Changes in ventricular repolarization during acidosis and low-flow ischemia

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Myocardial ischemia, primarily a metabolic insult, is also defined by altered cardiac mechanical and electrical activity. We have investigated the metabolic contributions to the electrophysiological changes during low-flow ischemia (7.5% of the control flow) using 31P NMR spectroscopy to monitor metabolic parameters, suction electrodes to study epicardial monophasic action potentials, and 86Rb as a tracer for K+ equivalent efflux during low-flow ischemia in the Langendorff-perfused ferret heart. Shortening of the action potential duration at 90% repolarization (APD90) was most marked between 1 and 5 min after induction of ischemia, at which time it shortened from 261 ± 4 to 213 ± 8 ms. The period of marked APD90 shortening was accompanied by a fivefold increase in the rate of 86Rb efflux, both of which were inhibited by the ATP-sensitive K+ (KATP)-channel blockers glibenclamide and 5-hydroxydecanoate (5-HD), as well as by a significant fall in intracellular pH (pHi) from 7.14 ± 0.02 to 6.83 ± 0.03 but no change in intracellular ATP concentration ([ATP]). We therefore investigated whether a fall in pHi could be the metabolic change responsible for modulating cardiac KATP-channel activity in the intact heart during ischemia. Both metabolic (30 mM lactate added to extracellular solution) and respiratory (PCO2 increased to 15%) acidosis caused an initial shortening of APD90 to 112 ± 1.5 and 113 ± 0.9%, respectively, followed by shortening during continued acidosis to 106 ± 1.2 and 106 ± 1.4%, respectively. The shortening of APD90 during continued acidosis was inhibited by glibenclamide, consistent with acidosis causing activation of KATP channels at normal [ATP]. The similar responses to metabolic (induced by adding either l- or d-lactate) and respiratory acidosis suggest that lactate has no independent metabolic effect on action potential repolarization.

Changes in ventricular repolarization during acidosis and low-flow ischemia. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H551–H561, 1998.—Myocardial ischemia, primarily a metabolic insult, is also defined by altered cardiac mechanical and electrical activity. We have investigated the metabolic contributions to the electrophysiological changes during low-flow ischemia (7.5% of the control flow) using 31P NMR spectroscopy to monitor metabolic parameters, suction electrodes to study epicardial monophasic action potentials, and 86Rb as a tracer for K+ equivalent efflux during low-flow ischemia in the Langendorff-perfused ferret heart. Shortening of the action potential duration at 90% repolarization (APD90) was most marked between 1 and 5 min after induction of ischemia, at which time it shortened from 261 ± 4 to 213 ± 8 ms. The period of marked APD90 shortening was accompanied by a fivefold increase in the rate of 86Rb efflux, both of which were inhibited by the ATP-sensitive K+ (KATP)-channel blockers glibenclamide and 5-hydroxydecanoate (5-HD), as well as by a significant fall in intracellular pH (pHi) from 7.14 ± 0.02 to 6.83 ± 0.03 but no change in intracellular ATP concentration ([ATP]). We therefore investigated whether a fall in pHi could be the metabolic change responsible for modulating cardiac KATP-channel activity in the intact heart during ischemia. Both metabolic (30 mM lactate added to extracellular solution) and respiratory (PCO2 increased to 15%) acidosis caused an initial shortening of APD90 to 112 ± 1.5 and 113 ± 0.9%, respectively, followed by shortening during continued acidosis to 106 ± 1.2 and 106 ± 1.4%, respectively. The shortening of APD90 during continued acidosis was inhibited by glibenclamide, consistent with acidosis causing activation of KATP channels at normal [ATP]. The similar responses to metabolic (induced by adding either l- or d-lactate) and respiratory acidosis suggest that lactate has no independent metabolic effect on action potential repolarization.

It is well established that an increased efflux of potassium ions and their extracellular accumulation characterize myocardial ischemia (25) and that the resulting effects on action potential duration (APD), conduction velocity, and refractoriness (19) are likely to be key factors in the genesis of ischemia-associated ventricular arrhythmias (25). Shortening of APD is reduced or prevented by sulfonylureas such as glibenclamide, which has implicated the ATP-sensitive K+ (KATP) channel as a potential mediator of these events (14, 48, 51). KATP channels are classically activated by a fall in intracellular ATP concentration ([ATP]); however, during ischemia the apparent activation of KATP channels occurs within 2–5 min (33), which is well before there is any fall in bulk [ATP] (29). This has led to speculation that other factors must be responsible for activating KATP channels during ischemia, e.g., changes in intracellular ADP concentration ([ADP]), intracellular pH (pHi) or intracellular lactate concentration (reviewed in Ref. 19). Which, if any, of these factors are important remains to be determined.

In the first part of this study we investigated the correlation between metabolic status, potassium efflux, and APD during ischemia. The results from these studies suggested that acidosis may be a determinant of activation of KATP channels under these conditions. We therefore investigated the effect of acidosis on KATP-channel activation in the intact heart. We used the Langendorff-perfused heart because this preparation allows adequate reproduction of the ischemic insult (49) along with the serial measurement of essential parameters, i.e., metabolic status (using 31P NMR spectroscopy), K+ efflux (using the K+ congener 86Rb), and APD (recording monophasic action potentials (mAP) with suction electrodes; Ref. 8). The estimation of K+ equivalent flux requires a protocol that maintains flow, and therefore hearts have been subjected to global low-flow ischemia rather than absolute zero-flow ischemia. The contribution of KATP channels to K+ efflux and APD changes during ischemia and acidosis has been investigated by using glibenclamide (33) and 5-hydroxydecanoate (5-HD) (35).

Methods

Heart Preparation

The monitoring and preparation of the Langendorff-perfused ferret heart have been extensively described (1, 10, 29). In the present study male ferrets, 3–12 mo of age, were anesthetized with pentobarbital sodium (250 mg/kg ip; May and Baker, Dagenham, UK). Hearts were excised and arrested in ice-cold 25 mM HCO3 solution (see 25 mM HCO3 solution), and the aorta was cannulated. Hearts were then Langendorff perfused with 25 mM HCO3 solution at constant flow (5–6 ml g−1 min−1) at 30°C, providing a well-characterized, stable preparation (10, 45, 46). All solutions were passed through a 5-µm filter and were not recirculated, except during the loading of 86RbCl where recirculation was required to minimize the potential hazards associated with using radioactive solutions. The atria were removed, and the atrioventricular (AV) node was crushed to abolish AV conduction. Hearts were paced at 1 Hz using a 2-ms square wave stimulus via platinum electrodes inserted into the epicardium of the right ventricle, using twice the threshold voltage (typically 1.4%, respectively. The shortening of APD90 increased to 15% acidosis caused an initial shortening of APD90 to 112 ± 1.5 and 113 ± 0.9%, respectively, followed by shortening during continued acidosis to 106 ± 1.2 and 106 ± 1.4%, respectively. The shortening of APD90 during continued acidosis was inhibited by glibenclamide, consistent with acidosis causing activation of KATP channels at normal [ATP]. The similar responses to metabolic (induced by adding either l- or d-lactate) and respiratory acidosis suggest that lactate has no independent metabolic effect on action potential repolarization.
which pH was monitored using the chemical shift of the NMR signal for intracellular Pi ([Pi]i). KH$_2$PO$_4$ was removed from the perfusion solution and replaced isosmotically with KC$_2$ to ensure that the pH$_i$ signal was that due to intracellular Pi. Previous studies have shown that perfusion of ferret hearts with Pi-free medium for up to 4 h does not significantly alter cardiac mechanical performance or bioenergetic status (45). All perfusion reagents were analytical grade purity, Fluka Chemicals, Gillingham, UK). 5-HD (Research Biochemicals, Natick, MA) was dissolved in perfusion solutions at a concentration, 100 µM, that has been used in previous studies to achieve near-maximal inhibition of cardiac K$_{ATP}$ channel activity (35). Hearts were loaded with either of the drugs for 30 min before a period of ischemia.

**Experimental Protocols**

Ischemia. Hearts were subjected to low-flow (7.5%) or zero-flow ischemia for 30 min and then reperfused. In most experiments hearts were allowed to recover for 2 h after a first episode of 7.5% low-flow ischemia and then subjected to a second period of 7.5% low-flow ischemia. In control hearts (n = 4) with no other experimental interventions, the second episode of ischemia was not different from the first episode as indicated by similar resting (preechymic) phosphocreatine concentration ([PCr]) to [ATP] ratios and similar patterns of LVDP and APD change during both ischemia and reperfusion (data not shown).

Acidosis. Acidosis was induced by increasing either lactate (lactic acidosis) or CO$_2$ (respiratory acidosis) in the perfusate. The purpose of using the different methods for the induction of acidosis was to discriminate the potential independent effects of lactate and, as such, these experiments can never be pure interventions, i.e., lactic acidosis will invariably be associated with a "respiratory" component. The pH$_o$ during these interventions (6.85) was similar to the pH$_o$ during the first 10 min of low-flow ischemia in the Langendorff-perfused ferret heart. pH$_i$ was monitored using a standard pH electrode calibrated before each set of experiments. Hearts were perfused with the acidic solutions for 6 min. APD was monitored for 1-2 min before the acidosis intervention, for 6 min during acidosis, and for 6 min during recovery using the standard perfusion solution.

**31P NMR Spectroscopy**

31P NMR experiments were performed using a wide-bore spectrometer (see Heart Preparation) operated in the pulsed Fourier transform mode, with a dual-tuned (H1/31P) NMR coil, as described previously (10, 45). During normal perfusion (5-6 ml·g$^{-1}$·min$^{-1}$) spectra were acquired over 5 min, which was the minimum time required to obtain an adequate signal-to-noise ratio (>5:1) for P$_i$. During ischemia there is a rapid increase in P$_i$, which can reach up to 10-20 mM after 10 min ischemia in the Langendorff-perfused ferret heart (1) and, under these conditions, a sufficient signal-to-noise ratio could be obtained in spectra acquired over 15-60 s (45). pH$_i$ was calculated from the chemical shift of the P$_i$ resonance relative to the PCr resonance, and relative phosphate metabolite concentrations were determined from the areas of the 31P NMR resonances, taking into account the differential saturation of resonances caused by incomplete relaxation of magnetization at high pulsing frequencies (45). [ADP] was derived from the mass action equation of creatine kinase equilibrium, assuming that the total creatine concentration of 17 mM remained constant throughout the experiment and using an equilibrium constant for creatine kinase of 2 x 10$^9$ M$^{-1}$ at 30°C and 1.66 x 10$^9$ M$^{-1}$ at 37°C (47).

mAP

Complementary suction electrodes for recording mAP (8) were constructed using two 1-mm-diameter Ag-AgCl$_2$ electrodes with 2-mm separation sealed into a Perspex header. The suction electrode was apposed to the left ventricle with contact maintained using negative pressure (Charles Austen D7C pump, Merck, Poole, UK). Action potentials were amplified (Gould universal amplifier model 13-4615-58;
Gould Electronics, Ilford, UK) and recorded on both a Gould 2400S chart recorder and a 486 DX2 Viglen PC (Viglen, Alperton, UK) interfaced with a CED 1401-plus analog-to-digital converter operated using Spike 2 software (both from Cambridge Electronic Design, Cambridge, UK). The duration of the mAP at 90% repolarization (APD90) was calculated using a standard method (8) automated using a custom-written Quick Basic computer program. Occasionally, electrical alternans was seen in conjunction with mechanical alternans (see Heart Preparation); under such circumstances and as for LVDP, means of consecutive beats were calculated.

**86Rb Efflux**

After the 2-h loading period, hearts were perfused with standard HCO3 solution for 15 min to wash out any extracellular 86Rb before specific experimental interventions. All effluent from the start of the washout period to the end of ischemia was collected, over 15-s intervals, in separate aliquots for later counting using a beta liquid scintillation counter (Packard Tri Carb 460C, Packard Instruments). The time taken between the effluent leaving the heart and reaching the collection point was recorded in every experiment, both under resting conditions and during ischemia, using 0.5% bromophenol blue dye (Sigma Chemicals), and the time course for 86Rb efflux during ischemia was corrected accordingly.

The time constant for 86Rb efflux was calculated as the fractional efflux rate (FER) (17). Total activity in the heart at the beginning of the experiment was calculated from the sum of the cumulative loss of activity from the heart during the experiment and the residual activity in the heart at the end of the experiment. Residual activity was estimated at the end of experiments by dissolving a sample of left ventricular apex (2.7 ± 0.8% total heart weight, n = 14) in 5 ml of 1 N nitric acid for 1 h. The pH of the solution was then corrected, 20 ml of 0.1% Nonidet P-40 (BDH Laboratories, Poole, UK) were added, and the sample was centrifuged at 5,000 rpm for 10 min. Five milliliters of 15% trichloroacetic acid were added to precipitate the protein in the supernatant, and the sample was again centrifuged for 5 min. The resultant supernatant was divided into four aliquots with a 0.1-ml sample from each added to 5 ml of liquid scintillant for the beta scintillation counter. Dissolving the heart in this manner has been reported to result in recovery of ~95% of the residual activity in the sample (30). The mean of the four counts was corrected for the dilution factor and used to calculate the residual activity in the whole heart.

**Data Analysis**

All data were analyzed using Microsoft Excel version 5.0 (Microsoft). All results are means ± SE. Statistical comparisons were made using analysis of variance, and P values of <0.05 were taken to indicate significant differences (2).

**RESULTS**

**Low-Flow Ischemia in Perfused Heart**

Serial 31P NMR spectra obtained from a single heart before, during, and after 30 min of 7.5% low-flow ischemia at 30°C are shown in Fig. 1A, and the averaged results of changes in metabolic parameters and LVDP for all hearts (n = 6) subjected to 30 min of 7.5% low-flow ischemia are summarized in Fig. 1B. Under resting conditions the [PCr]-to-[ATP] ratio was 2.4 ± 0.2, which is similar to those reported previously in perfused ferret heart (1, 45). The insult was more severe at zero flow, compared with 7.5% low-flow ischemia (data not shown); nevertheless, the metabolic and functional changes indicate that 7.5% low flow produced significant ischemia (13). The 7.5% low-flow rate was the lowest flow rate at which accurate 86Rb efflux measurements could be made, so all subsequent ischemia experiments were carried out at this flow rate. The majority of subsequent experiments were carried out at 30°C rather than at 37°C. At 30°C the patterns of metabolic and contractile responses were similar albeit slightly less rapid than at 37°C (see Table 1). However, at 30°C AV nodal conduction was more easily obliterated and there were fewer spontaneous ventricular extra beats, which distort analysis of APD90.

To correlate changes in metabolic parameters with those in APD90, a series of experiments were carried out in which mAP were obtained from hearts perfused within the NMR spectrometer. The deterioration in the signal-to-noise ratio in the presence of the mAP electrode, however, precluded detailed simultaneous assessment of changes in phosphate metabolite concentrations, pHi, and APD (data not shown). Therefore, subsequent mAP experiments were performed on the bench under conditions identical to those of the NMR experiments.

**APD90 Changes During Low-Flow Ischemia**

A typical example of the time course of changes in APD90 during 7.5% low-flow ischemia at 30°C is illustrated in Fig. 2A, with individual mAP recordings and APD90 at the times indicated in Fig. 2A shown in Fig. 2B. The averaged values for 15 hearts are shown in Fig. 2C. Over the first minute there was a significant increase in APD90 from 236 ± 4 to 261 ± 4 ms (P < 0.01), followed by shortening that reached a nadir of 213 ± 8 ms (P < 0.01 compared with resting APD90). The mean time to peak shortening was 1.1 ± 0.1 min, and the mean time to nadir was 6.4 ± 0.5 min. Reperfusion also caused significant shortening of APD90 (see Fig. 2C) before recovery to pres ischemic values.

APD is temperature dependent and shortens as temperature increases (24). Initial APD shortening during ischemia has been proposed to be caused by decreased epicardial temperature accompanying diminished flow (6). This possibility was tested by comparing the electrophysiological responses of hearts covered with buffer solution in a warmed water jacket with those of hearts left uncovered. During uncovered experiments (n = 5), right ventricular endocardial temperature fell from 30.3 ± 0.2 to 29 ± 0.3°C (P < 0.05) during ischemia and APD90 increased to 113 ± 2% of baseline, whereas during covered experiments (n = 3) the temperature did not fall (control = 30.3 ± 0.2°C; ischemia = 30.3 ± 0.6°C) and APD90 increased to 109 ± 2% of baseline. Thus, in the uncovered experiments, it is possible that the 1°C decrease in temperature contributed to the initial APD90 shortening, although it cannot account for it entirely.
Ischemia resulted in an increase in the $^{86}$Rb FER. During the first minute of ischemia there was a small but insignificant increase in the $^{86}$Rb FER from $1.98 \pm 0.33^{10^{-3}}$ min$^{-1}$ to $2.26 \pm 0.40^{10^{-3}}$ min$^{-1}$ after 30 s and to $3.10 \pm 0.50^{10^{-3}}$ min$^{-1}$ after 1 min (see Fig. 3, A and B). Thereafter, there was a rapid increase, reaching $10.10 \pm 1.90^{10^{-3}}$ min$^{-1}$ after 5 min ($P < 0.05$ compared with the $^{86}$Rb FER at the onset of ischemia). The $^{86}$Rb FER then gradually declined but remained significantly greater than the FER at the onset of ischemia throughout the rest of the ischemic period.

Effect of Glibenclamide on APD$_{90}$ Shortening and $^{86}$Rb Efflux During Ischemia

Loading hearts with 10 µM glibenclamide for 30 min did not significantly affect the resting mechanical or electrophysiological properties of the heart; LVDP was $94 \pm 7\%$ of that before drug loading ($n = 6$ hearts; $P = 0.05$, 30°C vs. 37°C). The fall in LVDP during ischemia and recovery on reperfusion were unaffected by the presence of glibenclamide (data not shown), suggesting that glibenclamide did not significantly modify the severity of the ischemic insult. Glibenclamide did, however, abolish shortening of the action potential during ischemia, with APD$_{90}$ remaining significantly prolonged throughout ischemia compared with both preischemic values and with the APD$_{90}$ in the control group (see Fig. 4A). pHi was not measured after the addition of glibenclamide.
mide or 5-HD. There is, however, a well-described relationship between pH_i and LVDP (see Ref. 46 for discussion), and the fact that neither glibenclamide nor 5-HD had any significant effect on LVDP would be consistent with no significant pH_i effect.

Glibenclamide and ^86^Rb during ischemia. The resting ^86^Rb FER in the presence of glibenclamide (1.7 ± 0.6 × 10^{-3} min^{-1}; n = 6 hearts) was not significantly different from that under control conditions (1.9 ± 0.3 × 10^{-3} min^{-1}; n = 5 hearts). However, glibenclamide significantly attenuated the increase in ^86^Rb FER reaching a peak of 5.2 ± 1.2 × 10^{-3} min^{-1} in the presence of glibenclamide compared with a peak of 10.1 ± 1.9 × 10^{-3} min^{-1} in the absence of glibenclamide (P < 0.05). The ^86^Rb FER after 5 min, in the presence of glibenclamide, was nevertheless significantly higher than the values during the first 30 s of ischemia. There was also a gradual decline in the ^86^Rb FER in the glibenclamide-loaded hearts over the rest of the ischemic period. The significant attenuation of the increase in FER, in the presence of glibenclamide, correlated with the abolition of APD_{90} shortening (Fig. 4, A and B). Therefore, addition of glibenclamide reduced the ^86^Rb FER during ischemia by ~50% and abolished shortening of the APD_{90}.

Fig. 2. A: typical time course of changes in action potential duration at 90% repolarization (APD_{90}) during 30 min of 7.5% low-flow ischemia. B: example of monophasic action potential (mAP) morphology at time points indicated in A; APD_{90} increased from 232 ms (i) to 252 ms (ii) after 1.25 min of ischemia, then decreased to 210 ms (iii) after 7.5-min ischemia before recovering slightly to 225 ms (iv) at end of 30-min ischemia. C: averaged changes in APD_{90} during 30-min 7.5% low-flow ischemia (means ± SE; n = 15 hearts).

Fig. 3. A: typical time course, taken from a single experiment, of changes in ^86^Rb fractional efflux rate (FER) during the 5 min before ischemia, 30 min of 7.5% low-flow ischemia, and 10 min of reperfusion. Control flow rate, 42 ml/min, during washout and reperfusion, compared with 3 ml/min during ischemia, accounts for higher baseline ^86^Rb FER during washout and reperfusion compared with ischemic FER. During reperfusion there was a sharp increase in FER of ^86^Rb before a return to baseline washout values. This is likely to be the result of sudden washout of ^86^Rb accumulated in the extracellular space during ischemia (Refs. 50, 52) with further contributions after possible cell lysis caused by protracted ischemia (Ref. 22). B: changes in ^86^Rb FER during ischemia shown on 2 time scales to highlight initial delay before any rise in ^86^Rb FER followed by rapid rise and, finally, slower changes over 5–30 min of ischemia. ^86^Rb FER increased from 1.3 × 10^{-3} min^{-1} to 10.3 × 10^{-3} min^{-1} after 6.5 min of ischemia before declining to 6.1 × 10^{-3} min^{-1} at end of ischemia.
In view of the reported nonspecific effects of glibenclamide (21), we investigated the effects of 5-HD, another inhibitor of the KATP channel (35), on 86Rb efflux during ischemia. 5-HD did not affect baseline 86Rb FER ($1.5 \pm 0.5 \times 10^3$ min$^{-1}$) in 5-HD-loaded hearts ($n = 3$) compared with control hearts ($n = 5$). However, after 5 min of ischemia the 86Rb FER increased to only $3.6 \pm 1.2 \times 10^3$ min$^{-1}$ in the presence of 5-HD compared with $10.1 \pm 1.9 \times 10^3$ min$^{-1}$ in control hearts ($n = 5$). 5-HD abolished shortening of APD$_{90}$ during ischemia (data not shown).

5-HD and 86Rb efflux during ischemia. In view of the effects of 5-HD, another inhibitor of the K$_{ATP}$ channel (35), on 86Rb efflux during ischemia, we investigated the effects of 5-HD during 7.5% low-flow ischemia. 5-HD did not affect baseline 86Rb FER ($1.5 \pm 0.5 \times 10^3$ min$^{-1}$) in 5-HD-loaded hearts ($n = 3$) compared with 1.9 $\pm$ 0.3 $\times$ 10$^3$ min$^{-1}$ in control hearts ($n = 5$). However, after 5 min of ischemia the 86Rb FER increased to only $3.6 \pm 1.2 \times 10^3$ min$^{-1}$ in the presence of 5-HD compared with $10.1 \pm 1.9 \times 10^3$ min$^{-1}$ in control hearts ($P < 0.05$, see Fig. 4C). Once again, there was a gradual, although insignificant, decline in the 86Rb FER in both groups (see text for details).

APD$_{90}$, 86Rb Efflux, and Metabolic Parameters During Low-Flow Ischemia

The averaged changes in APD$_{90}$ during 7.5% low-flow ischemia in relation to the changes in 86Rb efflux, pH$_i$, and metabolites are illustrated in Fig. 5. The period of APD$_{90}$ shortening is closely correlated with a rapid rise in 86Rb FER as well as the rapid phase of fall in pH$_i$ from 7.14 to 6.8. The period of APD$_{90}$ shortening was also associated with increases in [P$_i$], and [ADP],
although the most significant changes in these parameters preceded the period of APD$_{90}$ shortening. There was no significant change in [ATP] during the 30-min 7.5% low-flow ischemic episode.

Because acidosis appeared to be the most significant metabolic change during the period of glibenclamide- and 5-HD-sensitive ischemic APD$_{90}$ shortening we investigated whether acidosis per se could contribute to activation of K$_{ATP}$ channels.

**Effect of Acidosis on APD$_{90}$**

Typical changes in LVDP in a Langendorff-perfused ferret heart during lactic acidosis are illustrated in Fig. 6A, the patterns of mAP change are shown in Fig. 6B, and the averaged results of nine experiments showing changes in APD$_{90}$ are shown in Fig. 6C. The APD$_{90}$ initially increased but thereafter decreased from a maximum of 112 ± 1.5% of baseline (after 1–1.5 min) to a minimum of 106 ± 1.2% at the end of acidosis (P < 0.05 compared with the mean maximum APD$_{90}$). LVDP fell rapidly to ~25% of control and showed no recovery during acidosis.

The effects of respiratory acidosis on APD$_{90}$ and LVDP were similar to those observed for lactic acidosis (compare Figs. 6C and 7). The mean maximum APD$_{90}$ during respiratory acidosis was 113 ± 0.9% of baseline (compared with 112 ± 1.5% for lactic acidosis), and the minimum APD$_{90}$ during the subsequent shortening of APD$_{90}$ was 106 ± 1.4% (compared with 106 ± 1.2% for lactic acidosis). The changes in LVDP were also similar during respiratory and lactic acidosis and were consistent with only partial recovery of pH$_i$ as previously documented (46).

**Effects of Glibenclamide on APD$_{90}$ During Acidosis**

The recovery of APD$_{90}$ during continued acidosis occurred despite there being no recovery in LVDP, consistent with APD$_{90}$ recovery being only partially dependent on pH$_i$. In view of previous studies suggesting that both intracellular protons and lactate can activate K$_{ATP}$ channels in isolated cells and membrane patches (4, 12, 23, 26), we investigated whether the recovery of APD$_{90}$ during continued acidosis was sensitive to the K$_{ATP}$-channel inhibitor glibenclamide.

Glibenclamide (10 µM) did not alter baseline LVDP, APD$_{90}$, or pH$_o$, and the fall in pH$_i$ and LVDP during acidosis was the same in glibenclamide and glibenclamide-free groups, suggesting that the change in pH$_i$ was similar in both groups (46) and that glibenclamide did not affect the rate of acid loading. The averaged changes in APD$_{90}$ during lactic acidosis with (n = 4 hearts) and without (n = 9 hearts) glibenclamide are shown in Fig. 8A. The initial increase in APD$_{90}$ was slightly greater in the presence of glibenclamide (114 ± 1.2% compared with 112 ± 1.5% in control hearts), but this difference was not statistically significant. However, glibenclamide abolished the subsequent recovery of APD$_{90}$ during continued acidosis (see Fig. 8B). Glibenclamide also reduced the extent of APD$_{90}$ shortening.
during continued acidosis induced by increasing \( \text{PCO}_2 \) (see Fig. 8B) although the response was less dramatic than that seen during lactic acidosis.

**Contribution of Lactate Metabolism**

To test whether lactate had an independent metabolic effect that would influence the change in APD\(_{90}\) during acidosis, we compared the effects of l-lactate and the nonmetabolized stereoisomer d-lactate on APD\(_{90}\) and LVDP in five hearts. The profile of change in APD\(_{90}\) and LVDP during d- and l-lactic acidosis were similar (data not shown). The slight difference between the effects of d-lactate and l-lactate on the rate of change of LVDP and APD\(_{90}\) is consistent with the slower rate of acid loading with d-lactate on account of its slower transport via the monocarboxylate-H\(^+\) cotransporter compared with l-lactate (38). This would therefore suggest that increased intracellular lactate does not alter metabolism in a way that has a significant effect on the change in APD\(_{90}\) during acidosis.

**DISCUSSION**

Ischemia is a metabolic insult functionally defined by the loss of normal cardiac mechanical and electrical activity. The relationship between the metabolic disturbances of ischemia and contractile dysfunction has been extensively investigated (29), with somewhat less interest in the metabolic contribution to the electrophysiological changes occurring during ischemia (19).

**Electrophysiological Changes and \(^{86}\text{Rb}\) Efflux During Ischemia**

Low-flow ischemia, in the perfused heart, caused an initial increase in APD\(_{90}\). The results presented suggest that a decrease in epicardial temperature may contribute to this phenomenon being additional to APD\(_{90}\) prolongation because of transient outward current (I\(_{\text{to}}\)) inhibition (49). The subsequent APD\(_{90}\) shortening was accompanied by a rapid increase in \(^{86}\text{Rb}\) FER. These patterns of response are broadly similar to those described previously (6, 7, 15).

Direct mechanistic analysis of these phenomena was not possible, because when we tried to obtain simultaneous metabolic and electrophysiological measurements in the perfused hearts there was an approxi-


**Fig. 8.** A: lactic acidosis in control (○, n = 9) vs. glibenclamide-loaded (□, n = 4) hearts. Loading hearts with 10 \( \mu \text{M} \) glibenclamide did not alter baseline APD\(_{90}\). During acidosis there was a similar increase in APD\(_{90}\) over the first 2 min of acidosis, but in presence of glibenclamide there was no subsequent shortening of APD\(_{90}\). Under these conditions recovery of APD\(_{90}\) during acidosis is glibenclamide sensitive. B: recovery of APD\(_{90}\) during both lactic (Lac) and respiratory (Resp) acidosis was significantly attenuated by glibenclamide (Glib). *P < 0.05 vs. no glibenclamide under each condition. Numbers in parentheses indicate numbers of hearts.

mately fivefold reduction in the signal-to-noise ratio of NMR spectra obtained when suction electrodes were attached to the heart (compare Figs. 1A and 2). These considerations precluded acquisition of NMR data of high temporal resolution with simultaneous mAP recordings. These problems may be surmounted by using alternative techniques to record mAP (8) or by considering the use of other electrophysiological techniques, e.g., activation recovery intervals (11, 32), and thereby improving the temporal resolution of NMR spectra. One further experimental design consideration is that the electrophysiological recordings were obtained from epicardium, whereas estimations of K\(^+\) efflux and metabolic parameters were obtained from the whole heart. In this regard, the patterns of ion channel expression in epicardial cells may not fully reflect global expression patterns (31) and should be considered in future studies in this area. Even taking these limitations into account, the results of the present study do allow the comparison of metabolic and electrophysiological recordings studied under identical conditions and enable us to define indirectly the metabolic determinants of the electrical disturbances of ischemia.

**Metabolic Determinants of APD Shortening**

APD shortening is thought to be a primary determinant of enhanced arrhythmogenesis during ischemia (19). Considerable data suggest that the accumulation of potassium in the extracellular space is an important determinant of APD shortening (50, 52). However, the close correlation between APD\(_{90}\) shortening and the phase of rapid increase in \(^{86}\text{Rb}\) efflux observed here is in agreement with previous studies (14, 48) and consistent with an increased net outward potassium current also mediating this effect. This hypothesis is further reinforced by our findings that both glibenclamide and 5-HD, inhibitors of K\(_{\text{ATP}}\) channels, caused reductions (50–65%) in \(^{86}\text{Rb}\) efflux and abolished APD\(_{90}\) shortening. Glibenclamide not only prevented ischemic APD\(_{90}\) shortening but also maintained APD\(_{90}\) prolongation throughout ischemia. Although inhibition of K\(_{\text{ATP}}\) channels would decrease the net outward current during ischemia and, by allowing a net inward current to predominate, cause APD\(_{90}\) prolongation, glibenclamide has also been found to inhibit other channels that may...
contribute to APD\textsubscript{90} shortening during ischemia, e.g., cAMP-activated chloride channels (44). These considerations may account for the greater effect of glibenclamide in preventing APD\textsubscript{90} shortening in comparison with its limited effect on the reduction of \textsuperscript{86}Rb efflux.

There is general agreement that activation of K\textsubscript{ATP} channels accounts for much of the K\textsuperscript{+} efflux and APD\textsubscript{90} shortening during ischemia; however, what activates these channels remains unresolved (43). K\textsubscript{ATP} channels are classically activated by a fall in [ATP] \textsubscript{i} (34); however, in our experiments, during the period of APD\textsubscript{90} shortening there was essentially no change in [ATP], (see Fig. 1B). [ADP], has also been implicated in the modulation of these channels (43), but in our study the most rapid changes in [ADP], occurred before APD\textsubscript{90} shortening and increased \textsuperscript{86}Rb efflux (see Fig. 5). These observations do not necessarily exclude a role for [ATP], or [ADP], but are also consistent with the involvement of other factors. One other such parameter that is known to change during the period of APD\textsubscript{90} shortening is [P\textsubscript{i}]. However, Lederer and Nichols (28) have shown that an [P\textsubscript{i}] of up to 20 mM does not appear to affect the channels. The most rapidly changing parameter during APD\textsubscript{90} shortening was pHi, (see Fig. 5). A fall in pHi, has been shown to cause K\textsubscript{ATP}-channel activation in isolated feline and guinea pig ventricular myocytes (4, 26), and these results, in combination with our own, suggested that it would be worth investigating further the role of acidosis in activating K\textsubscript{ATP} Channels in the intact heart.

Effect of Acidosis on APD

Acidosis is a characteristic feature of myocardial ischemia and significantly contributes to ischemic contractile failure (37). Acidosis also has important effects on cardiac electrophysiology and has been suggested to predispose both to reentry and to triggered arrhythmias (36). The mechanisms underlying these effects of acidosis on cardiac electrical activity, however, remain uncertain; for example, acidosis has been reported to produce both lengthening (9, 20, 42) and shortening (27, 40) of the action potential. Lactate, similarly, appears to have diverse effects on APD\textsubscript{90}, i.e., lengthening (41), shortening (16, 23, 39), or no effect at all (3). The reason for the discrepancies among these various studies may lie in the widely differing methodologies used. Therefore, in this study we examined the effects of both lactic and respiratory acidosis on the APD\textsubscript{90} in the intact heart, under conditions approximating the extent of both acidosis and lactate accumulation seen during low-flow ischemia in the same model.

In the isolated, perfused heart both respiratory and lactic acidosis caused an initial lengthening of APD\textsubscript{90}, reaching 112% of baseline in both groups over the first 1.5–2 min. One possible explanation for the initial increase in APD\textsubscript{90} could be inhibition of I\textsubscript{to}, under acidic conditions, as has been shown to occur during ischemia (49), although such inhibition has not been directly documented. After initial lengthening significant shortening of APD\textsubscript{90} was observed during continued acidosis. It has been proposed that pH\textsubscript{i} and lactate can activate K\textsubscript{ATP} channels. If this is the case then we would expect that addition of glibenclamide during acidosis would cause an increase in the initial lengthening of APD\textsubscript{90} and/or abolish the recovery of APD\textsubscript{90} during continued acidosis. There was a tendency, albeit not significant, toward increased initial APD\textsubscript{90} lengthening in the presence of glibenclamide (see Fig. 8A). The subsequent recovery of APD\textsubscript{90}, however, was significantly reduced by glibenclamide (see Fig. 8B). These data suggest that the recovery of APD\textsubscript{90} during acidosis is caused, at least in part, by activation of K\textsubscript{ATP} Channels. Furthermore, this is consistent with the data obtained in single cell studies that have shown that K\textsubscript{ATP} Channels are activated by intracellular acidosis (4, 26, 28) and intracellular lactate (12, 23).

Lactate Has No Discriminable Direct Metabolic Effects

Samara and Opie (39) suggested that lactate caused APD\textsubscript{90} shortening through inhibition of glycolysis. During lactic and respiratory acidosis, the initial APD\textsubscript{90} shortening and the degree of recovery during continued acidosis were similar (see Figs. 6C and 7). Furthermore, the effects of l-lactate and d-lactate were similar, suggesting that lactate did not exert any additional metabolic stress that affected the APD\textsubscript{90} beyond that present during respiratory acidosis. The slight difference between the effects of d- and l-lactate could be explained by the slower transport of d-lactate into the cell by the monocarboxylate-H\textsuperscript{+} cotransporter (5).

In conclusion, the dose temporal association between APD\textsubscript{90} shortening and the rapid increase in \textsuperscript{86}Rb efflux, both of which are sensitive to inhibition by glibenclamide, supports the hypothesis that activation of K\textsubscript{ATP} channels underlies APD\textsubscript{90} shortening during ischemia. This activation occurs when bulk [ATP], remains unchanged but closely correlates with a rapid decline in pH\textsubscript{i} that may be the metabolic determinant underlying K\textsubscript{ATP}-channel activation during prolonged ischemia. The glibenclamide-sensitive shortening of APD\textsubscript{90} observed during prolonged acidosis (whether metabolic or respiratory in origin) further supports the hypothesis that acidosis is the link between ischemia and APD\textsubscript{90} shortening via K\textsubscript{ATP}-channel activation.

Received 4 November 1997; accepted in final form 21 April 1998.

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REFERENCES


