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MECHANISM OF POTASSIUM EFFLUX AND ACTION POTENTIAL SHORTENING DURING ISCHAEMIA IN ISOLATED MAMMALIAN CARDIAC MUSCLE

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SUMMARY

1. Ischaemia was simulated in the isolated sheep cardiac Purkinje fibre and guinea-pig papillary muscle by immersing the preparations in paraffin oil. Ion-selective microelectrodes recorded potassium (K_s^+) and pH (pH_s) in the thin film of Tyrode solution trapped at the fibre surface while other microelectrodes recorded intracellular pH (pH_i), membrane potential and action potentials (AP) (evoked by field stimulation), or membrane current (two-microelectrode voltage clamp in shortened Purkinje fibres). Twitch tension was also monitored. The paraffin oil model reproduced the salient characteristics of myocardial ischaemia, i.e. a decrease of twitch tension; a decrease of pH_i and pH_s; a rise in K_s^+ (by 2–3 mM); a depolarization of diastolic membrane potential; considerable shortening of the AP (up to 30% within 4 min).

2. The sulphonylurea compounds, glibenclamide (200 μM) and tolbutamide (1 mM), known inhibitors of the K_{ATP} channel, completely blocked the ischaemic rise of K_s^+ and prevented AP shortening. Ischaemic tension decline was notably less pronounced in the presence of sulphonylureas.

3. The ischaemic increase of slope conductance (Purkinje fibre) was prevented by 1 mM-tolbutamide and 200 μM-glibenclamide.

4. Sulphonylureas did not affect resting membrane potential, the AP or the current–voltage relationship under non-ischaemic conditions (this also indicates that ischaemic K_s^+ accumulation is not fuelled by the background K^+ current [i_{K1}] which was shown, as expected, to be Ba^{2+} sensitive).

5. In a normally perfused preparation, reducing intracellular ATP by inhibiting glycolysis with 2-deoxyglucose (DOG) produced a similar AP shortening plus a membrane hyperpolarization, both of which were inhibited by tolbutamide or glibenclamide. The AP shortening was not related uniquely to the fall of pH_i observed under these conditions since experimentally reducing pH_i (by reducing pH_o in the absence of DOG) *lengthened* rather than shortened the AP.

6. The possibility that the ischaemic rise in K_s^+ might be the *cause* of AP shortening was excluded by the observation that, in a normally perfused Purkinje fibre, experimentally reducing pH_i (by an amount similar to that seen during ischaemia) completely neutralized the AP-shortening effect of an elevated K_o^+ (from

4·5 to 6·5 mM). Furthermore, the sulphonylurea-sensitive AP shortening seen during DOG treatment (paragraph 5) could not have been associated with a K_s^+ rise since, in these particular experiments, the fibres were well perfused and diastolic membrane potential *hyperpolarized*.

7. Ischaemic K_s^+ accumulation was not affected by (i) the inhibitor of lactate transport, α-cyano-4-hydroxycinnamic acid (4 mM), (ii) a very high concentration of bumetanide (2 mM: a known high-affinity inhibitor of Na^+–K^+–$2Cl^-$ co-transport), and (iii) total Cl^- removal. This indicates that ischaemic K^+ efflux is not linked with anion movement.

8. We conclude that simulated ischaemia leads to K_s^+ accumulation through opening of sulphonylurea-sensitive K^+ channels, thus accounting for action potential shortening and for part of the decline in twitch tension during ischaemia.

INTRODUCTION

Cardiac ischaemia provokes an increase in potassium efflux from myocardial cells (for review see Kleber, 1984; Janse & Wit, 1989) and a shortening of the action potential duration (APD) (eg. McDonald, Hunter & MacLeod, 1971; Isenberg, Vereeke, van der Heyden & Carmeliet, 1983). Both phenomena have been closely associated with severe arrhythmias such as ventricular tachycardia and ventricular fibrillation which in many cases prove fatal. The extracellular accumulation of K^+ ions is also believed to be one of the major factors triggering pain during angina pectoris and acute myocardial infarction (e.g. Procacci & Zoppi, 1984). The investigation of ischaemic K^+ efflux began more than 40 years ago (Lowry, Krayer, Hastings & Tucker, 1942; Harris, Bisteni, Russell, Brigham & Firestone, 1954) but its mechanism has not so far been elucidated.

One possible mechanism is that K^+ efflux requires the simultaneous efflux of an anion such as Cl^- or lactate (Kleber, 1983; Gaspardone, Shine, Seabrooke & Poole-Wilson, 1986), perhaps via a co-transport carrier mechanism. An alternative possibility is that additional K^+ channels open up during ischaemia, thus accelerating the passive diffusive leak of K^+ from the cell (Vleugels, Vereeke & Carmeliet, 1980). In the present work we have simulated ischaemia in isolated papillary muscles (guinea-pig) and Purkinje fibres (sheep) by immersing them in paraffin oil while recording K_s^+ (extracellular, surface K^+), pH_s (surface pH) and pH_i (intracellular pH) using ion-selective microelectrodes.

We find that, as reported previously (Gasser & Vaughan-Jones, 1989), extracellular K^+ accumulation during simulated ischaemia can be completely blocked by the sulphonylurea compounds tolbutamide and glibenclamide, which are known blockers of the ATP-sensitive K^+ channel in a variety of cells (Ashcroft, 1988) including cardiac muscle (Noma, 1983; Noma & Shibasaki, 1985). We observe that these compounds prevent the shortening of APD and the increase in membrane conductance that occurs during the first few minutes of ischaemia. Furthermore, we show that sulphonylureas do *not* inhibit K^+ efflux via an effect on the normal background leak current, I_{K1}. This is strong evidence that the opening of a specific class of K^+ channel provides the route for accelerated K^+ efflux during ischaemia, a conclusion also reached recently by Kantor, Coetzee, Carmeliet, Dennis & Opie (1990) using measurements of K^+ wash-out from ischaemic Langendorf hearts.

Finally, we investigate but reject the possibility that the K^+ efflux is linked with transmembrane anion movement.

METHODS

Preparations. Guinea-pig papillary muscles and sheep Purkinje fibres were used. Experiments were performed on papillary muscles from the right ventricle of guinea-pig (animals approximately 400 g of either sex). For *papillary muscles*, the animal was killed by cervical dislocation and exsanguination and the heart quickly removed and washed in Tyrode solution equilibrated with 100% O_2. The right ventricle was opened near the intraventricular septum and a suitable uniform papillary muscle chosen, of diameter 1·0–2·0 mm. The base of the preparation was electrically isolated by a tight knot of surgical thread, used also for pinning the preparation in the experimental chamber. The preparation was partially immobilized by stretching it lightly over a narrow bridge made of four fine micropins (100 μm diameter; see Bountra & Vaughan-Jones, 1989). A conventional microelectrode was then inserted into a superficial cell in the region of muscle above this narrow bridge and pH-sensitive plus K^+-selective microelectrodes were pressed on the surface of the preparation, about 100 μm from the conventional microelectrode (see Fig. 1). The free tendinous end of the papillary muscle was connected via a hook to a force transducer (based on an Akers strain gauge). Total force development in such a preparation is considerably less than in an unrestrained muscle (Bountra & Vaughan-Jones, 1989). For *sheep Purkinje fibres*, sheep hearts were collected from a local abattoir and free-running fibres were removed, effectively shortened to < 2 mm in length (by squeezing the fibre at both ends using forceps) and mounted in the perfusion chamber as described previously (e.g. Bountra & Vaughan-Jones, 1989).

Solutions. The preparations were continuously superfused with Tyrode solution. The solution was pumped to the experimental chamber (about 1·5 ml/min; bath volume = 150 μl), passing through a heat exchanger, before reaching the preparation. The heating configuration was such that, in any experiment, temperature was maintained constant at 37 ± 0·5 °C. The superfusate was equilibrated with either 100% O_2 in 20 mM-HEPES-buffered solution or with 5% CO_2 plus 95% O_2 in 23 mM-bicarbonate-buffered solution. Solutions in the experimental chamber could be exchanged completely within 20–30 s (measured by using a K^+- and a pH-selective microelectrode in the chamber while reducing the K^+ activity and the pH of the perfusate; 90% exchange was recorded after ~ 20 s). The normal Tyrode solution consisted of (mM): NaCl, 140; KCl, 4·5; $CaCl_2$, 2·5; $MgCl_2$, 1·0; glucose, 10·0; HEPES, 20. The solution was adjusted to pH 7·4 by titration using 4 M-NaOH. CO_2–bicarbonate-buffered solutions were modified from our normal Tyrode solution and contained: NaCl, 127; $NaHCO_3$, 23; HEPES, 0; all other constituents were unchanged. Tolbutamide (Sigma chemicals) was dissolved in 0·3 ml dimethyl sulphoxide (DMSO) to make a solution (0·33 M) which was added to 1 l of Tyrode solution such that the concentration of DMSO did not exceed 0·3% in the final solution. The same procedure was applied when glibenclamide was used. α-Cyano-4-hydroxycinnamic acid (Sigma) was dissolved (4 mM) in Tyrode solution by warming it to approximately 50 °C, pH was then readjusted after 2 h. Bumetanide (2 mM; Leo Pharmaceuticals) and Ba^{2+} (0·3 mM) were added to Tyrode solution. L(+)-Lactic acid (Sigma) was added to Tyrode solution at a concentration of 25 mM without osmotic compensation. 2-Deoxyglucose (DOG; Sigma) was added (10 mM) to Tyrode solution, in place of glucose.

Fabrication of ion-selective microelectrodes. We used borosilicate tubing, 1·5 mm o.d., 1 mm i.d. Electrodes were pulled with a conventional microelectrode puller. They were constructed as described previously (Vaughan-Jones, 1988). Briefly, electrodes were silanized using trimethyl-dimethyl silylamine, they were then bevelled dry to an outer tip diameter of approximately 1·5–2 μm (for surface use) or < 1·0 μm (for intracellular use) (for a review of construction of ion-selective microelectrodes see also Gasser, 1988). The bevelled electrode was then filled with the ion-selective carrier plus electrode-filling solution (NaCl, 100 mM; KCl, 4·5 mM; HEPES, 10 mM buffered to pH 7·5 with NaOH). For pH electrodes, we used the H^+-selective ionophore of Ammann and co-workers (Ammann, Lanter, Steiner, Schulthess, Shijo & Simon, 1981*a*). For K^+, we used a valinomycin cocktail (Ammann, Lanter, Steiner, Erne & Simon, 1981*b*). Short-column electrodes were used to obtain low-noise recordings (Vaughan-Jones & Kaila, 1986). Electrodes were calibrated before the experiments and those which produced less than 57 mV for one pH unit or one decade change in K^+ activity were discarded.

Electrical arrangements. For both surface and intracellular ion-activity measurements, single-barrelled microelectrodes were used. In the case of surface recording, the ion-selective

microelectrode signal was referred to a blunt (bevelled) 3 M-KCl-filled microelectrode located in a corner of the experimental chamber (Fig. 1). In the case of intracellular recording, the intracellular ion electrode was referred to an intracellular, conventional 3 M-KCl-filled microelectrode (Fig. 1). The ion-selective electrode signal was monitored with a varactor bridge diode (Analog Devices

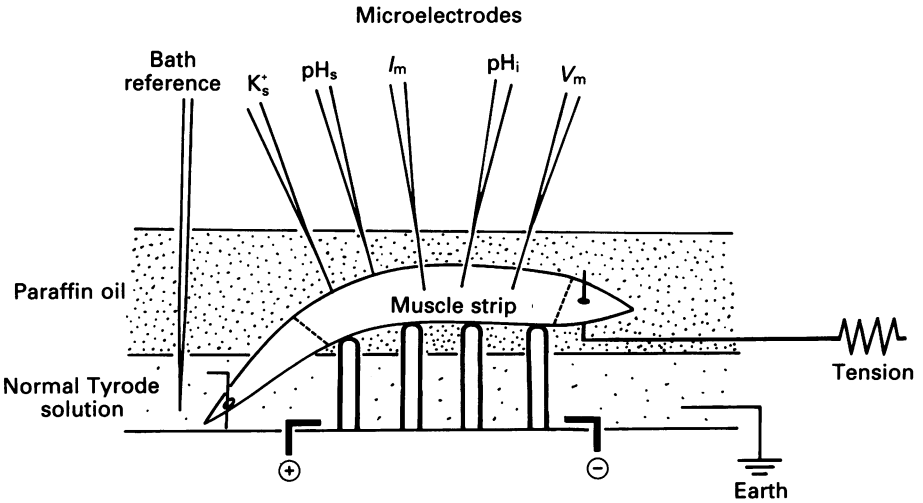

Fig. 1. Simulated ischaemia. Simplified diagram of the perfusion chamber. The different types of microelectrode are indicated; not all were necessarily used simultaneously in a given experiment. Ion-selective microelectrodes recorded surface K^+ and pH (K_s^+, pH_s) and intracellular pH (pH_i). Conventional intracellular microelectrodes were used to record membrane potential (V_m) and to pass current (I_m) into the preparation (when performing voltage-clamp experiments with shortened Purkinje fibres). The muscle strip (papillary muscle or Purkinje fibre; dashed lines represent ligatured or crushed areas as described in Methods) was laid across four supports (micropins, 100 μm diameter) and pinned at one end and attached to a force transducer at the other. Also shown are two electrodes (Pt) for field stimulation. The diagram illustrates an episode of simulated ischaemia, with the muscle immersed almost completely in a stationary pool of paraffin oil (heavy stippling) while perfusion of warm (37 °C) Tyrode solution (light stippling) continues at the base of the chamber.

311J) and both the bath reference (< 1 MΩ) and the conventional membrane potential-recording microelectrodes (4–5 MΩ) were connected to FET unity-gain, operational amplifiers (Signetics NES36H). All connections between electrodes and amplifiers were made via Ag–AgCl wires. In some experiments, an additional intracellular microelectrode was used to pass current so that the fibre could be voltage clamped.

Stimulation. To elicit twitch tension, the papillary muscle or Purkinje fibre was field stimulated using short square pulses (0·5–1 ms, 20 V) at 0·1, 0·2 or 0·5 Hz delivered between platinum electrodes in the experimental chamber.

Statistics. Mean values are quoted ± standard error of mean, $n = 3$ for each quoted experiment if not shown otherwise.

Simulated ischaemia. We used a modified version of the model first described by de Hemptinne & co-workers (de Hemptinne, Marrannes & Vanheel, 1982; Vanheel, Leybaert, de Hemptinne & Leusen, 1989). Ischaemia was simulated by immersing the preparation in paraffin oil as shown in Fig. 1. This was achieved by floating a drop of paraffin oil on top of the meniscus in the experimental chamber, while maintaining a constant solution flow. The level of the aqueous–oil interface in the bath was then reduced electronically using a servo-controlled level-device (Cannell & Lederer, 1986) until the bulk of the cardiac fibre was immersed in the oil. A small area of contact

between fibre and Tyrode solution remained, as shown in Fig. 1. This was essential in order to maintain electrical contact between the surface-ion microelectrode and the bath reference electrode. The flowing Tyrode solution at the base of the chamber also maintained total bath temperature at 37 °C (this was monitored in some experiments using a bead thermistor touching the fibre surface; surface temperature changes upon paraffin immersion were < 1 °C). The small area of Tyrode solution–fibre contact during simulated ischaemia was considered acceptable since the basic phenomena of cardiac ischaemia were readily observed (see Fig. 2 in Results). Moreover, we would argue that the contact area mimics a low background level of 'collateral flow' within the otherwise ischaemic tissue. We deal in detail with this feature in the Discussion, where we also consider the general validity of the model for simulating myocardial ischaemia.

In order to simulate reperfusion, the level of the aqueous–oil interface in the bath was raised again electronically until the fibre was completely reimmersed in well-oxygenated flowing Tyrode solution.

RESULTS

Simulated ischaemia in isolated cardiac muscle

Figure 2 shows the effects of simulated ischaemia in the sheep Purkinje fibre. Similar results were found in guinea-pig papillary muscle (see Figs 5 and 7). Figure 2*A* shows that, over a 5 min period, there was a decrease in developed tension and a rise of extracellular surface K^+ (K_s^+) from 4·5 to 5·8 mM. The rise in K_s^+ was paralleled by a depolarization of diastolic membrane potential (~ 5 mV) and by a 40% shortening of action potential duration (for convenience, 80% APD (APD_{80}) has been plotted *versus* time in Fig. 2*A*; original action potential recordings are illustrated in Fig. 4*B*). Figure 2*B* shows that ischaemia also produced a fall of surface pH (pH_s) and intracellular pH (pH_i). Terminating the ischaemic period (by elevating the solution level in the chamber thus removing the layer of paraffin oil) reversed all of the above changes. Note that the recovery of tension showed signs of a transient overshoot. This was observed to a variable degree in both Purkinje fibres and papillary muscles (see e.g. Figs 4*B* and 7). The recovery of pH_i was not a monotonic process, reperfusion consistently produced a further fall in pH_i followed by an increase to pre-ischaemic levels. Data obtained during ischaemia in seventeen Purkinje fibres and eight papillary muscles are pooled in Table 1 which quantifies changes in those parameters illustrated in Fig. 2. The changes are similar to those observed previously during various simulated ischaemic manoeuvres induced either in whole perfused heart (Kloner & Braunwald, 1980; Weiss & Shine, 1982), isolated cardiac muscle (de Hemptinne *et al.* 1982; Janse & Wit, 1989) or single ventricular myocytes (Lederer, Nichols & Smith, 1989; Isenberg *et al.* 1983).

Figure 3 shows that the ischaemic rise of K_s^+ occurred in both CO_2–HCO_3^- and HEPES-buffered media. However, the fall of pH_s and pH_i has been reported to be ~ 50% smaller when using CO_2–HCO_3-buffered media, presumably because of an increase in intracellular and extracellular H^+ buffering power (Vanheel *et al.* 1989).

The changes of K_s^+, pH_s and pH_i are qualitatively similar in quiescent and driven preparations (Figs 2, 4 and 7). Weiss & Shine (1982) have shown that the rise of K_s^+ during ischaemia is frequency modulated but, in the present work, we have not attempted to establish any quantitative differences introduced by pacing at different rates.

The results shown in Figs 2 and 3 as well as in Table 1 allow us to conclude that our experimental model can reproduce successfully the salient characteristics of

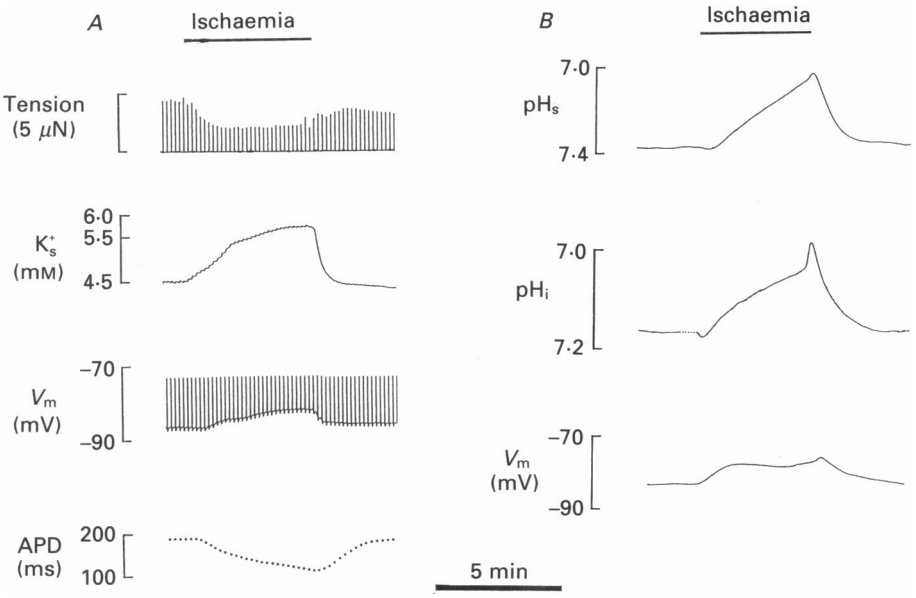

Fig. 2. Basic features of simulated ischaemia. *A*, a stimulated sheep Purkinje fibre (field stimulated, 0·1 Hz). Traces show time course changes of (from top to bottom) (i) twitch tension (recorded at 0·1–10 Hz in order to facilitate comparison of successive twitches, this protocol is also used in subsequent figures); (ii) surface K^+ (K_s^+); (iii) membrane potential (note peaks of action potentials are off-scale); and (iv) replot of action potential duration (80 %) during simulated ischaemia (ischaemia indicated by bar at top of figure). *B*, a quiescent Purkinje fibre subjected to simulated ischaemia. Traces show pH_s (top), pH_i (middle) and membrane potential (bottom).

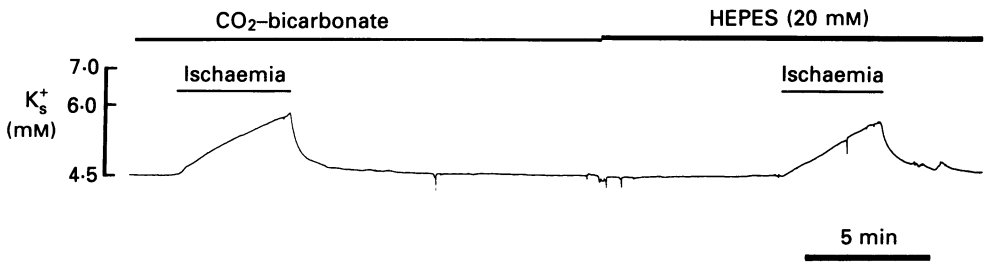

Fig. 3. Measurement of K_s^+ in a quiescent guinea-pig papillary muscle during two identically timed episodes of ischaemia, firstly in a 5 % CO_2–23 mM-HCO_3, and then in 20 mM-HEPES-buffered solution (pH_o, 7·4 throughout). The ischaemic, extracellular K^+ accumulation occurs regardless of which buffer solution is used.

TABLE 1. Values of pH_i, pH_s, K_s^+ and APD in quiescent or stimulated (0·5 Hz) guinea-pig papillary muscle and sheep Purkinje fibres before and during simulated ischaemia (4 min duration)

	Control	Ischaemia (after 4 min)
pH_i	7·12 ± 0·01	7·03 ± 0·02 (n = 3)
pH_s	7·31 ± 0·01	7·03 ± 0·02 (n = 8)
K_s^+	4·5	7·40 ± 0·56 (n = 8)
APD_{80}	100 % (= 192 ± 18 ms)	72·6 ± 5·6 % (n = 6)

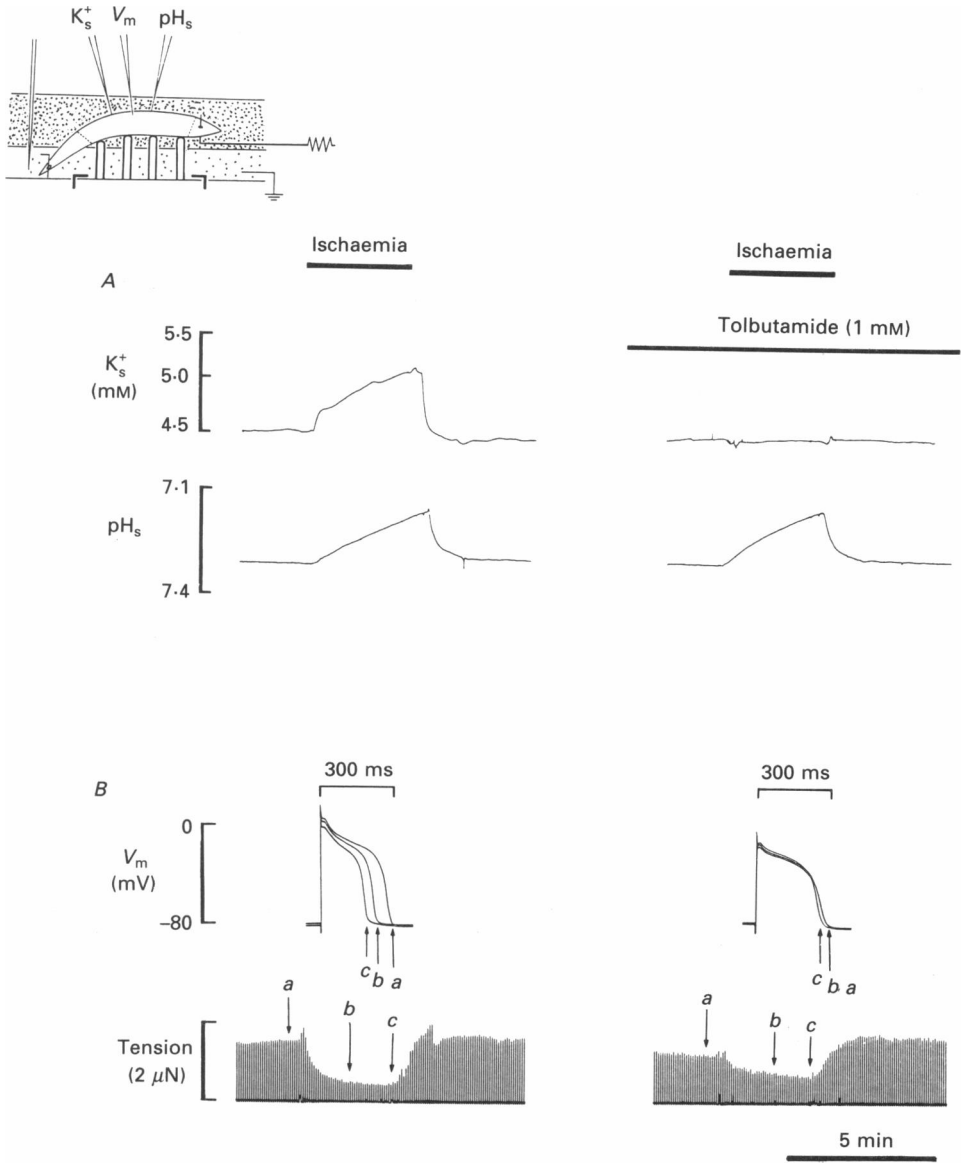

Fig. 4. Effect of tolbutamide during simulated ischaemia in the Purkinje fibre, *A*, tolbutamide (1 mM) completely abolishes the ischaemic K_s^+ accumulation whereas the fall of pH_s is unaffected. Left- and right-hand panels from same experiment. Tolbutamide added for 30 min before beginning of right-hand panel. *B*, left-hand panel, decline of twitch tension and shortening of action potential during simulated ischaemia. Lettered arrows on tension trace refer to times when action potentials were sampled as shown above. Right-hand panel, simulated ischaemia in the presence of 1 mM-tolbutamide (added 10 min before beginning of traces) in the same fibre. Note the lack of shortening of action potential. The ischaemic intervals in *B* were timed to equal those shown in *A*. Fibre stimulated at 0·2 Hz. Inset above *A* shows experimental arrangement in perfusion chamber (cf. Fig. 1).

myocardial ischaemia. We have therefore used the model to investigate the mechanism of K_s^+ accumulation.

Effect of sulphonylureas on K_s^+ accumulation and APD

Figure 4*A* shows that, in the Purkinje fibre, the sulphonylurea drug, tolbutamide (1 mM) completely inhibited the ischaemic rise of K_s^+ while exerting no effect upon

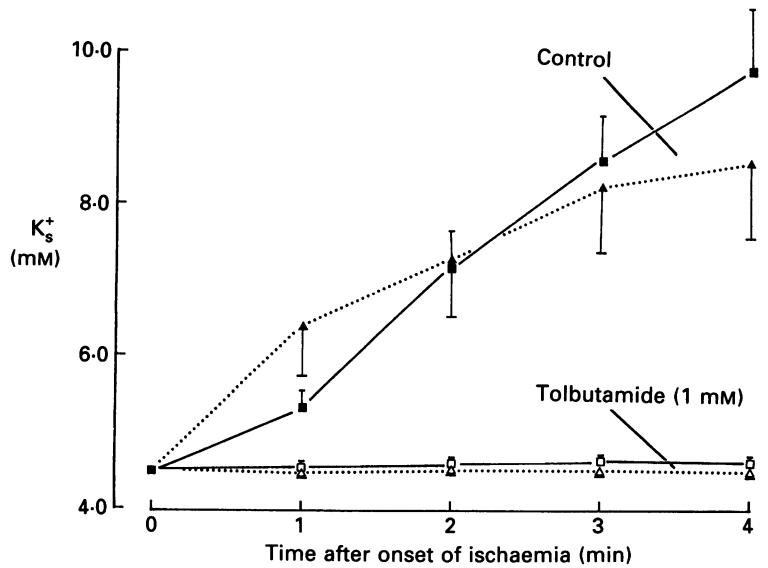

Fig. 5. Time course of K_s^+ accumulation in stimulated fibres (field stimulated, 0·1 Hz) during simulated ischaemia in presence (open symbols) and absence (filled symbols) of tolbutamide (1 mM). Each point shows mean ± S.E.M. of three fibres. Triangles and dotted lines, data from Purkinje fibres. Square symbols and continuous lines, data from papillary muscles. Data from experiments similar to Figs 4 and 7.

the fall of surface pH. Figure 4*B* and *C* shows that tolbutamide also prevented the shortening of APD that occurs during ischaemia. Interestingly, we found that tolbutamide exerted no effect upon the resting potential or APD when applied under well-perfused, control conditions (Fig. 13*A*). The effect of the drug on both APD shortening and K_s^+ accumulation implies that they are linked in some way, and the fact that tolbutamide is known to inhibit ATP-sensitive K^+ channels (K_{ATP} channels) in myocardial tissue (Noma, 1983; Noma & Shibasaki, 1985; Ashcroft, 1988) suggests that these channels may be involved.

Figure 5 shows a plot of the time course of K_s^+ accumulation observed in six experiments (three guinea-pig papillary muscles, three Purkinje fibres) in the presence and absence of tolbutamide (1 mM). The rise in K_s^+, which was similar in both preparations, was practically abolished by tolbutamide. Figure 6 also pools data from three Purkinje fibres and three papillary muscles and shows that the progressive reduction of APD_{80} (up to 30% reduction) during ischaemia was prevented by tolbutamide. In the presence of the drug, APD_{80} shortened by only 2·5% during the first 4 min of ischaemia.

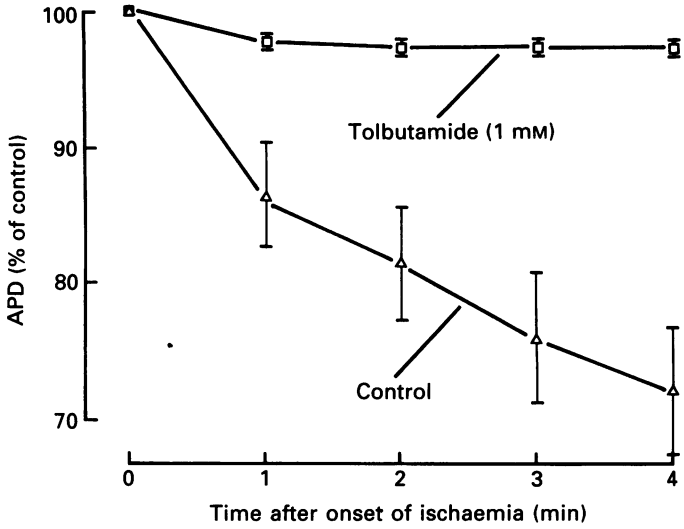

Fig. 6. Ischaemic action potential shortening is inhibited by tolbutamide. Each point is mean ± S.E.M. of six fibres (three Purkinje fibres and three papillary muscles) for APD_{80} (80% repolarization). Data from experiments similar to Figs 4 and 7. Control APD_{80} = 192 ± 18 ms.

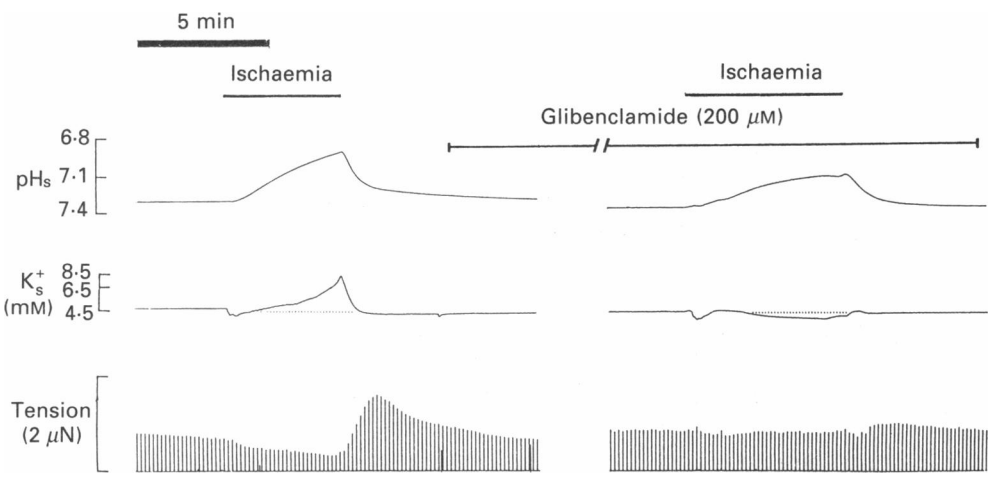

Fig. 7. Decrease in pH_s and rise in K_s^+ in an isolated guinea-pig papillary muscle during simulated ischaemia. The increase in K_s^+ is prevented by 200 μM-glibenclamide. Note that the fall in twitch tension during ischaemia is attenuated in the presence of glibenclamide. The gap represents 50 min. Twitch elicited by field stimulation (0·1 Hz).

Figure 7 shows the effect of another sulphonylurea compound, glibenclamide (200 μM), applied to papillary muscle. This drug is known to block K_{ATP} channels with a much higher affinity than tolbutamide (Fosset, de Weille, Green, Schmid-Antomarchi & Lazdunski, 1988; Zunkler, Leuzen, Manner, Panten & Trube, 1989). Once again, it inhibited the rise of K_s^+ while having little effect upon pH_s changes. It

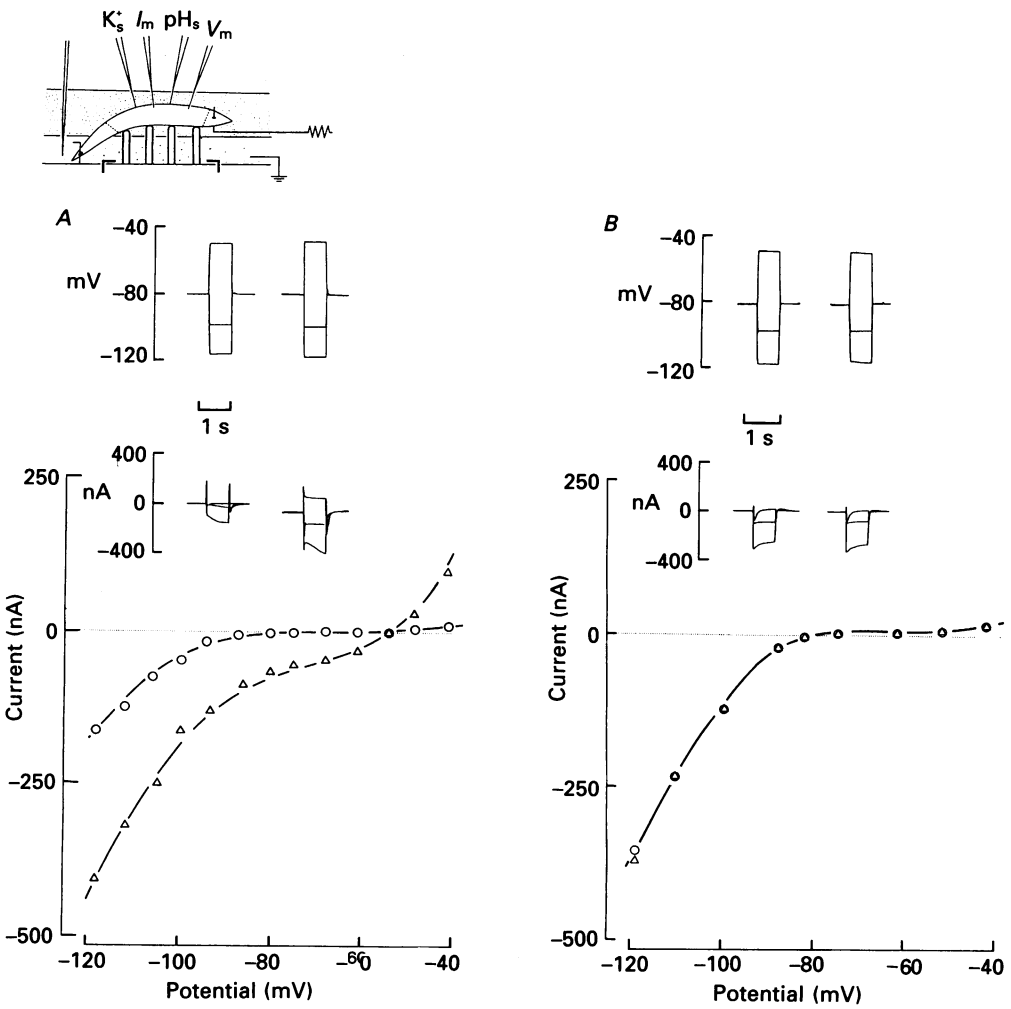

Fig. 8. Slope conductance of the Purkinje fibre increases during ischaemia and this increase is inhibited by tolbutamide (1 mM). *A*, isochronal current–voltage relationship of a voltage-clamped Purkinje fibre under control conditions (○) and after 8 min of simulated ischaemia (△). Current measurements taken at end of 700 ms voltage pulses from holding potential of −80 mV. Superimposed sample voltage and current records shown above graph: left, control; right, during ischaemia. *B*, isochronal current–voltage relationships (700 ms clamp pulses from holding potential of −82 mV) in a fibre treated with tolbutamide (1 mM; applied 10 min before *I–V* determination). ○, *I–V* in tolbutamide; △, *I–V* in tolbutamide after 10 min of simulated ischaemia. Superimposed sample records shown above graph: left, control with tolbutamide; right, in ischaemia with tolbutamide. Experimental arrangements in perfusion chamber are shown at top left. K_s^+ and pH_s were also recorded (but not shown). K_s^+ rose from 4·5 to 6·7 mM during control ischaemia (i.e. experiment in *A*); the rise of K_s^+ was inhibited fully by tolbutamide (i.e. experiment in *B*). In all cases an ischaemic fall of pH_s was detected.

is notable that, in glibenclamide, K_s^+ even appeared to *fall* slightly during ischaemia but this was not observed consistently in other fibres. It is also notable that the decline of tension during ischaemia was greatly attenuated in the presence of

glibenclamide. The protective effect on tension of sulphonylureas shown in Fig. 7 is the most extreme case that we observed. More usually tension declined during ischaemia, but the decline was less prominent in the presence of either glibenclamide or tolbutamide, as illustrated in Fig. 4. We return to this point in the Discussion.

Changes of conductance during ischaemia

Membrane K^+ conductance is reported to increase during complete metabolic blockade and during hypoxia (Isenberg *et al.* 1983). We therefore investigated if the current–voltage relationship of the Purkinje fibre changed during our model of simulated ischaemia. Figure 8*A* shows that after 8 min of ischaemia a considerable increase in slope conductance was evident at all potentials in the range −120 to −40 mV. Note that there was an almost threefold increase of *outward current* at −40 mV. A similar result was seen in two other fibres. This increase of outward current at depolarized potentials will therefore contribute to AP-shortening during ischaemia. In contrast Fig. 8*B* shows that in a fibre pre-treated with tolbutamide (1 mM) there was no change in the *I–V* relationship after 8 min of simulated ischaemia (a similar result was obtained in two other fibres). Furthermore, tolbutamide exerted *no* effect upon the *I–V* curve when applied under control (non-ischaemic) conditions (Fig. 14*B*). We have also observed that pre-treatment of a Purkinje fibre with glibenclamide (200 μM) prevents significant changes to the *I–V* curve during simulated ischaemia (three fibres, not shown).

Because elevating extracellular K^+ can, itself, increase K^+ conductance in cardiac tissue (by activating the anomalous rectifier current, i_{K1}; DiFrancesco & Noble, 1985) it is difficult to decide in Fig. 8 if an increase in K_s^+ is the cause of the observed increase in slope conductance or whether an increase in K_s^+ is secondary to increased membrane K^+ conductance (activated by some other factor), i.e. whether K_s^+ accumulation is the *cause* or the *effect* of an increased K^+ conductance. We address this problem later in the Results section. Nevertheless, Fig. 8 shows that the major changes of slope conductance during simulated ischaemia are readily suppressed by sulphonylureas.

Effects of ischaemia upon membrane potential

The membrane potential (V_m) tends to depolarize during myocardial ischaemia. In six experiments (three Purkinje fibres and three papillary muscles), V_m depolarized by ~ 5 mV after 2 min of ischaemia (from $-84 \pm 4{\cdot}0$ to -79 ± 4 mV) and ~ 9 mV after 4 min (to $-75 \pm 4{\cdot}1$ mV). Figure 9*A* replots data obtained in an experiment where V_m and K_s^+ were recorded simultaneously (Purkinje fibre). It is clear that the time course of depolarization of V_m was paralleled by the increase in K_s^+. We have attempted in Fig. 9*A* to estimate the change in the K^+ equilibrium potential (E_K) induced by the elevation of K_s^+. E_K was calculated from the Nernst equation, using the measured value of extracellular K^+ activity, a_K^s (assuming a K^+ activity coefficient of 0·75) and assuming a constant value of a_K^i during the 4 min of simulated ischaemia. The value of a_K^i was estimated from the initial control value of V_m by using the graph of V_m *versus* $\log_{10} a_K$ constructed previously by Sheu, Korth, Lathrop & Fozzard (1980) whose results were also in accordance with those of Miura, Hoffmann & Rosen (1977). The changes in E_K and V_m have been plotted on the same axes in Fig. 9*A*. Both E_K and V_m progressively depolarize by similar amounts during the ischaemic episode and

at all times E_K remains about 10 mV negative to V_m. Figure 9*B* summarizes data obtained from all six experiments. In this graph $(V_m - E_K)$ has been plotted *versus* time. It can be seen that the estimated value of E_K always remained negative to V_m, although the difference $(V_m - E_K)$ may have declined a little during ischaemia (recall

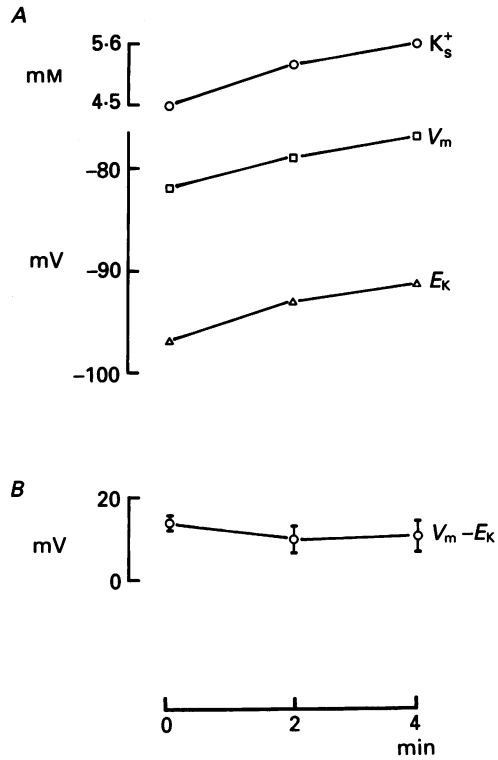

Fig. 9. *A*, values of K_s^+ (○) and membrane potential (□) are plotted *versus* time for an ischaemic episode in a Purkinje fibre. E_K for this experiment has been calculated from the value of intracellular potassium (K_i^+) for Purkinje fibres (from Sheu *et al.* 1980) and extracellular potassium (K_s^+) under the assumption that K_i^+ remains constant during the brief ischaemic episode. See text for details. *B*, calculated values for $(V_m - E_K)$ during ischaemia. Data pooled from three Purkinje fibres plus three papillary muscles.

that V_m was itself depolarizing by an average of 9 mV during this period). Figure 9 thus indicates that the membrane depolarization during ischaemia can be attributed to the depolarization of E_K caused by extracellular K^+ accumulation.

Note that if a K^+ conductance (g_K) progressively increases during ischaemia then the depolarization of V_m in response to elevated K_s^+ will be expected to become increasingly smaller than the depolarization of E_K. This is because (i) increasing g_K will *hyperpolarize* V_m (towards E_K) whereas (ii) raising K_s^+ will *depolarize* both V_m and E_K. If both events occur simultaneously then their effect on V_m will tend to cancel. The dominant feature determining V_m during ischaemia must therefore be the rise of K_s^+ since a clear depolarization of V_m is evident. This is also reasonable on theoretical grounds. One may calculate (constant-field equation, assuming initial pNa/pK ratio of 0·02) that in the face of (say) a 50% increase in membrane permeability to K^+(pK) at constant pNa one would anticipate a depolarization of V_m of $\sim$ 7 mV in response to a depolarization of E_K of 9 mV caused by elevation of K_s^+.

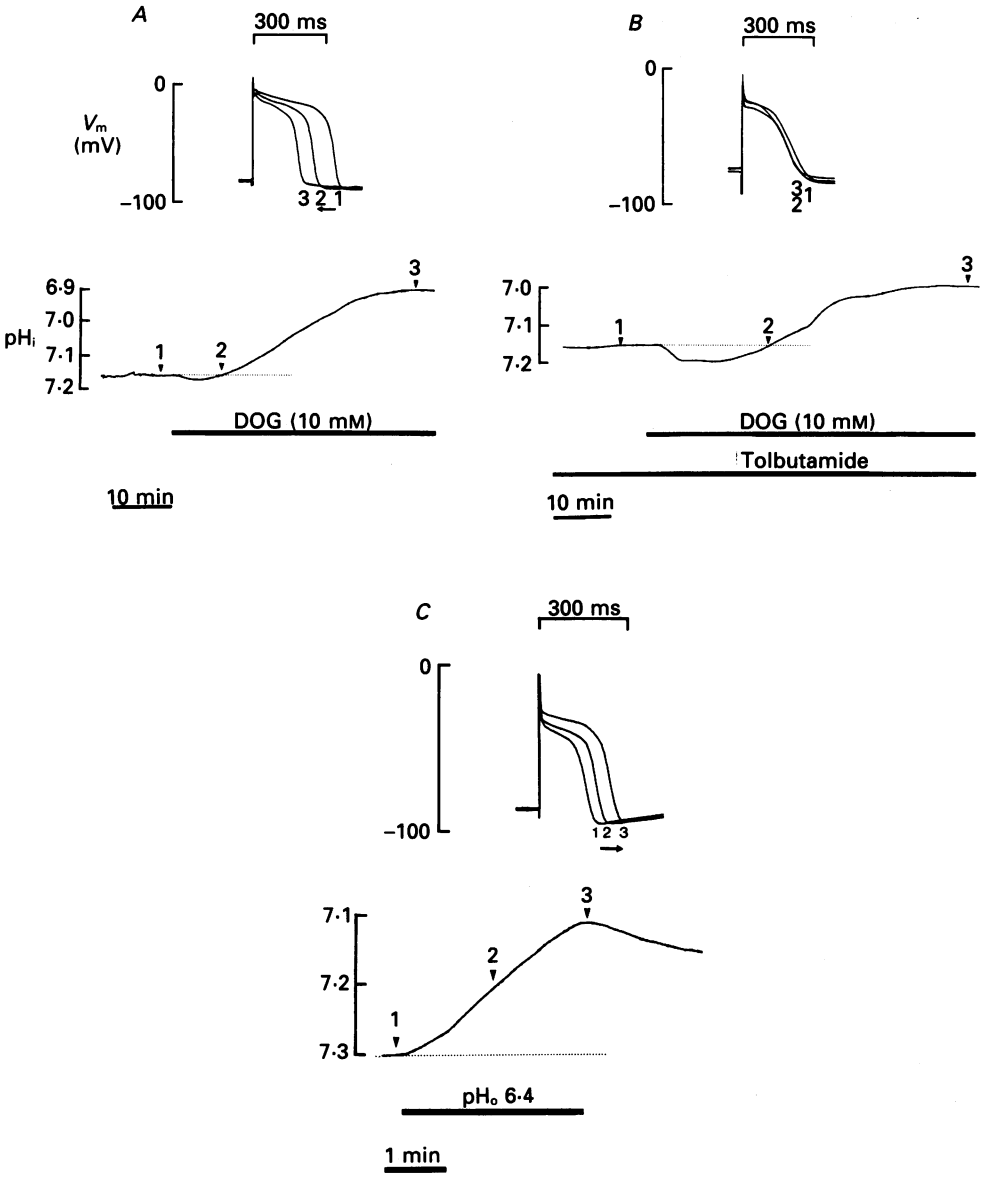

Fig. 10. Purkinje fibre. Inhibition of glycolysis (with 10 mM-2-deoxyglucose (DOG)) produces tolbutamide-sensitive action potential shortening and intracellular acidosis. Intracellular acidosis alone produces action potential *lengthening*. Traces in *A*, *B* and *C* show sample action potentials (top) recorded at times indicated (at arrows) on the pH_i trace (bottom). Fibres stimulated at 0·1 Hz (*A* and *B*) and 0·5 Hz (*C*). Bars indicate periods of application of DOG, tolbutamide or 6·4 pH_o Tyrode solution. Normal pH_o 7·40. Each panel shows a different experiment.

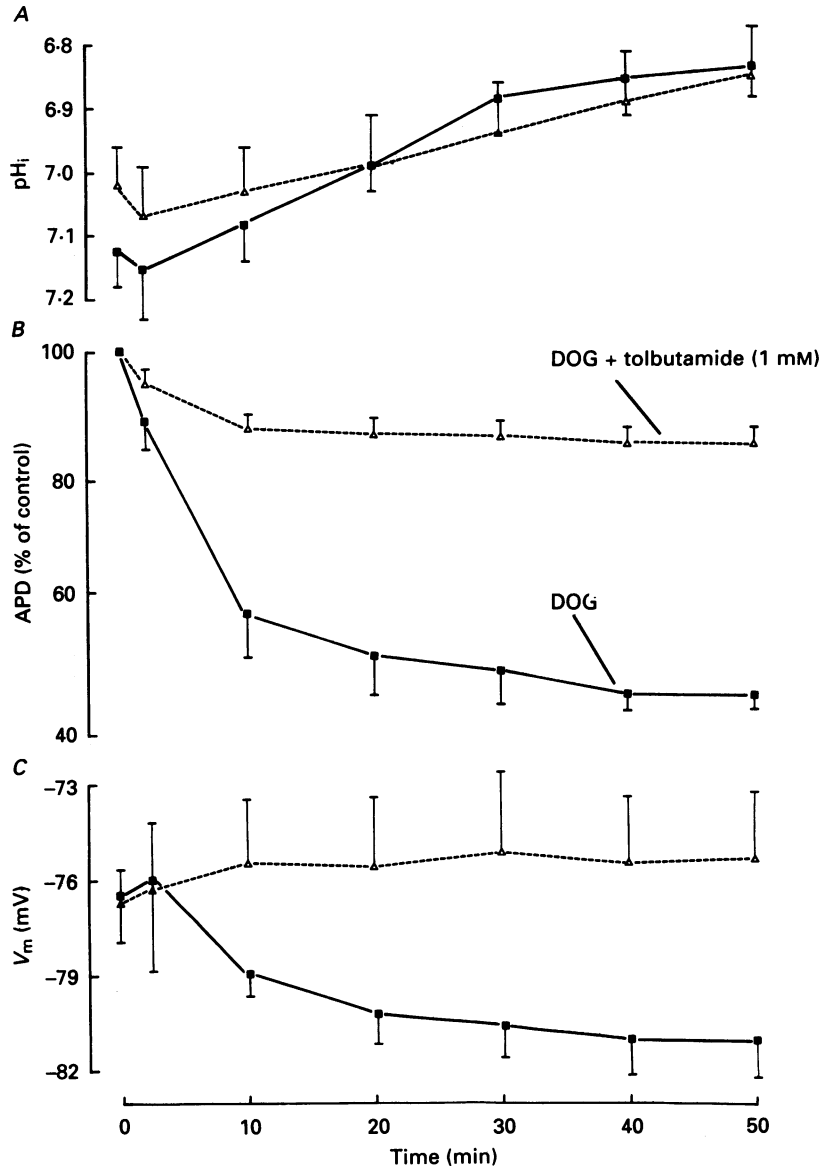

Fig. 11. Data pooled (means ± S.E.M.) from seven experiments (Purkinje fibres) like those shown in Fig. 10*A* and *B*. Graphs show time course of pH_i changes in 10 mM-DOG (*A*), shortening of action potential (APD_{80}, expressed as percentage of control) (*B*) and time course of resting membrane potential changes (C). Fibres stimulated at 0·1 Hz. Continuous lines are for DOG ($n = 4$); dashed lines are for DOG + 1 mM tolbutamide ($n = 3$).

Effect of tolbutamide during metabolic blockade

Many of the effects of myocardial ischaemia can be mimicked by subjecting well-perfused tissue to metabolic blockade. Figure 10*A* shows, for example, that inhibiting glycolysis with 10 mM-2-deoxyglucose (in glucose-free Tyrode solution) leads to a

progressive shortening of the action potential. This is accompanied by an initial alkalosis followed by a sustained acidosis of 0·2 units (Bountra & Vaughan-Jones, 1989; Eisner, Nicholas, O'Neill, Smith & Valdeolmillos, 1989). The acidosis is believed to be due, at least in part, to net ATP hydrolysis and depletion. Consistent

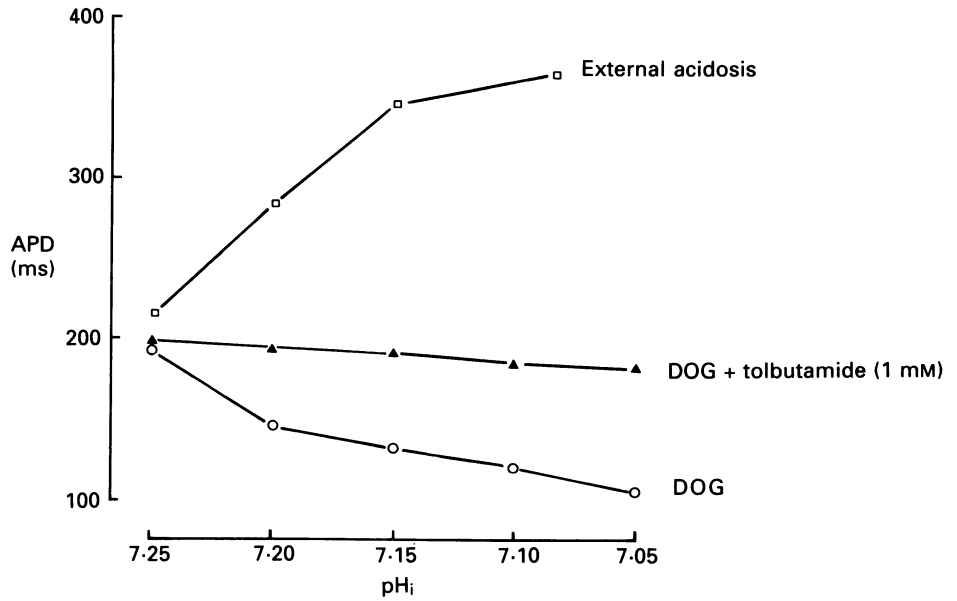

Fig. 12. Purkinje fibre. Relationship between pH_i and APD_{80} determined during reduction of pH_o to 6·4 (□). This is to be contrasted with relationships between APD and pH_i determined during application of 10 mM-DOG (○) and 10 mM-DOG + 1 mM-tolbutamide (▲).

with this, intracellular ATP falls in the Purkinje fibre by 60–70% after 40 min (Mei-Lin Wu & R. D. Vaughan-Jones, unpublished observations; ATP_i measured by luciferin–luciferase luminescence). Figure 10*B* shows that pre-treating a fibre with 1 mM-tolbutamide prevents the action potential shortening. Figure 11 summarizes results obtained from four Purkinje fibres treated with DOG. Over a 50 min period, APD shortened by only 10% in the presence of tolbutamide, compared with 50% in absence of the drug. This protective effect of tolbutamide upon APD is thus similar to that seen previously (Fig. 4) during simulated ischaemia. It is notable that, as shown in Fig. 11*C*, the diastolic membrane potential *hyperpolarized* by 4–5 mV in DOG and that this hyperpolarization was also inhibited by tolbutamide.

The magnitude and time course of pH_i changes during DOG treatment were unaffected by tolbutamide (Fig. 11*A*). This is of interest since APD is known to be sensitive to changes of pH_i which might therefore contribute to the effects of DOG seen in Figs 10 and 11. Figure 10*C* shows, however, that reduction of pH_o and pH_i cannot account for APD shortening during either DOG treatment or during

simulated ischaemia. Reducing pH_o from 7·4 to 6·4 led, within 3 min, to a 0·2 fall of pH_i and this was associated with an *increase* and not a decrease of APD. The time course of lengthening of APD shown in Fig. 10*C* follows the time course of fall of pH_i. In Fig. 12, using data from Fig. 10*C*, we have plotted the relationship between APD

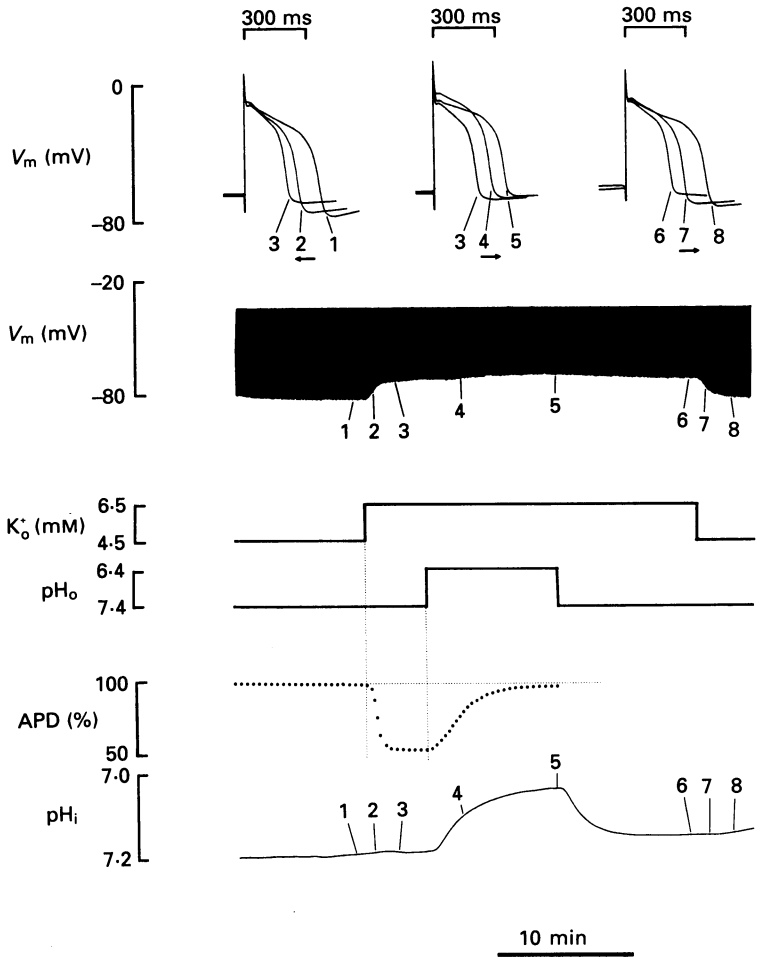

Fig. 13. Purkinje fibre stimulated at 0·5 Hz. A rise in K_s^+ from 4·5 to 6·5 mM (indicated towards the centre of the figure) depolarizes diastolic potential (the thickened V_m trace; action potential peaks are off-scale) and shortens APD (sample action potentials are superimposed at the top of the figure for the times indicated on the V_m and pH_i traces). The percentage change in APD_{80} is replotted (●) *versus* time, towards the bottom of the figure. The shortening of APD in 6·5 K_o^+ can be completely neutralized by reducing pH_i (bottom trace) by 0·2 units. Note that APD_{80} and pH_i change in parallel. The pH_i was changed by reducing pH_o to 6·40 as indicated. Thus raising K_o^+ by 2 mM *plus* reducing pH_i by 0·2 units has no major effect on APD in the steady state (cf. AP 1 with AP 5). These changes in K_o^+ and pH_i are comparable to those recorded during simulated ischaemia.

and pH_i (□) and, for comparison, we have also plotted APD *versus* pH_i observed during DOG treatment (○) and during DOG treatment in the presence of tolbutamide (▲). It is apparent that a tolbutamide-sensitive shortening of APD

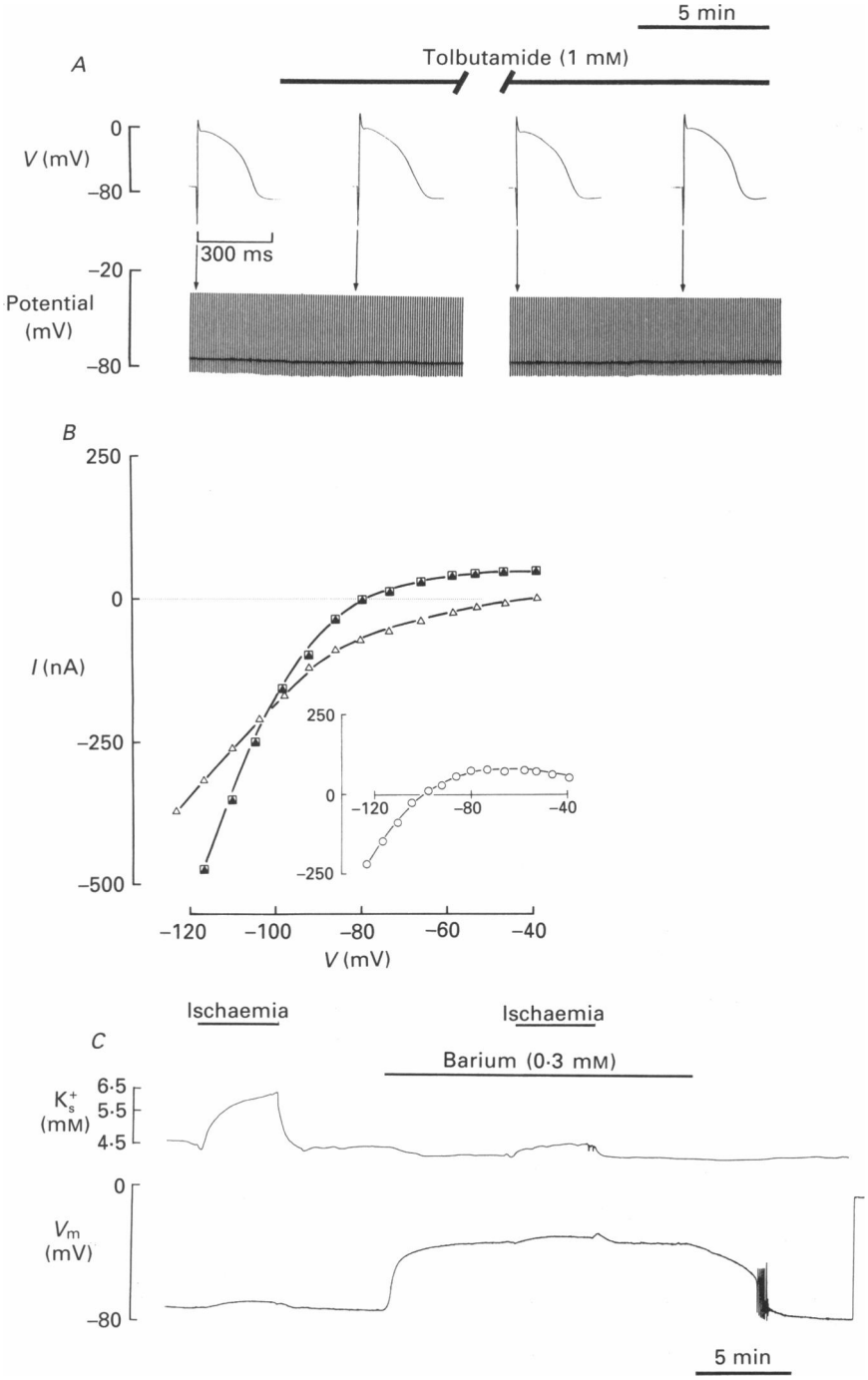

Fig. 14. Purkinje fibre. Tolbutamide (1 mM) has no effect on normal action potentials (*A*; fibre stimulated at 0·5 Hz; gap in traces represents 40 min) and no effect upon the isochronal current–voltage relationship (*B*; □, control; ▲, 1 mM-tolbutamide; *I–V* recorded after 500 ms voltage-clamp pulses from holding potential of −80 mV). In contrast, Ba^{2+} reduces slope conductance (*B*; △, in 300 μM-Ba^{2+}; inset shows (○) Ba^{2+}-sensitive *I–V* relationship, i.e. difference between top two curves). Ba^{2+} also depolarizes resting membrane potential and reduces ischaemic K_s^+ accumulation (*C*).

occurs during DOG treatment *despite* the development of acidosis which, of itself, would be expected to lengthen APD.

Ischaemic K_s^+ accumulation is not produced by background K^+ efflux (i_{K1})

In this section, we investigate the possibility that the time-independent background current (i_{K1}) could be responsible for the observed phenomena. At rest, potassium ions diffuse from the cell via the background K^+ conductance (a significant portion of this outward current will be i_{K1}). It is conceivable that, in simulated ischaemia, this simple efflux fuels K_s^+ accumulation (but note that, for K_s^+ accumulation to occur, passive K^+ efflux must exceed active K^+ reuptake). Because of the K_o^+ sensitivity of i_{K1} (e.g. Noble, 1979), extracellular K^+ accumulation could then conceivably act in a positive feedback manner to further increase i_{K1}. A 20% increase in background current can reduce APD by up to 50% (DiFrancesco & Noble, 1985). This shortening of APD would, in turn, reduce twitch tension (Lederer *et al.* 1989). Thus we would produce three main characteristics of myocardial ischaemia (K_s^+ accumulation, APD shortening and a fall in twitch tension) without having to postulate opening of K_{ATP} channels.

There are strong reasons for rejecting this model as the mechanism for contractile failure during ischaemia. Firstly, raising K_o^+ from 4·5 to 6·5 mM (equivalent to the early ischaemic rise of K_s^+) has only a small shortening effect on APD in guinea-pig papillary muscle (< 5%; not shown), much less than the shortening seen during simulated ischaemia (up to 30% shortening after 4 min; Fig. 6). As shown below, the shortening effect of K_o^+ upon APD is much greater in the Purkinje fibre. We find, however, that raising K_o^+ does *not* reduce APD in this latter tissue when it is accompanied, as in ischaemia, with a fall in both pH_i and pH_s. This is illustrated in Fig. 13. This shows that the APD of a well-perfused Purkinje fibre is reduced by 45% upon elevating K_o^+ from 4·5 to 6·5 mM. If, however, pH_o is then reduced (7·4 to 6·4), APD starts to lengthen again (note that lengthening follows the fall of pH_i rather than pH_o) such that, in the new steady state, APD has returned to the control value observed at the beginning of the experiment. This experiment therefore shows that K_s^+ accumulation *per se*, when it is accompanied by a fall of pH_i (similar to that observed during ischaemia), has little effect upon APD (cf. also, Brown, Cohen & Noble (1978) for antagonist effects of H^+ and K^+ ions on membrane current). The profoundly shortened APD observed in both papillary muscle and the Purkinje fibre during simulated ischaemia is therefore unlikely to be a secondary consequence of K_s^+ accumulation.

A further reason for rejecting K_s^+ accumulation via i_{K1} as the prime cause of APD shortening is shown in Fig. 14. This figure demonstrates that, under non-ischaemic conditions, tolbutamide has no effect upon resting membrane potential or upon the action potential (Fig. 14*A*) and no effect upon the isochronal *I–V* relationship of the Purkinje fibre. In comparison, external Ba^{2+} (a blocker of i_{K1}; e.g. Noble, 1983; Sakmann & Trube, 1984) produces a large depolarization (Fig. 14*C*) and a flattening of the *I–V* relationship (Fig. 14*B*). The Ba^{2+}-sensitive *I–V* relationship is indicated in the inset to Fig. 14*B* and displays characteristic inward rectification. Since tolbutamide inhibits both APD shortening and K_s^+ accumulation during simulated ischaemia, it cannot be acting via inhibition of i_{K1} or indeed of other 'non-ischaemic' K^+ currents since this would also require that the drug affect the electrical properties

and AP of the normal cell (clearly this does *not* occur). The APD shortening, K_s^+ accumulation, and increased slope conductance during simulated ischaemia are therefore likely to be caused primarily by opening of a specific class of tolbutamide-sensitive K^+ channel. The above analysis does not exclude contributions to K^+ efflux

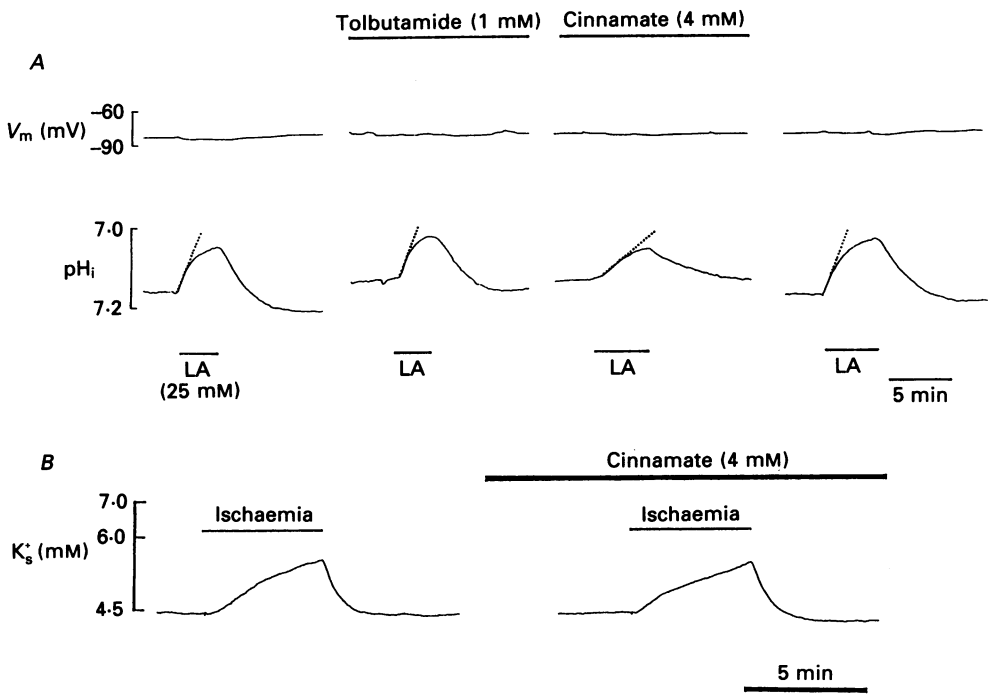

Fig. 15. *A*, Purkinje fibre. α-Cyano-4-hydroxycinnamate (4 mM) (a lactate-transport inhibitor) reduces fall of pH_i induced by 25 mM-external lactate (LA) whereas 1 mM-tolbutamide has no effect. Note that both tolbutamide and cinnamate reduce steady-state pH_i. Gap in traces represents ~ 15 min in each case. *B*, lack of effect of α-cyano-4-hydroxycinnamate upon ischaemic K_s^+ accumulation in a guinea-pig papillary muscle. Gap in traces represents 20 min.

from the Ba^{2+}-insensitive i_f current. This current, however, is *absent* in ventricular tissue (i.e. in the present work, papillary muscle) but sulphonylurea-sensitive K_s^+ accumulation still occurs. This suggests that, in the Purkinje fibre as well as ventricular tissue, i_f is unlikely to be the prime pathway for ischaemic K^+ efflux.

Finally, Fig. 14*C* shows that Ba^{2+} partly inhibits K_s^+ accumulation during simulated ischaemia. Thus, as well as inhibiting i_{K1}, Ba^{2+} also seems to inhibit the ischaemic K^+ efflux pathway. This would be consistent with previous observations of inhibition by Ba^{2+} of K_{ATP} channels in skeletal muscle (Castle & Haylett, 1987) and ventricular myocytes (Findlay, 1987).

Is K^+ efflux anion dependent?

In this section we test the hypothesis that accelerated K^+ efflux during ischaemia is obligatorily linked to anion movement.

Effect of inhibiting lactate efflux

The K^+ efflux has been suggested to be linked to lactate efflux which also increases during ischaemia (Gaspardone *et al.* 1986). Lactate efflux occurs to a large extent via a transmembrane carrier inhibitable by α-cyano-4-hydroxy-cinnamic acid (αHC)

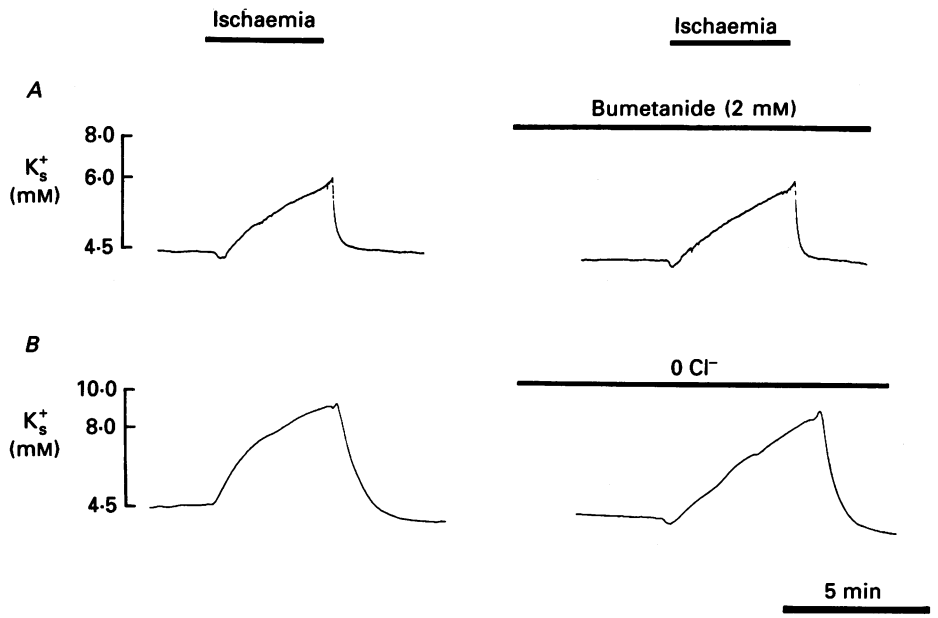

Fig. 16. *A*, lack of effect of bumetanide (2 mM) on ischaemia-induced K_s^+ accumulation. *B*, lack of effect of Cl^--free solution (Cl^- replaced by glucuronate). Gap in traces represents 12 min in *A* and 50 min in *B*. Both experiments performed on quiescent Purkinje fibres.

(Halestrap & Denton, 1974; de Hemptinne, Marrannes & Vanheel, 1983). Figure 15*A* shows that, although αHC inhibits the lactate carrier, tolbutamide (1 mM) does not, suggesting that lactate and K^+ movement must occur via independent pathways. The carrier is revealed by adding 25 mM-sodium lactate to the perfusate and recording a fall of pH_i. This occurs because lactate is co-transported into the cell with H^+ ions. Upon removing external lactate, the system is reversed so that pH_i recovers. The drug, αHC, reduces the rate of intracellular acidosis caused by lactate addition indicating an inhibitory effect upon the co-transporter. Tolbutamide does not influence the rate of acidosis indicating a lack of inhibition. Similarly, αHC does not inhibit K_s^+ accumulation during simulated ischaemia as shown in Fig. 15*B*. We conclude therefore that ischaemic K^+ efflux is not linked directly to lactate efflux.

Effect of inhibiting Cl^- efflux

Ischaemic K^+ might be linked to Cl^- efflux, perhaps via a KCl or even a Na^+–K^+–$2Cl^-$ co-transport system (Kleber, 1983, 1984; Janse & Wit, 1989). Figure 16*A* shows that a very high dose of bumetanide, a high-affinity inhibitor of Na^+–K^+–$2Cl^-$ co-transport with some inhibitory potency for KCl transport (Ellory & Stewart, 1982) has no effect on K_s^+ accumulation. Indeed K_s^+ accumulation can

proceed in the total absence of Cl^- as shown in Fig. 16*B* (Cl_o^- replaced by glucuronate). In this experiment Cl_o^- was removed for 1 h before testing the effect of simulated ischaemia, a time sufficient for complete removal of intracellular Cl^- (Vaughan-Jones, 1979).

We conclude that K^+ efflux during ischaemia is not linked with anion movement.

DISCUSSION

Our main finding is that extracellular K^+ accumulation during simulated myocardial ischaemia is fully inhibited by sulphonylurea compounds which are inhibitors of the K_{ATP} channel. These compounds also greatly reduce action potential shortening during either simulated ischaemia or during glycolytic blockade (induced by addition of DOG) and they prevent the large increase of slope conductance in the Purkinje fibre during simulated ischaemia. We conclude that K_s^+ accumulation is caused by an increased diffusive leak of K^+ from cardiac cells, most probably via K_{ATP} channels that open during ischaemia. The lack of effect of tolbutamide or glibenclamide upon the action potential or upon the steady-state *I–V* relationship suggests that these channels are not significantly activated in the non-ischaemic state (it also indicates that tolbutamide does not affect other background K^+ currents such as i_{K1}). Finally, our experiments indicate that K_s^+ accumulation is not linked obligatorily to the efflux of anions such as Cl^- or lactate, in contrast to conclusions drawn in previous work (Gaspardone *et al.* 1986). Although both anion and K^+ efflux seem to occur during ischaemia, each flux must be via an independent pathway.

The experimental model of ischaemia

Before considering the significance of our findings, we must consider the validity of our experimental approach. Our model of simulated ischaemia was first developed by de Hemptinne & co-workers (de Hemptinne *et al.* 1982). We have applied it to the sheep Purkinje fibre and guinea-pig papillary muscle and, in both tissues, obtain very similar results, suggesting that the mechanism of K_s^+ accumulation is not unique to a particular region of the heart. As well as simulating K_s^+ accumulation, the model reproduces a wide variety of other phenomena witnessed clinically (Kloner & Braunwald, 1980; Poole-Wilson, 1984; Janse & Wit, 1989), experimentally in whole hearts (Kloner & Braunwald, 1980; Weiss & Shine, 1982), and in isolated tissue and single ventricular myocytes (de Hemptinne *et al.* 1982; Isenberg *et al.* 1983; Stern, Chien, Capogrossi, Pelto & Lakatta, 1985; Allen & Orchard, 1987; Lederer *et al.* 1989). Notable among these other phenomena are a reversible decrease in contraction, action potential shortening, diastolic depolarization, a fall of pH_i and a fall of pH_s.

It might be argued that the model is inadequate since the covering of paraffin oil does not provide a truly closed system for solutes such as O_2 and CO_2. This would seem to be a specious argument since, even in the ischaemic heart, O_2 and CO_2 can move across cell membranes and within available extracellular spaces. In the clinical case, for example, a low, albeit inadequate, supply of O_2 will be provided by diffusion from adjacent non-ischaemic areas. Nevertheless the clinical situation may still be more hypoxic than in our experimental model. We have not, in the present work, attempted to record P_{O_2} levels at the membrane surface and so cannot address this

question directly. However, Vanheel *et al.* (1989) have measured P_{O_2} levels in their ischaemic model, which is essentially the same as ours, and have shown that P_{O_2} drops by $> 90\%$ during the ischaemic episode.

A further criticism of our model is that a small portion of the isolated tissue remains permanently in contact with the perfusion chamber (see Fig. 1). Thus the tissue is not uniformly ischaemic. This limited contact is necessary for our electrode measurements (see Methods). The local area of tissue where our ion measurements are made, however, is well covered with mineral oil and totally devoid of flowing solution. We would argue that the small area of solution contact effectively mimics a small degree of collateral flow. This is always present in real ischaemia, the flow coming either from collateral vessels or diffusion from the ventricular cavum through the subendothelial layer. Therefore, as in our experimental model, there can be an electrical connection between ischaemic and adjacent non-ischaemic areas in the heart. Indeed, real ischaemia produced by coronary spasm or thrombosis because of recurrent episodes of spontaneous reperfusion (Gasser & Dienstl, 1986; Hackett, Davies, Chierchia & Maseri, 1987) is less severe than the global ischaemia produced by stopping the flow in a Langendorf-perfused heart or in an arterially perfused septum (Weiss & Shine, 1982; Kleber, 1983). Therefore a maintained, minimized, collateral flow approaches more closely the real ischaemic condition.

Finally, our surface ion measurements are made at discrete points using microelectrodes. The recordings may not be representative of extracellular changes elsewhere within the tissue. For example, an ion-selective electrode might, itself, create an artificial cleft with limited diffusion so that surface ionic changes may become exaggerated. While we cannot dismiss this argument entirely there are reasons for believing that it is not a major problem. Firstly, the recordings of K_s^+ changes correlate well with recordings of membrane potential changes made at a different spot (200–300 μM away; Fig. 9). This suggests that the recorded K_s^+ changes are reasonable reflections of changes elsewhere. Secondly, Bountra, Hilgemann & Vaughan-Jones (1988) have shown that pH_s changes recorded with an ion-selective microelectrode within a Purkinje fibre agree remarkably well in time course and magnitude with those recorded using an extracellular pH-sensitive dye (Phenol Red) which diffuses throughout the whole extracellular cleft system. This again suggests that surface ion electrode recordings furnish realistic information concerning the extracellular space. The K_s^+ changes reported here also agree well with those reported previously for perfused ventricular septum (Weiss & Shine, 1982) suggesting that experimental variation introduced by the electrode technique in different preparations is small.

Advantage of the experimental model

It is perhaps worth emphasizing that the present model of simulated ischaemia offers a significant advantage over previously used models. It is a model based upon a multicellular preparation. Real myocardial ischaemia is a phenomenon of multicellular tissue with restricted diffusion within its complex extracellular spaces. This is emphasised in the present work by the fact that many of the phenomena observed in simulated ischaemia are associated with significant changes in extracellular ion levels. In contrast, work on ischaemia in, for example, single isolated myocytes cannot be expected to reproduce accurately the mechanisms of

myocardial ischaemia. An isolated cell is ideally perfused with a less complex extracellular space which cannot easily be rendered ischaemic. There will therefore be little or no extracellular accumulation of ions and metabolic waste products. Alternative approaches to mimic ischaemia in isolated cells have relied upon various methods of metabolic blockade (so called 'chemical ischaemia' e.g. Allen, Morris, Orchard & Pirolo, 1985; Eisner *et al.* 1989; Lederer *et al.* 1989). While these may be helpful in understanding some ischaemic mechanisms, they may give a distorted picture. For example, Allen & Orchard (1983) have shown that systolic Ca_i^{2+} transients *decrease* in well-perfused isolated papillary muscles subjected to complete metabolic blockade (hypoxia plus DOG or hypoxia plus glycogen depletion) whereas, during a more realistic mechanical simulation of ischaemia, Ca_i^{2+} transients actually *increase* (Allen, Lee & Smith, 1989). Nevertheless a comparison can be useful between mechanically simulated ischaemia (e.g. the paraffin oil technique) in multicellular tissue and 'chemical ischaemia' (i.e. metabolic blockade) induced either in multicellular or single-cell preparations. In the present work, for example, both approaches produced a tolbutamide-sensitive shortening of the action potential. Since K_s^+ accumulation during paraffin-simulated ischaemia was also tolbutamide-inhibited, it is likely that both the mechanical (paraffin) and the chemical (DOG) simulations of ischaemia lead readily to opening of K_{ATP} channels.

Finally the present model of simulated ischaemia is more accessible to experimental manipulation than previously adopted models using perfused whole hearts or septa. Multi-ion recordings can be made, in some cases under voltage-clamp conditions (shortened Purkinje fibres). The present model should therefore be extremely useful in future investigations of myocardial ischaemia.

Is K_s^+ accumulation mediated via a modulated K^+ efflux or influx?

If a tissue's intracellular K^+ levels are in a steady state with extracellular levels then passive K^+ efflux will be balanced by an equal K^+ reuptake, most likely via the Na^+–K^+ pump. Reducing the size of the extracellular space would not be expected to raise K_s^+ unless either K^+ efflux increased or K^+ reuptake decreased. The observed rise of K_s^+ therefore suggests that either (or both) K^+ efflux and K^+ influx must change during ischaemia. Although a slowing of Na^+–K^+ pumping has, in the past, been postulated as a mechanism for K_s^+ accumulation, this is now considered unlikely, at least in the early stages of ischaemia since intracellular ATP levels (normally > 5 mM) should still be sufficient to fuel the pump ($K_{0.5}^{ATP}$ of pump is $\sim 100\ \mu$M although this may rise during ischaemia, see Allen & Orchard, 1987). Experimental evidence has been provided that K_s^+ accumulation is not due to a failure of the Na^+–K^+ pump (e.g. Kleber, 1983; Rau, Shine & Langer, 1977). The latter authors showed, for example, that K^+ loss during the first hour of hypoxia could be completely accounted for by an increase in K^+ efflux without evidence of suppressed K^+ influx. Na^+–K^+ pump slowing certainly seems *not* to be the mechanism of K_s^+ accumulation in the present work, simply because sulphonylurea compounds completely inhibit K_s^+ accumulation. In order to reconcile this observation with the pump-slowing model, one would have to propose a pump-protective property of sulphonylureas during ischaemia which seems inherently unlikely. The early K^+ efflux has also been shown not to be due to K^+ leakage caused by cell membrane damage (Poole-Wilson, 1984) nor is it a result of a change in

osmolarity in the extracellular space (Kleber, 1983). We are left then with an acceleration of K^+ efflux via a specific pathway. Although we have not identified ischaemic opening of K_{ATP} channels using patch-clamp techniques, it is difficult to argue that the channel is *not* involved. Sulphonylureas are known inhibitors of the channel in cardiac muscle and the fact that the compounds do not affect the normal action potential or *I–V* relationship (Fig. 13) suggests that they are not significant inhibitors of other K^+ channels (e.g. i_{K1}, or other background K^+ currents) encountered under non-ischaemic conditions. This argues strongly that the increase of K_s^+ during ischaemia (Fig. 6) is largely due to opening of the K_{ATP} channel. A similar conclusion has been drawn recently (Lederer *et al.* 1989) for cause of the K^+ conductance increase in ventricular myocytes exposed to complete metabolic blockade with DOG+CN^- and the accelerated K^+ efflux in the ischaemic, isolated (Langendorf) heart (Kantor *et al.* 1990).

An alternative channel for K^+ efflux is the recently described fatty acid (arachidonic acid)-activated K^+ channel (Kim & Clapham, 1989; Ordway, Walsh & Singer, 1989) especially since intracellular free fatty acids increase during ischaemia. Notably, the opening of this K^+ channel is enhanced by a fall of pH_i (and pH_i falls during ischaemia, Fig. 2) and so it could conceivably contribute to ischaemic K^+ efflux. The present work indicates that a necessary requirement of this channel would be an inhibition by sulphonylureas. Unfortunately, this has not been tested as yet in any preparation and so a sulphonylurea inhibition of novel K^+ channels other than the K_{ATP} channel should not be excluded. The important point to note is that our data clearly implicate the opening of a class of K^+ channel during ischaemia that does not contribute to the normal action potential or K^+ balance of the cardiac cell.

Finally, it might be said that in the present work, we have used unusually high doses of tolbutamide (1 mM) and glibenclamide (200 μM). We would point out that in heart, high concentrations of tolbutamide are necessary for maximal inhibition of K_{ATP} channels (e.g. Belles, Heschler & Trube (1987) find that 0·4 mM-tolbutamide is required for 50% inhibition). We have not tested the effects of lower doses of glibenclamide.

Stimulus for increased K^+ conductance in ischaemia

$[ATP]_i$ reduction as a stimulus for action potential shortening has been an attractive possibility for many years (e.g. McDonald *et al.* 1971). Carmeliet and co-workers (Isenberg *et al.* 1983) identified that metabolic inhibition produces an increase in a time-independent K^+ conductance, but only recently has it been shown that action potential shortening during $[ATP]_i$ depletion can be reduced by sulphonylureas (Fosset *et al.* 1988; Lederer *et al.* 1989).

The main argument against opening of the K_{ATP} channel, at least in the initial stages of ischaemia, is that intracellular [ATP] has not decreased sufficiently, so that the channels would remain inhibited. Resting [ATP] is 5–10 mM (Matthews, Radda & Taylor, 1981; Allen *et al.* 1985; Ashcroft, 1988) and the K_i^+ for the K_{ATP} channel in isolated patches is $\sim$ 100 μM-ATP. This problem has been discussed extensively for a variety of cell types where the channel exists (Noma, 1983; Ashcroft, 1988) and no satisfactory solution has yet been provided. One possible explanation is that compartmentalization of $[ATP]_i$ may mean that the subsarcolemmal ATP concentration that inhibits the channel is very much lower than the measured whole-

cell level (Aw & Jones, 1985; Miller & Horowitz, 1986). Alternatively other intracellular factors, not available in isolated patch experiments, may regulate the ATP sensitivity *in situ*. One such factor could be pH. Although intracellular H^+ ions block the K_{ATP} channel in pancreatic β-cells, they *promote* opening in skeletal muscle cells (Davies, 1990) and, to a lesser extent, in ventricular myocytes (Lederer & Nichols, 1989). It is notable that both pH_i *and* pH_s decrease during myocardial ischaemia (Figs 2, 4 and 7). This increasing acidity might therefore increase the probability of K^+ efflux during ischaemia in cardiac tissue. While we have shown that pH changes during ischaemia are independent of K_s^+ accumulation (i.e. blocking the latter does not affect the former), we have yet to demonstrate that the reverse scenario is true, i.e. that K_s^+ changes can occur in the absence of pH changes. This must now be tested. The pH changes *per se* are unlikely, however, to be the sole trigger for the K_{ATP} channel opening during ischaemia since reducing pH_i and pH_o in non-ischaemic tissue *prolongs* rather than shortens APD (Figs 10, 12 and 14), presumably due to an inhibitory effect of H^+ ions on other repolarizing currents.

Whatever the trigger for K^+ channel opening, it would appear to be rapid following the onset of simulated ischaemia. In our own work, tolbutamide-sensitive K_s^+ accumulation is detectable (Figs 2–5 and 7) within 30 s, which is in accordance with the findings of others, who detected K^+ accumulation as early as 15 s after onset of ischaemia (Kleber, 1984). The time course for shortening of APD during our simulated ischaemia was also reasonably rapid, 30% shortening was seen after about 4 min (Fig. 6). This is similar to the time course of shortening that we observed in perfused fibres exposed to glycolytic inhibitors (Fig. 11) a condition also likely to lead to K_{ATP} channel opening (cf. Lederer *et al.* 1989). We therefore conclude that both glycolytic blockade and paraffin-simulated ischaemia readily open sulphonlurea-sensitive channels. Furthermore, the similarity of our results with Purkinje fibres and papillary muscle indicates that these K^+ channels exist *in the fast conduction system*, as well as in ventricular myocardium.

Effect of sulphonylureas on developed tension during ischaemia

The failure of developed tension during ischaemia has been attributed recently to failure of the action potential (Lederer *et al.* 1989). Before this occurs, the time course of decline of tension correlates well with the progressive shortening of the action potential. Since a sulphonylurea can militate against such shortening, one would expect that it would also lessen the early decline of tension. This is what we observe, although the degree of protection of contraction by the drug can vary somewhat (cf. Fig. 4 with Fig. 7). We have not attempted to quantify the degree of protection since it is possible that those small areas of cardiac tissue that remain in contact with the perfusate (see Fig. 1) during simulated ischaemia may remain functional and contribute to tension development thus off-setting the ischaemic decline of force. Nevertheless the attenuated decline seen in the presence of sulphonylureas indicates, qualitatively, an element of inotropic protection.

Clinical implications

An intriguing question is whether increased g_K and consequent K_s^+ accumulation during myocardial ischaemia (MI) is physiologically beneficial or whether it is merely a pathological occurrence. Because AP shortening leads to twitch failure, the

mechanism would, at first sight, seem to be pathological. Nevertheless, MI is often local and transient so that twitch failure could, in fact, be of benefit since it would reduce ATP_i consumption within the affected area while those non-ischaemic areas would continue to contract normally. MI-induced failure of the action potential due to high g_K would also render the non-working area electrically silent, thus lessening the probability of arrhythmic disturbance. Interestingly, K_s^+ accumulation could be beneficial for two further reasons: (i) it is likely to be one factor triggering pain (angina pectoris) during myocardial ischaemia (Procacci & Zoppi, 1984), thus serving to limit physical activity and O_2 demand, and (ii) if K_s^+ accumulation is transmitted to the lumen of coronary vessels, it may serve to decrease coronary vascular resistance and counteract any original coronary vasospasm. This role of K^+ in controlling local coronary flow remains controversial, but there is certainly evidence that a moderately elevated plasma K^+ can, at least transiently, dilate coronary vessels (Berne & Rubio, 1979). Therefore the early K^+ efflux might contribute (together with local acidity and other vasodilator agents such as endothelial-derived compounds) to the vasodilative component of the rhythmic, oscillatory reperfusion recently reported during MI (Gasser & Dienstl, 1986; Hacket *et al.* 1987). There are obvious disadvantages, however, of APD shortening and K_s^+ accumulation during MI, such as the possibility, during the intermediate stages of K_s^+ accumulation, of provoking malignant arrhythmias like ventricular tachycardia and fibrillation (Harris, 1966; Janse & Wit, 1989; Kantor *et al.* 1990; but see Ferrier, Moffat & Lukas, 1985).

Sulphonylureas are prescribed clinically as antidiabetic agents since they stimulate insulin release (by inhibiting K_{ATP} channels in the pancreatic β-cell thus producing depolarization; see Ashcroft, 1988). It is of interest to note that patients treated with sulphonylureas have an increased incidence of cardiovascular mortality (Levey, Lasseter & Palmer, 1974). The reason for this is not known but pharmacological inhibition by sulphonylureas of cardiac K_s^+ accumulation during MI could be potentially dangerous since it might decrease the development of pain (e.g. during physical exercise) without decreasing the intensity of the ischaemic episode, (a clinical condition known as 'silent ischaemia'). This could result in uncontrolled over-exertion in a patient, thus enhancing cardiovascular risk. The possible beneficial *versus* deleterious clinical effects of pharmacologically controlling MI-induced K^+ release and APD shortening must therefore remain speculative.

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