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# A $K_{ATP}$ channel opener inhibited myocardial reperfusion action potential shortening and arrhythmias

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**Abstract**

Low concentrations of certain $K_{ATP}$ channel openers have been reported to exert a moderate inhibitory effect on arrhythmias during post-ischaemic early myocardial reperfusion, but the accompanying effects on the time course of changes in action potentials in intact hearts have not yet been studied. We report that in rat isolated hearts, reperfusion following 10 min of regional no-flow ischaemia was associated with both an acute, marked, but transient, shortening of ventricular repolarisation (by 63%) during reperfusion, and a high incidence (90%) of ventricular tachyarrhythmias. The $K_{ATP}$ channel opener Ro 31-6930 [2-(6-cyano-2,2-dimethyl-2*H*-1-benzopyran-4-yl)-pyridine 1-oxide], delivered prior to ischaemia at a relatively low concentration (0.5 μM), significantly reduced the incidence and duration of reperfusion arrhythmias, and prevented the associated acute action potential shortening during reperfusion, each in a glibenclamide (1 μM)-sensitive manner ($P < 0.05$, $n = 10–15$ hearts). This was associated with a moderate and non-arrhythmogenic action potential shortening during ischaemia (a potentially "cardioprotective" effect). However, these data highlight the potential harm these drugs may cause, since a higher concentration of Ro 31-6930 caused marked shortening of action potentials and significant pro-arrhythmia during ischaemia. 

*Keywords:* $K^+$ channel, ATP-sensitive; Reperfusion; Heart, Isolated; Action potential; Ventricular arrhythmia

## 1. Introduction

Cardiac adenosine triphosphate-sensitive $K^+$ ($K_{ATP}$) channels, first identified in the sarcolemma (Noma, 1983) and more recently in the mitochondria (Inoue et al., 1991), are thought to remain closed under physiological conditions. It is well recognised that under certain pathological conditions such as myocardial ischaemia, however, opening of sarcolemmal $K_{ATP}$ channels may contribute to the shortening of action potential duration (Wilde and Janse, 1994; Wilde, 1997). We recently reported (Workman et al., 2000) that in the rat isolated heart, ischaemia, in the presence (but not the absence) of pharmacological $K_{ATP}$ activation, caused action potential shortening of a sufficient degree to cause ventricular tachyarrhythmias.

It is also recognised that $K_{ATP}$ activation-induced action potential shortening, under certain conditions, may help to protect the myocardium from the injury caused by excessive $Ca^{2+}$ influx and ATP consumption (see Grover and Garlid, 2000 for recent review). Several studies have highlighted the protective effects on the myocardium of pharmacological $K_{ATP}$ activation. In many cases, such protection was demonstrated during post-ischaemic reperfusion. For example, $K_{ATP}$ openers have been shown, during reperfusion, to improve recovery of contractile function (Docherty et al., 1997) to restrain the rise in the intracellular $Ca^{2+}$ concentration, $[Ca^{2+}]_i$ (Behling and Malone, 1995), to enhance the restoration of high-energy phosphates (Tanonaka et al., 1999), as well as limiting the extent of myocardial infarction after reperfusion (Grover et al., 1990). The $K_{ATP}$ openers also exerted effects during the preceding ischaemic episode, such as a reduction in $[Ca^{2+}]_i$ (Behling and Malone, 1995) and an attenuation of ATP loss (Docherty et al., 1997). In other studies, such effects have been correlated with a shortening of repolarisation during ischaemia (Jiang et al., 1994; Tanaka et al., 1996).

Experimental myocardial reperfusion is invariably accompanied by a high incidence of ventricular tachyarrhythmias. These are often most severe after episodes of ischaemia too short to produce myocardial necrosis, e.g. after 10 min in the rat (Hearse and Tosaki, 1988). Reperfu-

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sion arrhythmias are also considered to be important clinically, as highlighted by reports of patients in whom serious ventricular arrhythmias occurred shortly after spontaneous relief of episodes of coronary spasm-induced silent ischaemia (Tzivoni et al., 1983), or in whom persistent arrhythmias followed successful thrombolysis (Goldberg et al., 1983), or during reperfusion of the whole heart after a period of surgically induced ischaemic arrest (Rubin et al., 1985). Despite the potential importance of post-ischaemic reperfusion arrhythmias, the effects of pharmacological $K_{ATP}$ activation on these arrhythmias have been reported infrequently, especially in the absence of arrhythmias during the preceding phase of ischaemia (Ferdinandy et al., 1995; Tosaki et al., 1993; Tanaka et al., 1996). The rat isolated perfused heart has been used extensively to study the pharmacological modulation of arrhythmias. With this model, Ferdinandy et al. (1995) demonstrated that a relatively low concentration of the $K_{ATP}$ opener cromakalim, delivered prior to ischaemia, was anti-arrhythmic during reperfusion, despite the fact that higher concentrations caused marked pro-arrhythmia during ischaemia. These findings were consistent with earlier preliminary data that we presented (Workman et al., 1994) from a similar model, but using a different $K_{ATP}$ opener.

The electrophysiological mechanisms underlying reperfusion arrhythmias are not fully understood, but it has been suggested that a majority may be initiated by non-reentrant mechanisms, such as triggered activity due to afterdepolarisations (Pogwizd and Corr, 1987), but are then maintained primarily by reentry, promoted in part by action potential shortening (Coronel et al., 1992). However, it is recognised that both mechanisms may be involved in the generation as well as in the maintenance of reperfusion arrhythmias (Ferrier et al., 1990; Kaplinsky et al., 1981; Vera et al., 1995). The time course of action potential changes that occur during post-ischaemic reperfusion has been studied previously in intact hearts of several species. It is generally accepted that there is a rapid and transient shortening of repolarisation in pigs (Coronel et al., 1992), guinea pigs (Culling et al., 1984; Penny and Sheridan, 1983) and rabbits (Montrucchio et al., 1989). Only limited data is available from the rat (Perchenet and Kreher, 1995) but in that study, action potentials were recorded only after 1 min of reperfusion and beyond, but repolarisation also transiently shortened. In each of these studies, reperfusion arrhythmias only occurred if (and often immediately after) the action potentials had shortened. Moreover, when reperfusion arrhythmias were inhibited, either by shortening the ischaemic insult (Coronel et al., 1992; Culling et al., 1984) or by means of ischaemic preconditioning (Coronel et al., 1992; Perchenet and Kreher, 1995), or with pharmacological agents, e.g. with sotalol (Culling et al., 1984), there was an accompanying inhibition of the reperfusion-induced acute action potential shortening.

We therefore hypothesised that the anti-arrhythmic effect during reperfusion of $K_{ATP}$ openers delivered at low concentration prior to ischaemia, as seen in the rat (Ferdinandy et al., 1995; Workman et al., 1994), might involve some alteration to the time course of associated changes in action potential repolarisation. To our knowledge, the effect of $K_{ATP}$ modulators on the action potential duration during reperfusion has not been reported previously in intact hearts. Here we report, using rat isolated hearts, the effects of pharmacological $K_{ATP}$ activation and blockade on the acute changes in repolarisation and associated arrhythmias that occur during the phase of post-ischaemic reperfusion.

## 2. Materials and methods

### *2.1. Action potential and cardiac electrogram recording in isolated rat hearts*

This investigation conforms with the Guidance on the Operation of the Animals (Scientific Procedures) Act 1986. Hearts isolated from Sprague–Dawley rats (weighing approximately 500 g) were retrogradely perfused via the aorta at a head of pressure of either 0.66, 1.0 or 1.33 m $H_2O$, with a physiological salt solution containing (mM) NaCl (136.9), glucose (11.1), $MgCl_2$ (1.0), $NaH_2PO_4$ (0.3), $NaHCO_3$ (11.9), $CaCl_2$ (0.9) and KCl (5.0), which was bubbled with 95% $O_2$ and 5% $CO_2$ (pH 7.4). Monophasic action potentials were recorded continuously from the left ventricular epicardial wall using a custom-built suction electrode, as described previously (Workman et al., 2000). The cardiac electrogram was recorded in separate hearts, using wire electrodes inserted into the right atrium and ventricular apex. Each preparation was continuously enclosed in a thermostatically controlled jacket. The epicardial temperature, measured using a thermistor probe, was maintained at 36–37°C. All voltage signals were low-pass filtered at 5 kHz, digitised at a minimum of 3 kHz, and stored for later analysis at high time resolution, using a Gould Windograph chart recorder. The action potential duration was measured at 50% and 80% repolarisation ($APD_{50}$ and $APD_{80}$, respectively). Ventricular tachycardia and ventricular fibrillation were categorised using the guidelines of the Lambeth Conventions (Walker et al., 1988), and combined as ventricular tachyarrhythmia. The heart rate was calculated from the cardiac electrogram each minute, by measuring the number of QRS complexes that occurred during the preceding 20-s interval.

### *2.2. Experimental protocols*

All hearts were allowed to stabilise in the physiological salt solution for 10 min. When required, the perfusate was then switched to one containing drug(s), using independent perfusion lines, which were selected using a multi-way tap situated above the aorta. All drugs were administered prior to ischaemia only. Hearts were administered either the

$K_{ATP}$ opener Ro 31-6930 ([2-(6-cyano-2,2-dimethyl-2*H*-1-benzopyran-4-yl)-pyridine 1-oxide], Paciorek et al., 1990; kindly donated by Roche Research Centre, Herts), the $K_{ATP}$ blocker glibenclamide (Sigma), or a combination of these. Ro 31-6930 is structurally related to the benzopyran $K_{ATP}$ opener cromakalim, but has a much higher biological potency (Edwards et al., 1991). Its $K_{ATP}$ opening activity has been reported previously in a variety of experimental models (Bott et al., 1992; Griffin and Scott, 1994; Finta et al., 1993), including cardiac tissue, in which 3 μM Ro 31-6930 caused action potential shortening, which was reversed both by 100 μM ATP and 0.3–3 μM glibenclamide (Bott et al., 1992). Ro 31-6930 was freshly dissolved in $H_2O$ prior to each days' experiments, and used at final perfusate concentrations of between 0.05 and 5 μM. Glibenclamide was also freshly dissolved, from a stock solution previously prepared in dimethyl sulphoxide and stored at 4°C. The final perfusate concentration of glibenclamide was 1 μM, with 0.002% dimethyl sulphoxide. Coronary flow rate was measured continuously by timed collections of coronary effluent. In preliminary experiments using a perfusion head of pressure of 1 m, Ro 31-6930 (0.05–5 μM) significantly increased the coronary flow rate, and maximally at 0.05 μM, by 37% ($P < 0.05$, $n = 10$). Glibenclamide, on the other hand, reduced coronary flow at this pressure by 39% ($P < 0.05$, $n = 10$). Therefore, in all hearts used to measure electrical activity the perfusion head was set to 1.0 m in the absence of drugs, 0.66 m when using Ro 31-6930, and 1.33 m with glibenclamide. This equalised the mean coronary flow rate between groups, and permitted the investigation of electrical effects of these drugs without significant interference from effects on coronary flow. After starting perfusion with drugs, baseline electrical recordings were made every minute for 15 min. A silk ligature, which had been placed around the left main coronary artery immediately prior to cannulation, was then tightened using a custom-made device, which permitted rapid and remote closing and release of the ligature without disturbing temperature control. Electrical recordings were then made at minute intervals during a 10-min period of regional ischaemia. This duration was chosen since it produces a maximal incidence and duration of ventricular arrhythmias during post-ischaemic reperfusion in this model (Hearse and Tosaki, 1988), and also since it does not result in myocardial necrosis. The ligature was then opened, allowing left ventricular reperfusion, for a further 10 min. Hearts were referred to as being "in ventricular tachyarrhythmia" at each data collection time point, if the cardiac electrogram displayed any episode of ventricular tachyarrhythmia in the 1-min interval prior to that point. Hearts may not, therefore, have been in arrhythmia at the time of data collection. Indeed, no heart was arrhythmic immediately prior to reperfusion, apart from in the group treated with a high concentration (5 μM) of Ro 31-6930. Action potential recordings were made at 1-min intervals during reperfusion except for the first 2.5 min, when data was obtained every 10 s. At the end of each experiment, the zone of non-perfused muscle was delineated using reverse staining with Patent Blue V 1240 (4 g $l^{-1}$) to assess the extent and the wet weight of previously ischaemic muscle, and to confirm that the suction electrode had been located centrally in the non-perfused muscle zone.

### 2.3. Data analysis and statistics

All values were expressed as means ± standard error (S.E.). At each data collection point, the mean action potential duration was calculated only when a minimum of four action potentials per group of hearts could be recorded. Ventricular tachyarrhythmias often interfered with action potential measurement during reperfusion. Incidences of arrhythmias were compared using a $\chi^2$ test (with Yate's Correction). All data on action potentials, arrhythmia duration, heart rate and coronary flow rate were first subjected to a variance ratio test. Data were then compared using either a Student's *t*-test (two-tailed) or a Mann Whitney test (for non-parametric analysis), as appropriate. $P < 0.05$ was considered to be statistically significant.

## 3. Results

### 3.1. Reperfusion-induced arrhythmias were attenuated by a low concentration of a $K_{ATP}$ opener

Myocardial reperfusion, following a 10-min period of ligation of the left main coronary artery, was accompanied by a high incidence of ventricular tachyarrhythmias. Fig. 1(A) shows the time course of changes in the incidence of such arrhythmias prior to, and during the 10-min reperfusion phase. In control hearts ($n = 10$), there were no ventricular tachyarrhythmias during ischaemia, but within 1 min of reperfusion, 90% of hearts were in ventricular tachyarrhythmia. Frequently, this was initially paroxysmal. Mean onset time of the first episode of arrhythmia was 18 ± 8 s. The incidence of arrhythmias then settled at 50% after 6 min of reperfusion. The total duration of ventricular tachyarrhythmia was 331 ± 85 s during the first 10 min of reperfusion (Fig. 1(B)). The coronary flow rate, which had fallen significantly during coronary ligation to 71% of the pre-ischaemic value (10.6 ± 0.7 ml $min^{-1}$ $g^{-1}$ ventricular weight), increased in all hearts within 5 s of opening the ligature, and was 11.6 ± 1.0 ml $min^{-1}$ $g^{-1}$ ventricular weight after 1 min of reperfusion.

The $K_{ATP}$ opener, Ro 31-6930, delivered prior to ischaemia at the relatively low concentration of 0.5 μM, significantly reduced the incidence of ventricular tachyarrhythmias during the first 3 min of post-ischaemic reperfusion (Fig. 1(A)), and was not significantly arrhythmogenic during ischaemia. Indeed, no heart was in arrhythmia immediately prior to reperfusion in this group.

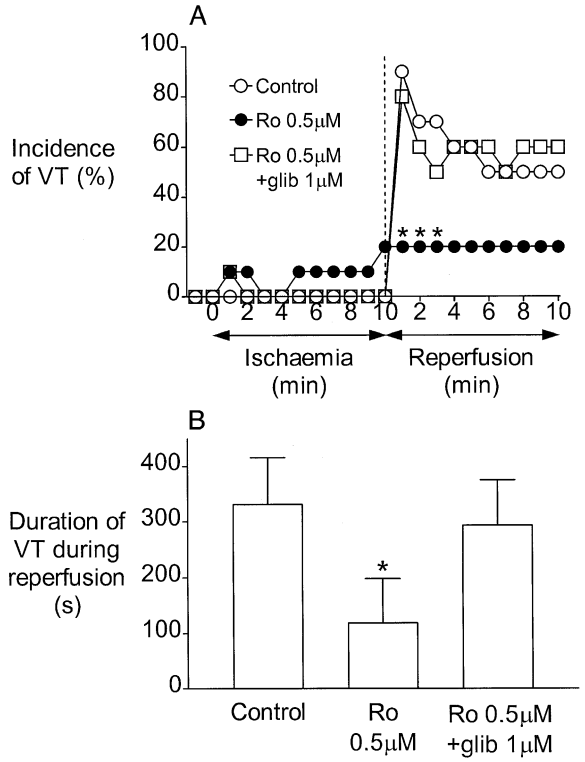


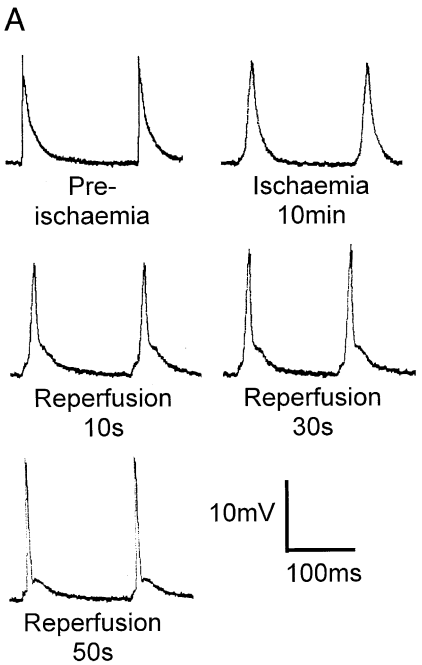

Fig. 1. Effect of pharmacological $K_{ATP}$ activation with a low dose of Ro 31-6930, on reperfusion-induced ventricular arrhythmias. (A) Incidence of ventricular tachyarrhythmias during left ventricular regional ischaemia and post-ischaemic reperfusion. The incidence of reperfusion-induced arrhythmia showed a glibenclamide (glib)-sensitive reduction with 0.5 μM Ro 31-6930 (Ro). Values are means ($n = 10$ hearts per group); (*) significant difference from control value at each time point. (B) Total duration of ventricular tachyarrhythmia during the 10-min reperfusion phase in the same hearts. This showed a shortening effect on ventricular tachyarrhythmia, of $K_{ATP}$ activation by Ro 31-6930 delivered prior to ischaemia. Values are means, error bars denote S.E.; (*) significant difference from control.

The maximal effect of Ro 31-6930 occurred during the first minute of reperfusion, with a reduction in the incidence of ventricular tachyarrhythmias from 90% to 20%. However, significant inhibition of arrhythmias by this drug was restricted to the early phase of reperfusion. Nevertheless, Ro 31-6930 at 0.5 μM also caused a significant reduction in the total duration of ventricular tachyarrhythmias, during the first 10 min of reperfusion by 64% (Fig. 1(B)). Ro 31-6930 at the even lower concentration of 0.05 μM had no effect on the incidence of reperfusion arrhythmias (80%, $n = 10$ hearts). The effect on reperfusion arrhythmias of a higher concentration of Ro 31-6930 (5 μM), also delivered prior to ischaemia, could not be assessed since it was significantly pro-arrhythmic during ischaemia at this concentration, causing ventricular tachyarrhythmias in the 10th minute in 9/10 hearts. Moreover, the arrhythmias persisted into reperfusion in each case. This result emphasises the recognised pro-arrhythmic effect during ischaemia of $K_{ATP}$ openers, when used at high concentration. In the single heart that was not in ventricular tachyarrhythmia at the end of ischaemia with 5 μM Ro 31-6930, no arrhythmias developed during reperfusion. Ro 31-6930 had no significant effect on the time of onset of arrhythmias during reperfusion at any concentration tested. The mean coronary flow rates prior to, during and following ischaemia, were not significantly different from control values in any of the Ro 31-6930-treated groups (owing to the adjustment of perfusion pressure detailed in the Methods), and so the effects of the $K_{ATP}$ opener on ventricular tachyarrhythmias were, therefore, unlikely to have been obscured or complicated by its vasodilator action.

The $K_{ATP}$ blocker glibenclamide (1 μM) abolished the inhibitory effect of 0.5 μM Ro 31-6930 on ventricular arrhythmias, whilst having no significant effect alone, on either the incidence or duration of arrhythmias prior to or during reperfusion ($n = 10$ hearts). Fig. 1 shows that concomitant glibenclamide restored both the incidence of reperfusion arrhythmias (as well as the time course of their change) and the total duration of these arrhythmias. At the end of each experiment, the wet weight of the region of muscle that had been made ischaemic was measured. This was found to be insignificantly different from the control value ($0.67 \pm 0.03$ g, or 52% of the total ventricular weight) in each of the drug-treated groups of hearts. In addition, the heart rate, at $237 \pm 14$ beats $min^{-1}$ prior to ischaemia in the control group, did not differ significantly in any of the drug-treated groups, at any time point before or during ischaemia.

### *3.2. Post-ischaemic reperfusion caused rapid and transient action potential shortening*

Representative action potentials recorded from the left ventricular epicardium are shown in Fig. 2(A). Prior to ischaemia, the $APD_{80}$ was 38 ms, and after 10 min of regional ischaemia, the $APD_{80}$ was 39 ms (see upper traces), confirming the previously reported lack of action potential shortening during early ischaemia in the rat (Workman et al., 2000). In contrast, post-ischaemic reperfusion was accompanied by a rapid and dramatic shortening of repolarisation (Fig. 2(A), lower traces), with the $APD_{80}$ reaching a nadir of 23 ms, in this case, after 50 s. This was often associated with a pronounced (near complete) phase 1 repolarisation, as illustrated in the bottom trace of Fig. 2(A). In 2/15 hearts, however, the action potential transiently lengthened after 20-s reperfusion. An example, which may represent action potentials displaying subthreshold early afterdepolarisations, is shown in the upper trace of Fig. 2(B). In this case, the $APD_{80}$ after 20 s of reperfusion was 79 ms. By 40 s of reperfusion, the $APD_{80}$ had declined to 48 ms. Unfortunately, it became immeasurable beyond 40 s due to the onset of chaotic electrical activity, characteristic of ventricular tachyarrhythmia (bottom trace of Fig. 2(B)). Chaotic electrical activity appeared (and therefore interfered with action potential measurement) at various times during reperfu-



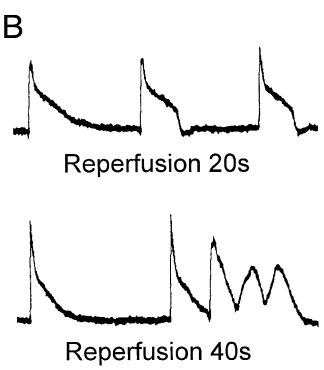




Fig. 2. Action potential changes produced by 10 min of regional ischaemia and reperfusion. (A) Representative suction electrode-recorded action potential traces from the left ventricular epicardial wall of a single heart, with a characteristically short repolarisation phase prior to ischaemia and a lack of action potential shortening after 10 min of ischaemia (upper panels), but with a brisk shortening during reperfusion (maximal after approximately 50 s). Recording times are shown beneath each trace. (B) Traces from a different heart during reperfusion, illustrating the occasional and early presence of "long-footed" action potentials (upper panel) and the onset of ventricular tachyarrhythmia (lower panel). Calibration bars apply to all traces.

sion. Its appearance was not consistently preceded by either lengthening or shortening of repolarisation.

Fig. 3 shows the time course of changes in the mean $APD_{80}$ during post-ischaemic reperfusion, obtained from a group of 15 hearts. The associated marked but transient action potential prolongation (Fig. 3(A)), which occurred early during the preceding 10-min episode of ischaemia, was entirely characteristic of this model and has been described in detail elsewhere (Workman et al., 2000). It can be seen in Fig. 3(B) that there was marked, transient and significant shortening (below the pre-reperfusion value) of mean $APD_{80}$ values over the first 2.5 min of reperfusion. This shortening was maximal between 20 and 90 s of reperfusion in different hearts, reaching a nadir (with a shortening of 63% to $15 \pm 2$ ms) after approximately 40 s. The time course of changes in mean $APD_{50}$ was similar to that of $APD_{80}$, but $APD_{50}$-shortening did not reach statistical significance during reperfusion.

### *3.3. The low dose of a $K_{ATP}$ opener shortened action potentials during ischaemia and prevented further shortening during reperfusion*

We next examined the effect on epicardial action potentials of the $K_{ATP}$ opener Ro 31-6930 at a relatively low (but nevertheless, arrhythmia-inhibiting) concentration of 0.5 μM. There was no significant effect on repolarisation prior to ischaemia, but during ischaemia, Ro 31-6930 markedly attenuated the initial transient action potential lengthening, as reported in detail previously (Workman et

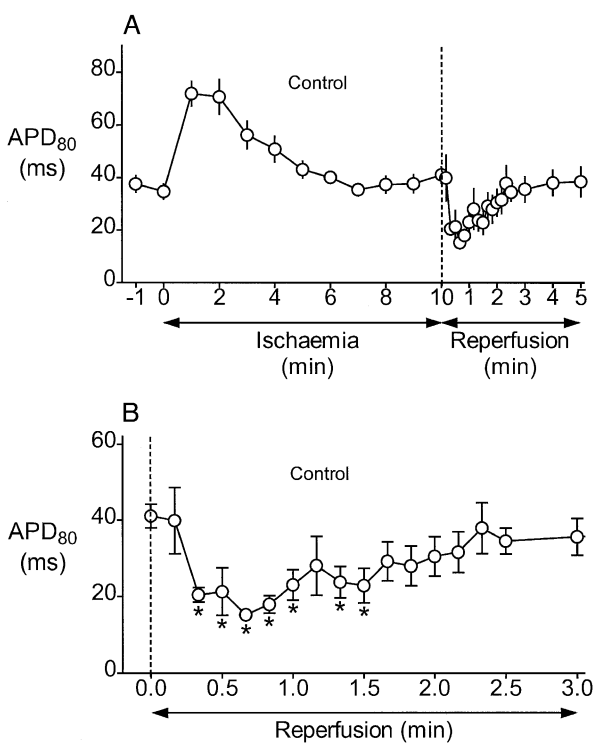



Fig. 3. Time course of changes in late repolarisation during regional ischaemia and reperfusion. (A) Left ventricular epicardial $APD_{80}$. This showed a marked but transient lengthening produced by 10 min of left coronary artery ligation, followed by a rapid shortening during left ventricular reperfusion. Values are means, error bars denote S.E. ($n = 15$ hearts, 6–15 and 4–11 action potentials prior to and during reperfusion, respectively). (B) Expanded time scale to show the changes in action potential duration during the first 3 min of post-ischaemic reperfusion. The first value (at time zero) was obtained immediately before releasing the coronary ligature. Asterisks indicate a significant difference from this value for subsequent data points.

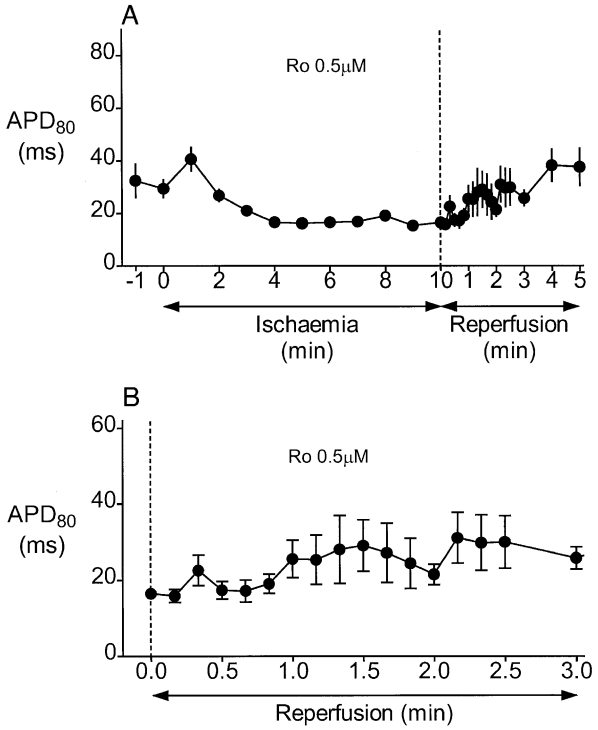



Fig. 4. Effect of a low concentration of a $K_{ATP}$ opener on repolarisation during ischaemia and reperfusion. (A) Effect of 0.5 μM Ro 31-6930 (Ro), delivered prior to ischaemia, on the time course of changes in $APD_{80}$, showing attenuation of the lengthening during ischaemia and a shortening after approximately 4 min of ischaemia. These effects were associated with an absence of further shortening of $APD_{80}$ during reperfusion. (B) Expanded time scale to show the changes in $APD_{80}$ during the first 3 min of reperfusion. There were no significant differences between the pre-reperfusion value (time zero) and all subsequent data points. Values are means, error bars denote S.E. ($n = 13$ hearts, 7–13 and 7–10 action potentials prior to and during reperfusion, respectively).

al., 2000). $APD_{80}$ was significantly shortened to below the pre-ischaemic value at each time point between 4 and 10 min of ischaemia (Fig. 4(A)). Additionally, this concentration of Ro 31-6930 caused significant shortening during ischaemia, of both the mean $APD_{50}$ and $APD_{80}$ values, at each time point compared to the control group. For example, at min 10 of ischaemia, $APD_{80}$ was $41.1 \pm 3.1$ and $16.5 \pm 1.3$ ms in the control and drug-treated groups, respectively ($P < 0.05$, $n = 15$ and 13 hearts).

In marked contrast to the situation in the control group (Fig. 3(B)), during post-ischaemic reperfusion, there was no further shortening of mean $APD_{80}$ values in hearts treated with the $K_{ATP}$ opener (Fig. 4(B)). In Ro 31-6930-treated hearts, a transient lengthening in action potentials was observed during reperfusion (possibly indicative of action potentials displaying subthreshold early afterdepolarisations). As before (see Fig. 2(B)), this was seen only in two hearts. In the first, this occurred after 10 s of reperfusion ($APD_{80}$ was 93 ms), and in the second, at both 70 and 90 s of reperfusion ($APD_{80}$: 83 and 89 ms, respectively). Therefore, the main and usual effect of Ro 31-6930 at 0.5 μM on action potentials during reperfusion was to prevent the normal early phase of shortening of repolarisation. This was associated with attenuation of the normal action potential prolongation seen during the preceding episode of ischaemia. As with the earlier experiments on arrhythmias, the coronary flow rate in hearts treated with Ro 31-6930 was not significantly different from that in the control group. The ability of the $K_{ATP}$ opener to prevent action potential shortening during reperfusion, therefore, was unlikely to have been obscured or complicated by its vasodilator action.

### *3.4. The $K_{ATP}$ blocker glibenclamide abolished the effects of a $K_{ATP}$ opener on repolarisation during ischaemia and reperfusion*

The $K_{ATP}$ blocker glibenclamide at 1 μM, a concentration which was shown earlier to abolish the inhibitory effect of Ro 31-6930 at 0.5 μM on ventricular tachyarrhythmias (Fig. 1), abolished the effects of this drug on ventricular action potentials during both reperfusion and the preceding phase of ischaemia. This can be seen by comparing Figs. 3, 4 and 5. When delivered alone, gliben-

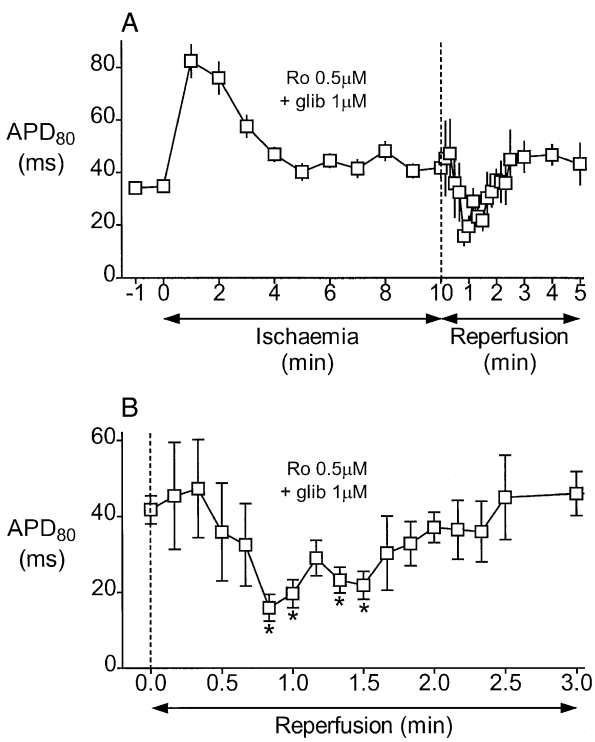



Fig. 5. Abolition by glibenclamide of $K_{ATP}$ opener effects during ischaemia and reperfusion. (A) Time course of changes in $APD_{80}$. Glibenclamide (glib) at 1 μM, delivered prior to ischaemia, completely reversed the effects of 0.5 μM Ro 31-6930 (Ro). Thus, there was a recovery of both action potential prolongation during ischaemia and rapid action potential shortening during reperfusion. (B) Expanded scale to show the detailed time course of changes in $APD_{80}$ during the first 3 min of reperfusion. This indicates a significant (*), marked but transient shortening below the pre-reperfusion value (0 min). Values are means, error bars denote S.E. ($n = 15$ hearts, 7–15 and 4–13 action potentials prior to and during reperfusion, respectively).

clamide had no effect on the action potential changes seen during ischaemia (as reported in detail elsewhere; Workman et al., 2000). Additionally, we now report that glibenclamide alone failed to modify the changes in repolarisation produced during reperfusion. Thus, the nadir of $APD_{80}$-shortening during reperfusion was similar in control and glibenclamide-treated hearts, at $15.3 \pm 1.5$ and $12.2 \pm 5.3$ ms, respectively ($n = 15$ hearts each). In both groups, this point was reached after 40 s of reperfusion.

Thus, glibenclamide at 1 μM, when delivered in combination with 0.5 μM Ro 31-6930 ($n = 15$ hearts), completely restored both transient action potential prolongation during ischaemia (as reported previously; Workman et al., 2000), and the transient action potential shortening normally seen during reperfusion. In the drug combination-treated group, in contrast to the group given Ro 31-6930 alone, there was marked prolongation of $APD_{80}$ early during ischaemia (compare Fig. 5(A) with Fig. 4(A)) and there was significant shortening of $APD_{80}$ early during reperfusion (compare Fig. 5(B) with Fig. 4(B)), as seen in the unmedicated group of controls (Fig. 3(B)).

## 4. Discussion

In this study, we have demonstrated that in rat isolated hearts, post-ischaemia reperfusion is associated with ventricular tachyarrhythmias. Using a $K_{ATP}$ opener at a low concentration delivered before ischaemia, we have also shown an inhibitory effect on these arrhythmias during early reperfusion. This was closely associated with prevention by the drug of the usual acute action potential shortening seen early during reperfusion in this model, which in turn was associated with an action potential shortening effect of the drug during ischaemia.

The significant reduction by 0.5 μM Ro 31-6930, in both the incidence and duration of ventricular tachyarrhythmias during reperfusion, was unlikely to have been complicated by this drug's well known vasodilator action since coronary flow was kept similar in the control and drug treatment groups. The $K_{ATP}$ blocker glibenclamide at 1 μM had no effect by itself on reperfusion ventricular tachyarrhythmias, and nevertheless abolished the inhibitory effect on arrhythmias shown by Ro 31-6930. By using glibenclamide at 1 μM, we sought to minimise the risk of producing actions additional to $K_{ATP}$ blockade. The ventricular A-kinase-dependent $Cl^-$ current, for example, is blocked with $> 1$ μM glibenclamide (Tominaga et al., 1992). The lack of effect of glibenclamide on reperfusion arrhythmias is in agreement with previous studies in rats (Bernauer, 1997; Ferdinandy et al., 1995). Nevertheless, 1 μM glibenclamide has been reported to reduce the duration of reperfusion ventricular fibrillation in this species (Bril et al., 1992).

Most $K_{ATP}$ openers are pro-arrhythmic during ischaemia (Chi et al., 1990; Wolleben et al., 1989). In agreement, we have previously demonstrated that Ro 31-6930, at relatively high concentration, exerted substantial and glibenclamide-sensitive pro-arrhythmic and action potential shortening effects during ischaemia (Workman et al., 2000). A shortening of the QT interval in the rat was demonstrated during ischaemia with a $K_{ATP}$ opener (Rees and Curtis, 1995b); and in canine epicardial tissue, shortening of repolarisation with pinacidil lead to arrhythmias with characteristics of reentry (Di Diego and Antzelevitch, 1993). Whilst several studies have shown that $K_{ATP}$ openers can prevent triggered activity induced by agents such as $Cs^+$ (D'Alonzo et al., 1993), there are few reports of any effect of $K_{ATP}$ openers on post-ischaemic reperfusion arrhythmias, particularly in the absence of any pro-arrhythmic effect demonstrable during the ischaemic episode. Nevertheless, the $K_{ATP}$ openers cromakalim, nicorandil and NIP-121 [((+)-7,8-dihydro-6,6-dimethyl-7-hyroxy-8-(2-oxo-1-piperidinyl)-6*H*-pyrano[2,3-f]benz-2,1,3-oxadiazole)], have each been reported to be anti-arrhythmic during reperfusion (Ferdinandy et al., 1995; Kempsford and Hawgood, 1989; Tanaka et al., 1996). Moreover, in close agreement with a previous report on rat hearts (Ferdinandy et al., 1995), the present data show that pharmacological $K_{ATP}$ activation may be both pro-arrhythmic during ischaemia and anti-arrhythmic during subsequent reperfusion, depending only upon the concentration of the $K_{ATP}$ opener used. Furthermore, the present data highlight that whilst a $K_{ATP}$ opener at low concentration could attenuate reperfusion arrhythmias without significant pro-arrhythmia during ischaemia, this should be interpreted cautiously due to the recognised significant risk of pro-arrhythmia with these drugs.

In the absence of drugs, ventricular action potentials recorded from intact hearts in the present study underwent a rapid, marked and significant but transient period of shortening during post-ischaemic reperfusion. The question arises, however, of how faithfully do suction electrode-recorded potentials reflect the time course of transmembrane action potentials recorded from the same region (Ino et al., 1988). Previously, we demonstrated the reliability of this recording method in our model (Workman et al., 2000). Moreover, a period of similar reperfusion-induced early action potential shortening has been reported by others in the hearts of various species, including pig (Coronel et al., 1992), guinea pig (Culling et al., 1984; Penny and Sheridan, 1983), rabbit (Montrucchio et al., 1989), and rat (Perchenet and Kreher, 1995). In each of those studies, however, repolarisation was measured first after a full 1 min of reperfusion. That is in contrast to the earlier action potential measurements that were possible in the present study. Nevertheless, in a previous rat study (Perchenet and Kreher, 1995), in close agreement with the present findings (Fig. 3(B)), some shortening in repolarisation was initially recorded during reperfusion (following 25 min of

ischaemia). It is noteworthy that in contrast to this early reperfusion-induced action potential shortening, the rat features a marked but transient lengthening during ischaemia (Workman et al., 2000; Perchenet and Kreher, 1995). We recently reported (Workman et al., 2000) that in Langendorff-perfused rat hearts, significant flow of $I_{KATP}$ may not occur early on during regional no-flow ischaemia, as used here. Nevertheless, it is acknowledged that $K_{ATP}$ channels may contribute to $K^+$ efflux during global low-flow ischaemia in this species (Kantor et al., 1990; Wolleben et al., 1989).

The rat has been used extensively to study the effects of pharmacological $K_{ATP}$ modulation on ventricular arrhythmias, and there is notable agreement between data obtained in this and other species. For example, during myocardial ischaemia, in dogs, rabbits, guinea-pigs and rats, $K_{ATP}$ openers are generally pro-arrhythmic (Chi et al., 1990; Bellemin-Baurreau et al., 1994; Workman et al., 2000) whilst $K_{ATP}$ blockers are anti-arrhythmic (Billman et al., 1993; Gwilt et al., 1992; Wolleben et al., 1989; Kantor et al., 1990; Rees and Curtis, 1995b). Moreover, in each of these species, reperfusion after only a short period of ischaemia is accompanied by both ventricular arrhythmias and acute action potential shortening. To our knowledge, however, the present study is the first to examine effects of $K_{ATP}$ modulators on action potentials during reperfusion in the rat. In hearts treated with Ro 31-6930 at a concentration which inhibited reperfusion arrhythmias (0.5 μM), there was a moderate, non-arrhythmogenic shortening of the $APD_{80}$ during ischaemia, as reported earlier (Workman et al., 2000). In these hearts, in marked contrast to the controls, however, there was no further shortening of repolarisation during reperfusion. In several previous studies of action potentials during post-ischaemic reperfusion, arrhythmias were only seen during reperfusion if (and often immediately after) repolarisation had shortened (Coronel et al., 1992; Culling et al., 1984; Montrucchio et al., 1989; Penny and Sheridan, 1983; Perchenet and Kreher, 1995). Moreover, those interventions that inhibited these arrhythmias, such as reducing the duration of prior ischaemia (Coronel et al., 1992), preceding it with an episode of ischaemic preconditioning (Coronel et al., 1992; Perchenet and Kreher, 1995) or using various pharmacological treatments (Culling et al., 1984), all resulted in an inhibition of the acute action potential shortening during reperfusion. Indeed, it has already been suggested that such shortening may provide the necessary substrate for reperfusion arrhythmias (Culling et al., 1984; Ferrier et al., 1990; Coronel et al., 1992). It is conceivable, therefore, that prevention by 0.5 μM Ro 31-6930 of the acute shortening of repolarisation during reperfusion, may have contributed to this drug's inhibitory effect on arrhythmias. Moreover, we have confirmed an involvement of $K_{ATP}$ channel activation in this effect on repolarisation, as well as in the associated arrhythmias, by abolishing both of these effects with glibenclamide. The latter drug on its own had no effect on either repolarisation or ventricular arrhythmias.

Reperfusion-induced shortening of action potentials has previously been correlated (Coronel et al., 1992) with a rapid washout of extracellular $K^+$. That ion is known to treble in concentration in the extracellular space during the preceding 10 min of ischaemia in the rat (Wilde et al., 1990). Reperfusion was reported to cause a rapid and transient undershoot in $[K^+]_o$ (Coronel et al., 1992). Activity of the $Na^+,K^+$ pump ($Na^+,K^+$-ATPase), which is thought to play a key role in the development of reperfusion arrhythmias (Tani and Neely, 1991), was markedly reduced after only 10 min of ischaemia in the rat (Avkiran et al., 1996). Reperfusion-induced $[K^+]_o$-undershoot is thought to result largely from recovery of $Na^+,K^+$-ATPase activity upon restoration of coronary flow (Coronel et al., 1992; Tani and Neely, 1991; Avkiran et al., 1996). This enzyme, whilst extruding the excess $(Na^+)_i$ that accumulated during ischaemia, would also rapidly remove $K^+$ from the extracellular space, possibly leading to a $[K^+]_o$ undershoot. Indeed, in pig hearts, such an effect on $[K^+]_o$ during reperfusion has been reported to cause hyperpolarisation, an action potential shortening and the attendant reentrant arrhythmogenesis (Coronel et al., 1992). Moreover, such an arrhythmogenesis occurred only in the presence of a reperfusion $[K^+]_o$ undershoot. Interventions, which attenuated or delayed the fall in $[K^+]_o$, also attenuated or delayed the arrhythmias. In that, as well as in other studies (Kaplinsky et al., 1981), the arrhythmias were deduced to have been of reentrant origin. That would be consistent with a shortening of the ERP, which also had been demonstrated previously during reperfusion (Naimi et al., 1977).

The ability of 0.5 μM Ro 31-6930 to shorten repolarisation during ischaemia via opening of sarcolemmal $K_{ATP}$ channels without causing concurrent arrhythmias would be expected to reduce the rise in $[Ca^{2+}]_i$ during ischaemia, a potentially "cardioprotective" effect. A close correlation has been reported between reductions in action potential duration and $[Ca^{2+}]_i$ by the $K_{ATP}$ opener lemakalim in guinea pig ventricular myocytes (Jiang et al., 1994). Likewise, there was attenuation of ischaemia-induced increases in $[Ca^{2+}]_i$ by cromakalim in intact rat hearts (Behling and Malone, 1995). It is presently unknown whether Ro 31-6930 opens the mitochondrial $K_{ATP}$ (mito$K_{ATP}$). Although such an effect might reduce $[Ca^{2+}]_i$ (Garlid, 2000; Sato and Marbán, 2000), that action alone would not be expected to shorten repolarisation in the way that we observed. A reduction in $[Ca^{2+}]_i$ during ischaemia would be expected to attenuate the rapid rise in $[Ca^{2+}]_i$ during reperfusion, as shown previously in the rat (Behling and Malone, 1995). This might inhibit afterdepolarisation formation during reperfusion. It might also attenuate other deleterious effects of $[Ca^{2+}]_i$ overload, which manifest during reperfusion. For example, a reduced rise in $[Ca^{2+}]_i$ during ischaemia would also limit $Na^+/Ca^{2+}$ exchange-

induced $(Na^+)_i$ accumulation (Tani and Neely, 1991). This may in turn attenuate the enhancement of $Na^+,K^+$-ATPase activity during reperfusion, and thus some of the acute shortening of repolarisation. This is supported by reports that different "cardioprotective" interventions, known to reduce $[Ca^{2+}]_i$ during ischaemia in the rat, such as ischaemic preconditioning and verapamil treatment, prevented action potential shortening during reperfusion (Perchenet and Kreher, 1995).

The following limitations of the present study must be accommodated when interpreting the data. (1) The inhibitory effect on arrhythmias of Ro 31-6930 was mild, and with a reduction in the incidence of ventricular tachyarrhythmias being restricted to the early phase of reperfusion. Whilst an effect during this phase is recognised to be important, owing to the normally high prevalence of tachyarrhythmias, it must be weighed against the established pro-arrhythmic effects of higher concentrations of $K_{ATP}$ openers. (2) This effect was observed at one concentration only. This may reflect the fact that only three concentrations were tested, chosen from a 100-fold range. More closely spaced concentrations in the vicinity of 0.5 μM would be needed before testing in-vivo. (3) This effect may have been due partly to $I_{KATP}$ flowing during reperfusion. The present study was not designed specifically to test this possibility, and future investigations, by perfusing hearts with Ro 31-6930 exclusively during the reperfusion phase, are warranted. (4) Future studies should also utilise $K_{ATP}$ modulators with an established selectivity for mito$K_{ATP}$ or sarcolemmal $K_{ATP}$, in order to investigate the relative involvement of such ion channels during reperfusion. (5) Whilst rat hearts share many electrophysiological similarities with those of other species, they also display some important differences, such as a relatively short action potential duration and a marked prolongation of repolarisation during ischaemia (Perchenet and Kreher, 1995; Workman et al., 2000; Rees and Curtis, 1995a; Ponce Zumino et al., 1998). Caution should therefore be exercised when extrapolating the present data, particularly regarding specific drug concentrations, to those from other species.

In summary, the present study, the first to our knowledge to examine effects of $K_{ATP}$ openers on acute action potential changes during reperfusion of intact hearts, suggests that in the rat, the inhibitory effect on arrhythmias during post-ischaemic reperfusion, obtained with a low dose of a $K_{ATP}$ opener delivered prior to ischaemia, may be associated with an attenuation of acute action potential shortening during reperfusion. This in turn may be related to a shortening of repolarisation during the preceding ischaemic episode; a possible "cardioprotective" effect. The results also emphasise the potential harm (pro-arrhythmia) that ischaemia may cause with $K_{ATP}$ openers, even when such drugs are anti-arrhythmic during subsequent reperfusion.

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