$ experiment in the presence of nifedipine, a drug which effectively blocks i_{si} (Lee & Tsien, 1983; Mitchell, Powell, Terrar & Twist, 1983). Nifedipine shortened the action potential and reduced peak force. Subsequent reduction of $[Na^+]_o$ caused a further shortening of the action potential, but relatively less than in the absence of nifedipine, and caused a relatively greater increase of peak

force (Fig. 6*B*). Reperfusion with solution containing 100 % Na^+ induced a prolongation of $a.p.d._{20}$ and reduction of peak force. The prolongation of $a.p.d._{50}$ however was absent and $a.p.d._{20}$ returned more rapidly to its steady-state value than in the absence of nifedipine (compare Fig. 6*A* and *B*). This result shows that the initial rapid prolongation of $a.p.d._{20}$ is independent of Ca^{2+} influx via i_{si}. The slow return of $a.p.d._{20}$ to its steady state in Fig. 6*A* probably was a secondary effect of the parallel slow return of $a.p.d._{50}$, i.e. of enhanced Ca^{2+} influx.

DISCUSSION

The present study confirms a positive correlation between $a.p.d._{20}$ and peak force in rat myocardium, as has been observed in myocardium of other mammals (Vaughan Williams, 1959; Sleator *et al.* 1964; Coraboeuf & Vassort, 1968; Ravens, 1983). This was established both during the negative force staircase following post-extrasystolic potentiation (Fig. 1) and during mechanical and electrical restitution (Fig. 2). According to current ideas force reflects the amount of Ca^{2+} released into the sarcoplasm (see reviews by Wohlfart & Noble, 1982; Chapman, 1983; Fabiato, 1983). The simplest explanation for the present results is that intracellular Ca^{2+} activates the contractile filaments and the current responsible for the slow phase of repolarization. The same conclusion was drawn by Mitchell *et al.* (1984) from the fact that in myocytes isolated from rat heart the slow phase of repolarization was abolished when intracellular Ca^{2+} was buffered by EGTA. According to Allen & Kurihara (1980) the transient elevation of $[Ca^{2+}]_i$ persists until the moment of peak force in rat myocardium. This coincides with the slow phase of repolarization (Fig. 1*A*).

The negative correlation between $a.p.d._{50}$ and peak force (Figs. 1, 2 and 6) is probably due to a negative feed-back mechanism. Intracellular Ca^{2+} is thought to either activate a repolarizing K^+ current or to inactivate i_{si}, which both result in a negative relationship between the plateau of the action potential ($a.p.d._{50}$) and peak force. This mechanism probably tends to decrease Ca^{2+} influx via i_{si} if $[Ca^{2+}]_i$ is high (for a review see Boyett & Jewell, 1980). It has been shown that inactivation of i_{si} in myocytes of rat heart is accelerated by intracellular Ca^{2+} (Mitchell *et al.* 1983; Josephson, Sanchez-Chapula & Brown, 1984).

Influence of ionic composition

By means of voltage-clamp experiments Simurda *et al.* (1981) observed an inward current, activated at membrane potentials negative to the threshold potential of i_{si}. It corresponded well to the slow phase of repolarization in cat and dog papillary muscles. As possible charge carriers for inward current must be considered the ions Na^+, Ca^{2+} and Cl^-. Our results indicate that the slow phase of repolarization is neither due to a non-inactivating component of the rapid Na^+ current nor to i_{si} for the following reasons. The spike of the action potential results from the rapid Na^+ current (Lee *et al.* 1979; Mitchell *et al.* 1984), and was abolished in sucrose solution but not in Li^+ solution, whereas the slow phase was reduced in both cases (Fig. 4). This indicates that manipulation of the rapid Na^+ current does not necessarily affect the slow phase, but obviously extracellular Na^+ is important for the slow phase. Since reduction of $[Na^+]_o$ had the same effect on the slow phase as reduction of both $[Na^+]_o$

and $[Cl^-]_o$ it is concluded that Cl^- is probably not important. Similar results have been described for isolated myocytes (Mitchell *et al.* 1984).

At constant peak force an increase of $[Ca^{2+}]_o$ caused a reduction of the slow phase of repolarization (Fig. 3). Thus, extracellular Ca^{2+} has an influence on the slow phase but the effect would have been opposite if Ca^{2+} was important as a charge carrier. Furthermore, nifedipine, which is known to block i_{si} (Mitchell *et al.* 1983; Lee & Tsien, 1983), abolished the plateau of the action potential but not the transient prolongation of the slow phase of repolarization (Fig. 6*B*). These results indicate that i_{si} is not involved in the genesis of the slow phase.

In low $[Na^+]_o$ the action potential shortened and peak force increased. The latter results from a rise of $[Ca^{2+}]_i$ induced by low $[Na^+]_o$, which is attributed to inhibition of Ca^{2+} extrusion via Na^+–Ca^{2+} exchange (Sheu & Fozzard, 1982; Allen *et al.* 1983). Reperfusion with normal solution induced a transient prolongation of the action potential and a reduction of peak force (Figs. 5 and 6). This may be related to the intracellular Na^+ depletion that has been described for myocardium in low $[Na^+]_o$ (Keenan & Niedergerke, 1967; Deitmer & Ellis, 1978; Sheu & Fozzard, 1982; Chapman, Coray & McGuigan, 1983). If this was the case in our preparations it suggests that lowered $[Na^+]_i$ causes prolongation of the slow phase of repolarization.

Thus according to our results the slow phase of repolarization is prolonged by an increase of $[Na^+]_o$ and probably of $[Ca^{2+}]_i$ and is shortened by an increase of $[Ca^{2+}]_o$ and probably of $[Na^+]_i$.

Possible hypotheses

Simurda *et al.* (1981) have suggested three possible explanations for the inward current underlying the slow phase of repolarization: (a) a current generated in the membranes of the sarcoplasmic reticulum during Ca^{2+} release, (b) a background current activated by the transient increase of intracellular Ca^{2+} concentration during contraction, and (c) electrogenic Na^+–Ca^{2+} exchange.

From the hypothesis (a) it follows that strong contractions should coincide with a large and long-lasting current, and thus it is in agreement with the positive a.p.d.$_{20}$–F relationship. The hypothesis however does not explain the sensitivity of the slow phase of repolarization to the extracellular Na^+ and Ca^{2+} concentrations. Furthermore, it is contradicted by our observation that in low $[Na^+]_o$ enhancement of peak force coincided with a reduction of the duration of this phase. Ca^{2+} release is therefore probably of minor importance in the genesis of the slow phase of repolarization. Recently, Colquhoun, Neher, Reuter & Stevens (1981) reported evidence for a cation channel, activated by intracellular Ca^{2+}, in the sarcolemma of cells isolated from neonatal rat heart. Both Na^+ and K^+ permeate through this channel and an inward current will be generated at negative membrane potentials. This current can be considered as a Ca^{2+}-activated background current (hypothesis (b)). Its properties may explain the positive a.p.d.$_{20}$–F relationship and the reduction of a.d.p.$_{20}$, in sucrose solution. However, it is difficult to explain the observed shortening of a.p.d.$_{20}$, by LiCl solution and high $[Ca^{2+}]_o$, unless Li^+ does not permeate through the cation channel and extracellular Ca^{2+} inactivates the channel. Furthermore, the current through the cation channel should depend predominantly on $[K^+]_i$ and $[Na^+]_o$. Therefore the transient increase of the slow phase of repolarization following low

$[Na^+]_o$ perfusion is difficult to reconcile with this hypothesis as it is unlikely that $[K^+]_i$ was affected to a large extent. The cation channel is thus a less likely candidate for mechanisms underlying the slow phase of repolarization.

Na^+–Ca^{2+} exchange

The Na^+–Ca^{2+} exchange hypothesis (c) offers an explanation for all results presented here. This mechanism is thought to exchange 1 Ca^{2+} for about 3 Na^+ (Sheu & Fozzard, 1982) across the sarcolemma. The unequal charge transport generates a transmembrane current. The exchange carrier can transport either ion in both directions, and net transport depends on intracellular and extracellular concentrations of Na^+ and Ca^{2+} and on membrane potential. Under normal conditions the mechanism serves to extrude Ca^{2+} from the cells against its electrochemical gradient driven by Na^+ moving down its electrochemical gradient (for reviews see Reuter, 1974; Mullins, 1979; Fozzard, 1979; Chapman, 1983). It is to be expected, therefore, that an increase of the $[Na^+]$ gradient will cause an increase of the exchange current and vice versa, while an increase of the $[Ca^{2+}]$ gradient will cause a decrease of the exchange current and vice versa (Mullins, 1979). The observed effects of $[Na^+]_o$ and $[Ca^{2+}]_o$ on the slow phase of repolarization, and the probable relationship with $[Ca^{2+}]_i$ and $[Na^+]_i$, as discussed above, correspond to the expected effects on Na^+–Ca^{2+} exchange during the action potential. Na^+–Ca^{2+} exchange has been proposed to be responsible for hyperpolarization in K^+- and Na^+-free medium (Coraboeuf, Gautier & Guiraudou, 1981), for the transient inward current which accompanies after-contractions (Kass, Tsien & Weingart, 1978; Arlock & Katzung, 1982; Lipsius & Gibbons, 1982), and for the inward current induced by caffeine (Clusin, Fischmeister & DeHaan, 1983). Model simulations predict an influence on the action potential (Di Francesco, Hart & Noble, 1982; Fischmeister & Vassort, 1981). The present results also strongly suggest that the slow phase of repolarization reflects electrogenic Na^+–Ca^{2+} exchange.

The balance between influx and efflux of Ca^{2+}

The transients observed following a period of low $[Na^+]_o$ can be explained as follows. At low $[Na^+]_o$ intracellular Ca^{2+} accumulation and Na^+ depletion occurs (Sheu & Fozzard, 1982). Upon reperfusion with normal $[Na^+]_o$, Ca^{2+} extrusion via Na^+–Ca^{2+} exchange is instantaneously enhanced, i.e. $a.p.d._{20}$ is instantaneously prolonged. Subsequently, the accumulated Ca^{2+} is rapidly extruded and peak force drops within a few beats. According to Wasserstrom, Schwartz & Fozzard (1983) slow changes in $[Na^+]_i$ will cause similar slow changes in twitch force. Thus, as a consequence of low $[Na^+]_i$, peak force drops to a subnormal level and gradually increases as $[Na^+]_i$ increases to its normal level. Such changes of $[Na^+]_i$ generally occur over periods up to 20 min in duration (Ellis, 1977; Eisner, Lederer & Vaughan-Jones, 1984), which is comparable to the slow increase of peak force presently reported.

It was discussed above that a reduction of peak force is associated with prolongation of $a.p.d._{50}$, and with prolonged Ca^{2+} influx via i_{si}. This large Ca^{2+} influx leads to a large Ca^{2+} efflux (prolonged $a.p.d._{20}$). Therefore, the transient prolongation of $a.p.d._{20}$ and $a.p.d._{50}$ and transient reduction of force last as long as $[Na^+]_i$ is low. It is, however, crucial to note that the prolongation of $a.p.d._{20}$ preceded the changes of peak force

and a.p.d.$_{50}$ by a few beats (arrows in Fig. 6*A*). The experiments with nifedipine support the above explanation. In the presence of this drug Ca^{2+} influx via i_{si} is blocked as reflected by a short a.p.d.$_{50}$ and the absence of changes of a.p.d.$_{50}$ upon return to normal $[Na^+]_o$. A.p.d.$_{20}$ was then only prolonged during the first strong contractions at normal $[Na^+]_o$. It is expected that changes of $[Na^+]_i$ are accompanied by changes in the activity of the Na^+–K^+ pump. The experiments with nifedipine indicate that this electrogenic pump influenced the results to a minor extent.

In conclusion, the slow phase of repolarization of the myocardial action potential is due to an inward current (Simurda *et al.* 1981) which flows as a result of a rise of intracellular Ca^{2+} and which is sensitive to $[Na^+]_o$ (Mitchell *et al.* 1984), $[Ca^{2+}]_o$ and $[Na^+]_i$. The Na^+–Ca^{2+} exchange hypothesis accounts for all the observed phenomena.

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