Do K_{ATP} channels open as a prominent and early feature during ischaemia in the Langendorff-perfused rat heart?

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Abbreviated title: Do K_{ATP} channels open during ischaemia in the isolated rat heart?

Abstract

The objective was to investigate whether myocardial adenosine triphosphate-sensitive K^+ (K_{ATP}) channels open during the first 10min of regional ischaemia in Langendorff-perfused rat hearts. Changes in monophasic action potentials and arrhythmias were studied during myocardial ischaemia in both the presence and absence of pharmacological K_{ATP} modulation. Ligation of the left main coronary artery for 10min did not shorten the action potential duration (APD). The APD_{50} and APD_{80} (15.5±1.0 and 38.1±2.3ms, respectively [mean±S.E., n=15 hearts], immediately prior to ligation) increased transiently during the first 4min of ligation (by 160 and 79% respectively, P<0.05), before returning to pre-ligation values, but without a significant below-baseline-shortening. The cardiac electrogram showed no accompanying ventricular tachyarrhythmia (VT). These results raised the possibility that the myocardial K_{ATP} channels had not opened during the ligation. The K_{ATP} opener Ro 31-6930 (0.5 and 5μM) shortened the APD_{50} and APD_{80} during coronary ligation, to significantly below both their control and pre-occlusion values (P<0.05), and caused a concentration-dependent increase in both the incidence and duration of VT during the ligation. Ro 31-6930 at 5μM also shortened APD_{50} and APD_{80} even before ligation (by 50 and 62% respectively, P<0.05), and abolished the normal APD-lengthening seen during ischaemia. The K_{ATP} blocker glibenclamide (1μM) abolished both the APD-shortening and pro-arrhythmic effects of the K_{ATP} opener, both before and during coronary ligation, yet when delivered on its own, at the same concentration which abolished the effects of K_{ATP} activation, it had no significant effect on the APD changes seen during the coronary ligation alone. These results suggest that, in Langendorff-perfused rat hearts in the absence of drugs, K_{ATP} channels do not open during early myocardial ischaemia.

Keywords

ATP-sensitive potassium channel; Ischaemia; Isolated heart; Action potential; Ventricular arrhythmias.

Introduction

In cardiac myocytes the normal intracellular ATP concentration keeps adenosine triphosphate-sensitive K^+ (K_{ATP}) channels in a closed state, but ATP removal can open them (25). Under certain conditions, this increases cellular K^+ efflux, with a profound alteration of normal cardiac electrical activity, such as shortening of the action potential duration (APD) (39). Shortening of the action potential caused by the K_{ATP} current (I_{KATP}) may protect the ischaemic myocardium by reducing intracellular Ca^{2+} overload and by conserving intracellular stores of ATP. However, by concomitantly shortening the refractory period, any APD reduction would favour reentrant arrhythmias (6,15). It should be noted that the newly recognised mitochondrial K_{ATP} channel, mitoK_{ATP} (which may open more readily than sarcolemmal K_{ATP}) also may been involved in myocardial protection, but this would not be expected to be reflected by changes in the APD (11). For reviews on the various electrical consequences of cardiac sarcolemmal K_{ATP} channel activation, see (2,24,39).

Several lines of evidence indicate that K_{ATP} channels can open within the first 5 to 10min of acute myocardial ischaemia, i.e.: before necrosis would be expected, but when lethal arrhythmias may occur. Thus, APD shortens within about 3min of ischaemia onset in several species, and is sensitive to K_{ATP} blockers in the intact hearts of dogs and rabbits (30). Additionally, K_{ATP} openers have been reported to cause, and K_{ATP} blockers to prevent, various ventricular arrhythmias during acute myocardial ischaemia in several species, including the rat (9,16).

However, ionic currents additional to I_{KATP} are known to participate in APD-shortening during early ischaemia (4), and, there is evidence to suggest that K_{ATP} activation may not be the main cause of such APD-shortening. For example, the reversal of APD-shortening in rabbits by the K_{ATP} blocker glibenclamide occurs well after 10min of ischaemia or hypoxia have elapsed (1,29), even though APD-shortening in this species occurs within 3min of ischaemia. Moreover, only partial reversal by glibenclamide of the APD-shortening effects of ischaemia or hypoxia have been observed (30). Indeed, K_{ATP} channels may not open at all under certain conditions. The intracellular ATP concentration remained in the millimolar range (perhaps as high as 10mM) for the first 10min in some studies (18), yet half maximal inhibition of K_{ATP} channels in excised membrane patches is known to occur at 17-25μM (17), and so the possibility remains that significant K_{ATP} activation may not occur during early ischaemia, even when taking into account the limitations involved in comparing data obtained using different experimental techniques (24).

The rat has been used extensively to investigate the effects of pharmacological K_{ATP} modulators on arrhythmogenesis. In this species, an accumulation of K^+ in the extracellular space has been detected after only 15s of ischaemia, with $[K^+]_o$ reaching 9mM after 5min. However, glibenclamide only partially altered this accumulation (with $[K^+]_o$ still reaching 8mM after 5min) and exerted even less of an effect up to 25min beyond this time (38). This suggested only a limited involvement of K_{ATP} channels during early ischaemia in this species. Moreover, the APD has been shown to increase markedly during the first few minutes of ischaemia in the rat (26-28,40), i.e.: at a time when any K_{ATP} activation might have been expected to shorten APD. Furthermore, the APD was shown not to shorten at all during global ischaemia but to exhibit transient lengthening during the first 5min, followed by a second increase during the following 15min (26). An activation of only 1% of the available K_{ATP} conductance should be sufficient to shorten APD by approximately 50% (24). We therefore hypothesised that K_{ATP} channels might not open during early ischaemia in this species. Ischaemic APD-lengthening (or a lack of APD-shortening) *per se* does not preclude concurrent K_{ATP} activation, and so data on the sensitivity of such changes to K_{ATP} blockers is required. There are currently no reports of investigations into the effects of K_{ATP} blockers on APD during ischaemia in intact rat hearts.

The aim of the present study, therefore, was to investigate whether K_{ATP} channels open during the first 10min of myocardial ischaemia in Langendorff-perfused rat hearts, by assessing the sensitivity to pharmacologic K_{ATP} modulation, of changes in action potentials and arrhythmias during regional ischaemia in these intact hearts.

Methods

Isolation and perfusion of rat hearts in-vitro

The investigation conforms with the *Guidance on the Operation of the Animals (Scientific Procedures) Act 1986*. In accordance with *Schedule 1*, male Sprague Dawley rats (350-650g) were killed by concussion, followed by exsanguination. The heart was surgically exposed and a silk suture (Type W593 Mersilk, Ethicon) was placed beneath the left main coronary artery. The aorta was then cannulated and the heart was rapidly excised and perfused in Langendorff mode at 35-37^oC (inside a water-heated covered glass chamber) 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), pH 7.4. This solution was bubbled with 95% O_2 plus 5% CO_2 and pre-filtered using a 5μm filter (Isopore SM, Millipore). The duration between concussion and exsanguination, and the start of Langendorff perfusion, was approximately 2-3min.

Drugs

A stock solution of the K_{ATP} opener Ro 31-6930 (Roche Research Centre) was dissolved in H_2O, and was freshly prepared before each experiment. A stock solution of the K_{ATP} blocker glibenclamide (Sigma) dissolved in dimethyl sulphoxide was prepared weekly and stored at 4^{o}C. These drugs were added to the physiological salt solution when required, prior to perfusing the hearts. Final concentrations of Ro 31-6930 were 50nM-10μM. Glibenclamide was used at 1μM, with 0.002% dimethyl sulphoxide in the final perfusate.

Electrical recording techniques

The cardiac electrogram (CEG) was recorded continuously using stainless steel wires inserted into the ventricular apex and the right atrium, respectively. Arrhythmias were monitored on a scroll-type storage oscilloscope (Cardiostore SEM 431, SE Labs), and categorised using the guidelines of the Lambeth Conventions (34). Ventricular tachycardia and ventricular fibrillation were combined as ventricular tachyarrhythmia (VT). The heart rate was calculated from the CEG each minute, by measuring the number of QRS complexes which occurred during a 20s interval. Action potentials were recorded, in separate groups of hearts, from the left ventricular epicardial wall, 4mm distal to the coronary ligature, using a custom made suction electrode (internal diameter 1mm, external diameter 1.3mm), evacuated to 200mm Hg below atmospheric pressure. Voltage signals were low-pass filtered at 5kHz, digitised and stored on the magnetic disc of a scroll-type oscilloscope (Windograph, Gould).

Experimental protocols

Hearts were allowed to stabilise for 10min by perfusing with the physiological salt solution. Recording of either the CEG or epicardial action potentials was then started. Perfusion fluid was switched to one containing drugs, if required, and baseline recordings continued for another 15min. The coronary ligature was then closed around the left main coronary artery. Electrical recordings were continued throughout a 10min period of ligation. This interval was chosen since it is substantially longer than that reported to be required for

ischaemic-K_{ATP} opening in many animal species, and would therefore aid comparison with those studies, and yet it would not be sufficiently long to cause myocardial necrosis. Coronary flow rate was measured by timed collection of coronary effluent. At the end of each experiment, the zone of non-perfused muscle was delineated using the technique of reverse staining, with Patent Blue V 1240 (at $4g.l^{-1}$). This produced a distinct zone of dye-stained (non-perfused) muscle (risk zone), which invariably extended over approximately 50% of the total ventricular surface, demonstrating the success of the coronary artery occlusion, and confirming the limited involvement of coronary collaterals in this species. Dye-stained and non-stained ventricular muscle masses were separated with scissors, blotted dry and weighed. In a separate group of 10 hearts, the suction electrode was replaced by a thermistor probe (Wayne Kerr), to measure the epicardial temperature in the ischaemic zone prior to and during coronary ligation.

Data analysis and statistics

APD measurements were excluded if the suction electrode was found to have not been located centrally in the non-perfused area of muscle. All values were expressed as means±standard error (S.E.). Incidences of arrhythmias were compared using a χ^2 test (with Yate's Correction). All data on arrhythmia duration, coronary flow rate and APD were subjected to a variance ratio test. Data were then compared using either a Student's *t* test or a Mann Whitney test (for non-parametric analysis), as appropriate. $P<0.05$ was considered significant.

Results

Acute left ventricular regional ischaemia did not cause arrhythmias in isolated rat hearts

Ligation of the left main coronary artery for 10min was not accompanied by any episode of VT in Langendorff-perfused rat hearts ($n=10$), despite producing a distinct zone of non-perfused muscle in each heart. The incidence of VT was zero during the 10min period prior to coronary occlusion. Occlusion abruptly reduced the total coronary flow rate from 10.6±0.7 to $6.7±0.6ml.min^{-1}.g^{-1}$ ventricular weight ($P<0.05$, $n=10$, perfusion pressure=100cm H_20). The mean wet weight of the muscular zone at risk of ischaemia, as revealed by staining, was 0.67±0.03g, or 52% of the total ventricular weight ($n=10$). The heart rate was 237±14

beats.min^{-1} 1 min before coronary occlusion. During occlusion, the heart rate fell progressively, and to significantly below the pre-occlusion value only after 10min, to 197±18 beats.min^{-1} (P<0.05).

The action potential duration did not shorten during coronary artery occlusion

Representative action potentials recorded from the left ventricular epicardium are shown in Fig. 1., with the typical action potential configuration (short duration) seen prior to ischaemia in Fig. 1.(a). The time course of changes in left ventricular action potentials recorded during the 10min period of coronary occlusion from the area at risk of ischaemia can be seen from the recordings in Fig. 1.(c). The APD at both 50 and 80% repolarisation (APD_{50} and APD_{80}, respectively) was measured at a high time resolution [e.g.: Fig. 1.(b)], and the mean time course of these changes was plotted graphically, as shown in the "control" curves of Fig. 2. Neither APD_{50}, nor APD_{80} shortened during occlusion. Rather, there was a transient (3-4min) increase in left ventricular APD at both measured levels of repolarisation, followed by a return to the pre-occlusion state in the last 4 or 5min of occlusion, with no sign of a significant below baseline shortening of APD. Immediately prior to the occlusion, APD_{50} and APD_{80} values were 15.5±1.0 and 38.1±2.3ms, respectively (n=15 hearts). After only 1min of occlusion, these had increased significantly, by 160 and 79%, respectively (P<0.05, n=15), before returning to the pre-ischaemic state. The APD increase was not associated with any change in epicardial surface temperature. Prior to ischaemia, this was 37.3±0.2^{o}C, and was not significantly different at the end of the period of ischaemia, at 36.6±0.2^{o}C (n=10). Action potential amplitude and duration were measured during prolonged perfusion in the absence of coronary artery occlusion in other hearts. At the start of the experiment, APD_{80} and action potential amplitude were 37.6±3.3ms and 12.3±1.8mV, respectively. After 45min of continuous perfusion, these values were not significantly different, at 34.1±3.6ms and 9.6±2.1mV, respectively. This demonstrates the substantial stability of the recordings, despite the application of prolonged suction.

Insert Fig.1.

A K_{ATP} blocker did not alter action potentials during a 10min coronary occlusion

The K_{ATP} blocker glibenclamide at a concentration of 1μM would be expected to have blocked open cardiac K_{ATP} channels, but it had no effect on the APD changes caused by 10min of coronary occlusion. This lack of effect of glibenclamide on both APD_{50} and APD_{80} is shown in Fig. 2. The control and drug-treatment experiments were performed contemporaneously. There was no significant difference in the APD_{50} or APD_{80}

values between these two groups at any time point during occlusion. Glibenclamide exerted a vasoconstrictor action in preliminary experiments, so perfusion pressure was raised to 133cm H_20 for recording action potentials in glibenclamide-treated hearts, but kept at 100cm H_20 in control hearts. This equalised coronary flow rates in the two groups, so that any effects of glibenclamide on APD were unlikely to have been obscured by its vasoconstrictor action.

Insert Fig.2.

A K_{ATP} opener caused VT during the coronary occlusion

The K_{ATP} opener Ro 31-6930 caused VT to occur during 10min of coronary artery occlusion, and its incidence and duration were increased in a concentration-dependent manner by this drug. This can be seen in Fig. 3., which shows the effects of Ro 31-6930 between 0.5 and 10μM. In each case, Ro 31-6930 was introduced into the perfusate 15min before the occlusion. VT only occurred to a significant extent (compared to controls) with high concentrations of Ro 31-6930, i.e.: 5 or 10μM (Fig. 3.). With Ro 31-6930 at 5μM, no VT was observed prior to coronary occlusion, but a significant proportion of hearts displayed VT after only 4min of the occlusion. However, at 10μM, Ro 31-6930 caused significant VT even prior to coronary occlusion, with all hearts displaying continuous VT within 3min of the occlusion. In preliminary experiments, Ro 31-6930 exerted a potent vasodilator action, even at 0.05μM. This effect was maximal at 0.5μM, causing an increase in the total coronary flow rate from 10.6±0.7 to 14.5±1.0ml.min^{-1}.g^{-1} ventricular weight (P<0.05, n=10). The perfusion pressure was therefore lowered to 66cm H_20 when recording the CEG from hearts treated with the K_{ATP} opener. This equalised the coronary flow rates between the control group and all the drug-treated groups. The effects of the K_{ATP} opener on APD were unlikely therefore to have been obscured by its vasodilator action. The heart rate in groups treated with Ro 31-6930 at 0.5 and 5μM was 225±20 and 246±16 beats.min^{-1}, respectively, 1 min before coronary occlusion. The heart rate did not differ significantly in either group, from that of the control group at any time point prior to or during the occlusion.

Pro-arrhythmic effects of the K_{ATP} opener were glibenclamide-sensitive

Glibenclamide, at the low concentration of 1μM abolished the pro-arrhythmic effects of Ro 31-6930 at each concentration tested. This effect of glibenclamide is clearly illustrated in Fig. 3., which shows both the incidence and duration of VT during ischaemia in groups of hearts perfused with various combinations of Ro 31-6930 and glibenclamide. Glibenclamide on its own (denoted in the histograms by 0μM Ro 31-6930 in

combination with 1μM glibenclamide) did not cause VT to a significant extent (compared to controls) either before or during coronary occlusion, but prevented the pro-arrhythmic effects of Ro 31-6930 both before and during the period of coronary occlusion, even when in combination with Ro 31-6930 at the high concentration of 10μM. The heart rate in those hearts treated with glibenclamide alone was 221±13 beats.min^{-1} prior to coronary occlusion, and did not differ significantly from that of the control group at any time point prior to, or during the occlusion. The perfusion pressure in these hearts was adjusted in the same way as with the action potential recording experiments above.

Insert Fig.3.

A K_{ATP} opener shortened action potentials during coronary occlusion

During a 10min coronary artery occlusion, the K_{ATP} opener Ro 31-6930 caused a concentration-dependent shortening of action potentials recorded from the area at risk of ischaemia. Fig. 4. shows the time course of the effects of a low concentration of Ro 31-6930 (0.5μM), on these left ventricular action potentials. This drug had no significant effect on either APD_{50} or APD_{80} prior to coronary occlusion, but it significantly shortened APD_{80} below the corresponding control values at each time point during the period of occlusion, and APD_{50} values at most time points studied (n=13 hearts, 7-13 action potentials, P<0.05). This effect of the K_{ATP} opener became evident after only 1min of coronary occlusion (Fig. 4.). Beyond 4min of coronary occlusion, Ro 31-6930 also shortened APD_{80} values below the pre-occlusion values at each time point (P<0.05). This contrasts markedly with the situation in the absence of the K_{ATP} opener.

Insert Fig.4.

Fig. 5. shows the effects of a high concentration of Ro 31-6930 (5μM), administered under similar conditions. During coronary occlusion, Ro 31-6930 at 5μM markedly shortened both APD_{50} and APD_{80} at each time point, compared to the corresponding control values [Fig. 5.(a)], and in this case, the drug abolished the transient APD-lengthening that was seen in the control group. As at the lower concentration (0.5μM), Ro 31-6930 at 5μM also significantly shortened action potentials below the pre-occlusion value seen during coronary occlusion (n=13, P<0.05). However, in contrast to the effects of the low concentration, the high concentration (5μM) of the K_{ATP} opener caused significant APD-shortening even before the occlusion, by 50 and 62%, for APD_{50} and APD_{80}, respectively (n=13, P<0.05). Again, none of these electrical effects of the K_{ATP} opener were likely to have been complicated by its vasodilator action, since the coronary flow rates were adjusted to not be significantly different in control and drug-treated groups. Fig. 6. summarises these concentration-

dependent effects of Ro 31-6930 in the two experimental phases. Significant shortening of APD prior to ischaemia only occurred with the K_{ATP} opener at high concentration, but the shortening of APD during ischaemia occurred at both 0.5 and 5μM. When APD_{80} shortened to much below 10ms, i.e.: during coronary occlusion in hearts treated with 5μM Ro 31-6930, paroxysms of VT became evident in the action potential recordings. After 5min of occlusion, it became impossible to measure APD due to continuous VT [Fig. 5.(b)]. This pro-arrhythmic effect of Ro 31-6930 at 5μM, and its association with a severely shortened APD_{80} are shown in Figs. 3. and 6.

Insert Fig 5.

Action potential shortening by the K_{ATP} opener was glibenclamide-sensitive

The K_{ATP} blocker glibenclamide abolished the effects on APD_{50} and APD_{80}, of both concentrations of the K_{ATP} opener Ro 31-6930, not only before, but also during coronary artery occlusion. Fig. 7.(a) shows that glibenclamide at the low concentration of 1μM completely prevented the shortening of APD_{80} that was caused by Ro 31-6930 at 0.5μM during the period of coronary occlusion (compare this with Fig. 4.). The complete reversal by 1μM glibenclamide of Ro 31-6930-induced APD-shortening was evident even when using 5μM Ro 31-6930 [Fig. 7.(b) compared with Fig. 5.(a)]. This reversal by glibenclamide of the effects of Ro 31-6930 on APD during coronary occlusion, i.e.: a restoration of the normal APD, is consistent with the prevention by glibenclamide of the pro-arrhythmic effects of the K_{ATP} opener seen in Fig. 3, and confirms the involvement of K_{ATP} activation in both of these effects.

Discussion

We have provided evidence to suggest that K_{ATP} channels do not open during the first 10min of myocardial regional ischaemia in Langendorff-perfused rat hearts. In agreement with several previous studies on rat isolated hearts (26-28,40), ischaemia did not shorten the action potential duration, thus raising the possibility that the K_{ATP} channels hadn't opened early on during the coronary artery occlusion. Our data obtained with pharmacologic K_{ATP} modulation supports this possibility, since the opening of K_{ATP} channels was able to cause marked APD-shortening during ischaemia [clearly detectable despite the normal (26-28,40) APD-lengthening seen in the absence of a drug], yet glibenclamide, which was able to completely abolish that

effect of the K_{ATP} opener (even after 10min of ischaemia) had no significant effect on the APD during that time, when it was delivered alone, and at the same concentration.

We used as an index of K_{ATP} activity, the sensitivity of the monophasic action potential (MAP) duration to pharmacologic K_{ATP} modulation, not, importantly, the ischaemic APD-change *per se*. Direct measurement of I_{KATP} (such as with voltage clamping of single channels) is unfeasible in the intact heart, and the latter model was used to study the electrical consequences of "true" myocardial ischaemia [as opposed to "simulated" ischaemia, which inevitably must be utilised when measuring single channel currents directly (37)]. The repolarisation time course of the MAP, such as that recorded using a suction electrode, accurately reproduces that of the action potential recorded with microelectrodes from the same region (14), and the constancy of our suction electrode recordings over a 45min experimental period demonstrates the reliability of the method in our model.

Insert Fig.6.

The time-course of the ischaemia-induced APD changes, namely, marked and transient lengthening, closely followed the reported transmembrane APD changes in rat isolated hearts during 25min of global low-flow ischaemia (26). The cause of the early transient APD increase is unknown. Mechanical changes, a fall in either temperature or intracellular pH, and the release of catecholamines has each been suggested (7), as well as blockade of both the inward rectifier (I_{K1}) (13,35) and transient outward (I_{TO}) (33) K^+ currents. It should be noted that such marked APD-prolongation is largely species-specific: in mammals larger than the rat, early ischaemic APD change is characterised mainly by prominent shortening. In the study of Henry *et al* (13), rat isolated ventricular myocytes were used, so coronary artery mechanical deflation was therefore absent, yet a similar transient APD-lengthening was demonstrated during simulated ischaemia. Moreover, it is important to note that a fall in temperature did not occur in the present study. Catecholamine release could only have been local (as opposed to systemic) in the present, *in-vitro*, model, and local release is known to be negligible during the first 10-15min of ischaemia (4). In the absence of the much larger, second phase accumulation of catecholamines which occurs *in-vivo* after 15min of ischaemia, catecholamine release would not have substantially influenced APD during ischaemia in the present experiments, which is consistent with the absence of heart rate increase during ischaemia. In contrast, acute blockade of I_{TO} (a major repolarising current in the rat ventricle) is a more likely candidate for the APD-prolongation (33), and such an ionic mechanism is consistent with a significant prolongation of the APD by 4-aminopyridine in this model (40) and could account

for a suppression of phase 1 repolarisation during ischaemia (Fig. 1c). Since the APD-lengthening during early ischaemia was abolished by the K_{ATP} opener Ro 31-6930 and this effect was glibenclamide-sensitive, this suggests that any non-I_{KATP}-induced changes that might tend to increase APD were unable to mask any concurrent effect of K_{ATP} opening on APD.

In the present study, the K_{ATP} opener Ro 31-6930 caused action potential shortening and VT during the 10min period of coronary occlusion, each in a concentration-dependent manner. Ro 31-6930 is related to the benzopyran K_{ATP} opener cromakalim, but has a non-chiral structure of much higher biological potency (8). Its K_{ATP} opening activity has previously been demonstrated in several experimental models (3,10,12), including cardiac tissue, in which 3μM Ro 31-6930 caused action potential shortening, which was reversed both by ATP (100μM) and glibenclamide (0.3-3μM) (3). It is noteworthy that we report a greater decrease in APD by 0.5μM Ro 31-6930 during early ischaemia, than prior to ischaemia. It has been shown previously that an increase in intracellular [ADP] reduced the sensitivity of K_{ATP} channels to block by ATP (17). Therefore, it might be speculated that such a biochemical event (or other, e.g.: an increased intracellular acidosis or accumulation of adenosine) might sensitise K_{ATP} to an opening action of Ro 31-6930, possibly as a result of reduced inhibitory effect of ATP on the K_{ATP} channel. The significant pro-arrhythmic effect of Ro 31-6930 during ischaemia is consistent with a pro-arrhythmic effect from cromakalim-induced K_{ATP} activation, seen in a previous study during a 10min period of occlusion in rat working hearts (9). The Ro 31-6930-induced VT that we observed even prior to ischaemia has also been seen by other workers with related drugs (6). Other studies on intact hearts have revealed significant K_{ATP} opener-induced arrhythmogenesis and a shortening of the QT interval (36) and refractory periods (1) during ischaemia. The implications of this potentially undesirable property for the therapeutic use of this group of drugs warrants further study.

Insert Fig.7.

The K_{ATP} blocker glibenclamide, at the relatively low concentration of 1μM, completely reversed the APD-shortening and pro-arrhythmic effects of the K_{ATP} opener Ro 31-6930 seen during 10 min of coronary artery occlusion. This, as well as confirming that these effects of Ro 31-6930 were due to K_{ATP} activation, suggested that if K_{ATP} activation should occur in the absence of a K_{ATP} opening drug, it would be detectable as APD-shortening which would be glibenclamide-sensitive. The main supporting evidence that the K_{ATP} channels had not opened during the occlusion alone, therefore, was provided by using glibenclamide alone,

since at the same concentration (1μM) which abolished the APD-shortening effects of the K_{ATP} opener, glibenclamide had no effect on the action potentials during the occlusion.

Previously reported electrical effects of glibenclamide during ischaemia or hypoxia have been variable. Moritani *et al* (22) demonstrated a complete suppression of APD-shortening during a 5min coronary occlusion in dogs *in-vivo*. Some attenuation by glibenclamide of APD-shortening during ischaemia (5,30), hypoxia (20,29) and dinitrophenol-induced metabolic blockade has also been reported (23). Glibenclamide had no effect until 45min of hypoxia had occurred in the rabbit, however (29), but some attenuation of the refractory period shortening occurred in a similar model (1). In canine ventricular isolated tissues, glibenclamide also was unable to restore the loss of the action potential plateau induced by simulated ischaemia (19).

The lack of effect of glibenclamide on the APD in the present study, is consistent with a study by Wilde *et al* (38) who have demonstrated that, in rat isolated hearts, extracellular K^+ accumulation (the earliest effect detected during ischaemia, and at least partly mediated by K^+ channels) also was insensitive to glibenclamide during early ischaemia.

Glibenclamide, although not entirely selective for K_{ATP} channels, is nevertheless acknowledged as one of the best presently available K_{ATP} blockers. However, it is acknowledged that, in some studies, doubts have been raised concerning the efficacy of glibenclamide to block K_{ATP} channels during ischaemia or hypoxia. Venkatesh and colleagues have suggested that an accumulation of cytosolic ADP, such as occurs early during ischaemia (when [ADP] rises rapidly to the 100μM range), might limit the ability of glibenclamide (even at 100μM) to block cellular K^+ loss in guinea pig ventricular myocytes during metabolic blockade (32). Use of the non-sulphonylurea 5-hydroxydecanoate (5-HD) might be considered in future studies, although it has been shown previously (22) that no additional K_{ATP} blocking effect of 5-HD occurred during coronary occlusion in dogs, to that produced by pre-treatment with glibenclamide. It is noteworthy that 5-HD has been reported to be selective for mitoK_{ATP} (21), and its use, therefore, may not be appropriate for investigating sarcolemmal K_{ATP} channels. Nevertheless, a study of effects of 5-HD on the electrophysiological responses to Ro 31-6930 in this model might be considered. In the present study, we found that 10μM glibenclamide produced quantitatively similar results to 1μM, and owing to the complete antagonism of Ro 31-6930's effects with 1μM and the reduced risk of actions additional to I_{KATP} blockade [such as block of cardiac ventricular A-kinase-dependent Cl^- current with >1μM glibenclamide (31)], we chose to do the majority of experiments with 1μM glibenclamide. The preservation of the K_{ATP} modulating drugs' effects was an important factor to consider in

our interpretation of the results, and in the present study, 1μM glibenclamide was able to entirely block effects of Ro 31-6930 on both APD and VT, during coronary occlusion, as well as before it, indicating its suitability to block K_{ATP} channels during ischaemia in the present model.

Limitations of the study

The following should be considered when interpreting our findings. Firstly, it is unknown to what depth of tissue MAPs are recorded, since these are injury currents flowing from an area of uninjured tissue surrounding depolarised cells beneath the electrode. Therefore, the MAPs recorded from the ischaemic zone during coronary ligation inevitably contained a component from the surrounding, normally perfused zone. Such a component should not seriously limit interpretation of the data, however, since it seems not to have been large enough to prevent clear detection of APD-shortening in the ischaemic zone with low dose Ro 31-6930, despite this concentration having been shown to have no effect on the APD in normally perfused muscle (and presumably, therefore, on non-ischaemic tissue during regional ischaemia). Secondly, it is unclear why 1μM glibenclamide caused a small degree of APD-shortening prior to ischaemia. The only expected effect on APD of blockade of any open K_{ATP} channels prior to ischaemia, would be to prolong, not shorten it, and so we cannot exclude the possibility that effects additional to K_{ATP} blockade were operating, and that these might have had a similar effect on the APD during ischaemia. In comparison, however, the pronounced, and 1μM glibenclamide-sensitive APD-shortening effects of the K_{ATP} opener seen during ischaemia, lend support to our conclusion that K_{ATP} had largely remained closed during ischaemia in the absence of drugs. Thirdly, the use of an *in-vitro* preparation coupled with a relatively short period of ischaemia and relatively high perfusate [KCl], whilst permitting action potential recording, is known to result in fewer and less severe episodes of VT than would be encountered *in-vivo* with a longer ischaemic insult, and may be associated with a sub-physiological heart rate. Finally, the fact that in a rat heart performing no external work, no APD-shortening was observed during a 10min period of ischaemia [and none would be expected even during 30min in this model (26)], does not preclude K_{ATP} activation prior to this in hearts of other species, and in those performing external work, either *in-vitro* or *in-vivo*. The present results, therefore, may be both model-, and species-dependent.

In summary, whilst caution must be exercised in interpreting a complex situation in a specific experimental model, our data suggest that cardiac K_{ATP} channels did not open as a prominent feature during the first 10min of myocardial regional ischaemia in the Langendorff-perfused rat heart.

Acknowledgements

We gratefully acknowledge Roche Research Centre, Herts for providing a Gould Windograph chart recorder and a generous supply of Ro 31-6930. We also thank Martin Edwards, Steven Liquorish, Anita O'Donoghue, Michael Ball and Elizabeth Palfreyman for technical assistance.

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Figure legends

Fig. 1.

Suction electrode-recorded action potentials from the left ventricular epicardial wall. (a) Typical configuration of a baseline action potential recording. The APD_{50} and APD_{80} were 14 and 45ms, respectively). (b) Time resolution of APD measurement method. A recording of an action potential with a short duration (from a heart treated with the K_{ATP} opener Ro 31-6930 at 5μM) illustrates the high time resolution obtained and required for accurate measurement of the APD_{50} (6.1ms in this case). (c) Change in APD during early ischaemia. Values

beneath each trace (recorded from the same heart) indicate the time (min) relative to occlusion of the left main coronary artery. The APD_{50} and APD_{80} (13 and 37ms, respectively, for the pre-occlusion trace) increased transiently during the first minutes of occlusion, before returning to approximate baseline levels (APD_{50} and APD_{80} =15 and 35ms, respectively, after 10min).

Fig. 2.

Time course of APD changes during coronary occlusion, and lack of effect of a K_{ATP} blocker. Following a 5min period of baseline recording, the left main coronary artery was occluded at time zero (indicated by vertical arrow), and recordings were taken continuously for a further 10min. Values (APD_{50} and APD_{80}) are means (with error bars denoting S.E.); *n*=15 hearts (6-15 action potentials) in both control and glibenclamide-treated groups. *'s denote a significant difference ($P<0.05$) from control values at each time point prior to and during coronary occlusion.

Fig. 3.

Reversal by glibenclamide of effects of a K_{ATP} opener on arrhythmias during coronary occlusion. Upper panel shows the incidence of ventricular tachyarrhythmias (VT) recorded during a 10min occlusion of the left main coronary artery. Lower panel shows the associated durations of VT. Values are means, with the S.E. of duration data indicated by error bars. NV=no value recorded, i.e.: zero. For groups of Ro 31-6930-treated hearts (*n*=7-10), *'s denote a significant difference ($P<0.05$) from the control value (i.e.: with Ro 31-6930 at 0μM). Hearts treated with glibenclamide (*n*=9-10 per group): both the incidence and duration of VT in hearts treated with glibenclamide alone (indicated by 0μM Ro 31-6930 in combination with 1μM glibenclamide) were not significantly different from the control values of 0% and 0s, respectively. There were no significant differences in either the incidence or duration of VT, between any of the drug-combination-treated groups.

Fig. 4.

Effects of a low concentration of a K_{ATP} opener on APD changes during coronary occlusion. Upper panel shows changes in APD_{50} and lower panel, in APD_{80}. Values are means (n=13 hearts, 7-13 action potentials), with error bars denoting S.E. In each heart, the left main coronary artery was occluded at time 0, indicated by

vertical arrow. *'s denote significant differences ($P<0.05$) from control values at each time point, prior to and during coronary occlusion.

Fig. 5.

Effects of a high concentration of a K_{ATP} opener on action potentials during coronary occlusion. (a) Effects of Ro 31-6930 (5μM) on APD. Values are means (n=13 hearts, 4-11 action potentials), with error bars denoting S.E. *'s denote significant differences ($P<0.05$) from control values at each time point, prior to and during coronary occlusion. APD could not be recorded beyond 4min of occlusion in hearts treated with Ro 31-6930 at 5μM, due to the appearance of continuous VT. (i) and (ii) indicate times at which traces in (b) were recorded. (b) Left ventricular epicardial voltage signals, sampled from a heart treated with 5μM Ro 31-6930. (i) Trace showing the last action potential recorded during ischaemia (APD_{50} and APD_{80} =4 & 9ms, respectively) prior to interruption of APD measurement by VT, which eventually caused complete degeneration of the signal (ii).

Fig. 6.

Concentration-dependent effects of Ro 31-6930 on APD prior to and during coronary occlusion. Effects of Ro 31-6930 on APD immediately prior to coronary occlusion (Pre-occlusion APD) are shown in comparison with the shortest mean APD recorded during the subsequent 10min occlusion. Values (APD_{80}) are means; error bars denote S.E. *'s = significant differences from the control group (Ro 31-6930 at 0μM) within each experimental phase. §'s = significant differences from the pre-occlusion value, for a given concentration of Ro 31-6930. Thus, Ro 31-6930 only shortened APD prior to occlusion, when delivered at 5μM, but shortened APD below the pre-occlusion value during occlusion, when delivered at both 0.5 and 5μM.

Fig. 7.

The abolition by glibenclamide of Ro 31-6930-induced changes in APD. (a) Effect of Ro 31-6930 (0.5μM)+glibenclamide (1μM). (b) Effect of Ro 31-6930 (5μM)+glibenclamide (1μM). Only changes in APD_{80} are shown. Values are means, with error bars denoting S.E. (n=15 hearts, 7-15 action potentials, for each control and drug combination-treated group). In each heart, the left main coronary artery was occluded at time zero, indicated on the time scales by the vertical arrows. Asterisks denote significant differences ($P<0.05$) from control values at each time point, prior to and during coronary occlusion.

Fig. 1.

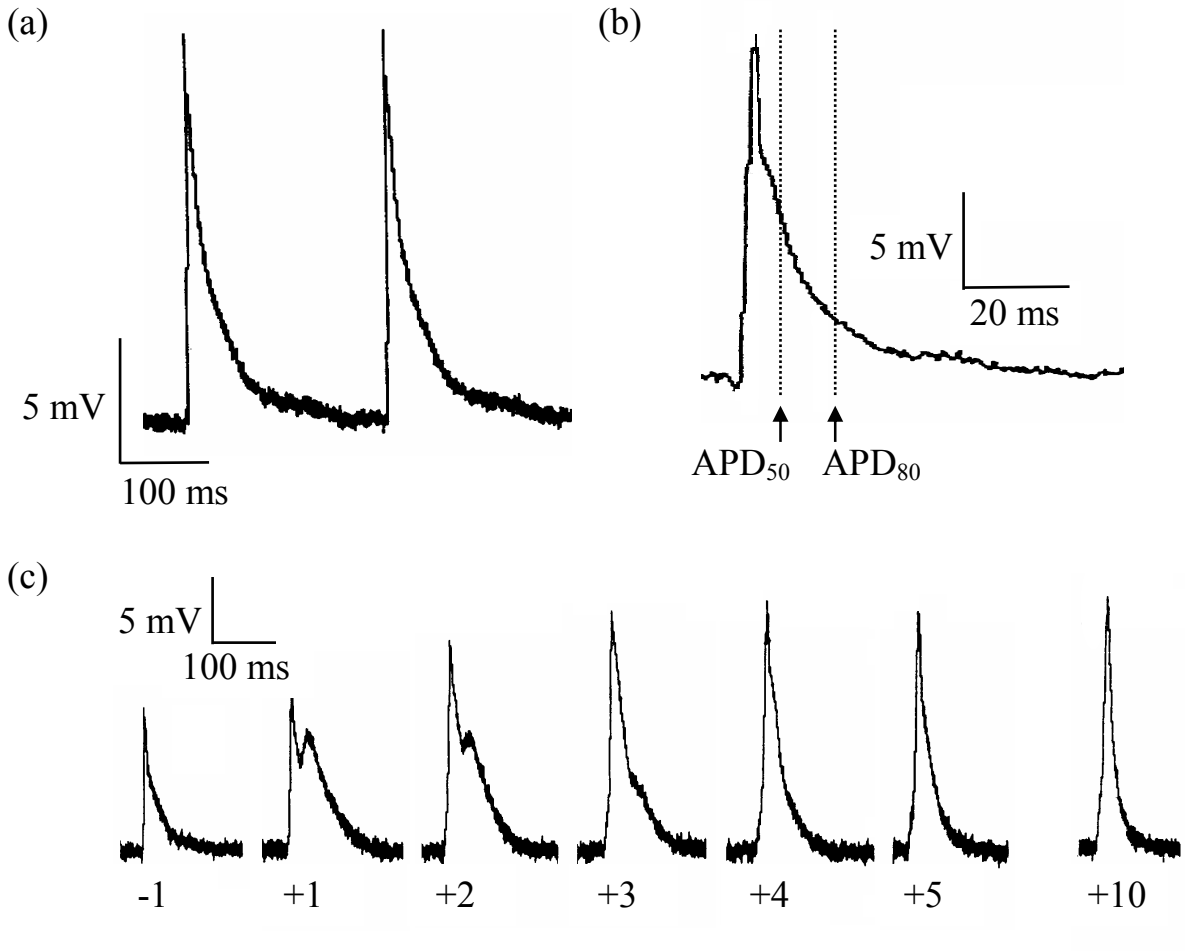


Fig. 2.

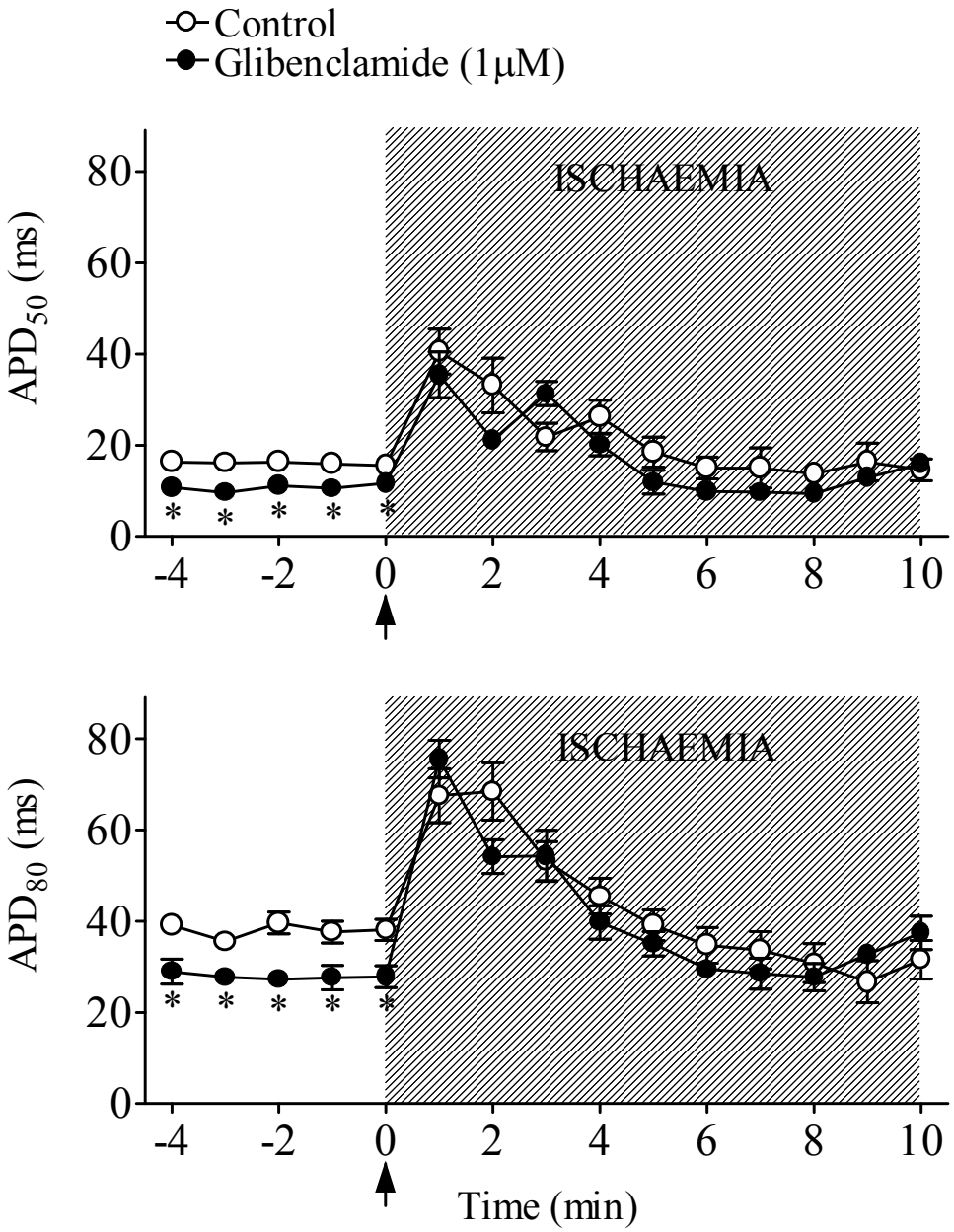

Control
Glibenclamide (1μM)
ISCHAEMIA
APD50 (ms)
APD80 (ms)
Time (min)
0
20
40
60
80
-4
-2
0
2
4
6
8
10

Fig. 3.

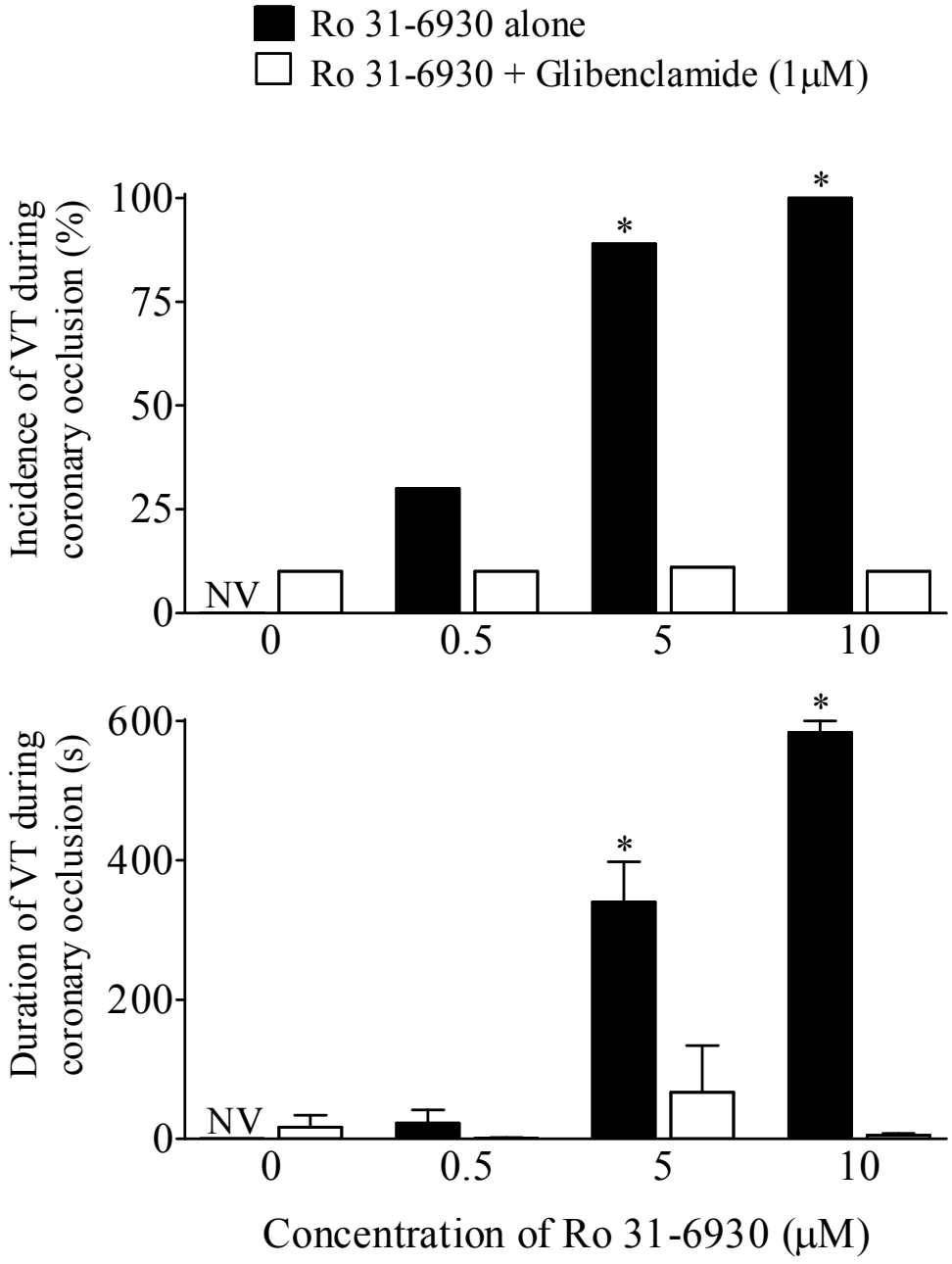

Ro 31-6930 alone
Ro 31-6930 + Glibenclamide (1μM)
Incidence of VT during coronary occlusion (%)
100
75
50
25
0
NV
0
0.5
5
10
*
*
Duration of VT during coronary occlusion (s)
600
400
200
0
NV
0
0.5
5
10
*
*
Concentration of Ro 31-6930 (μM)

Fig. 4.

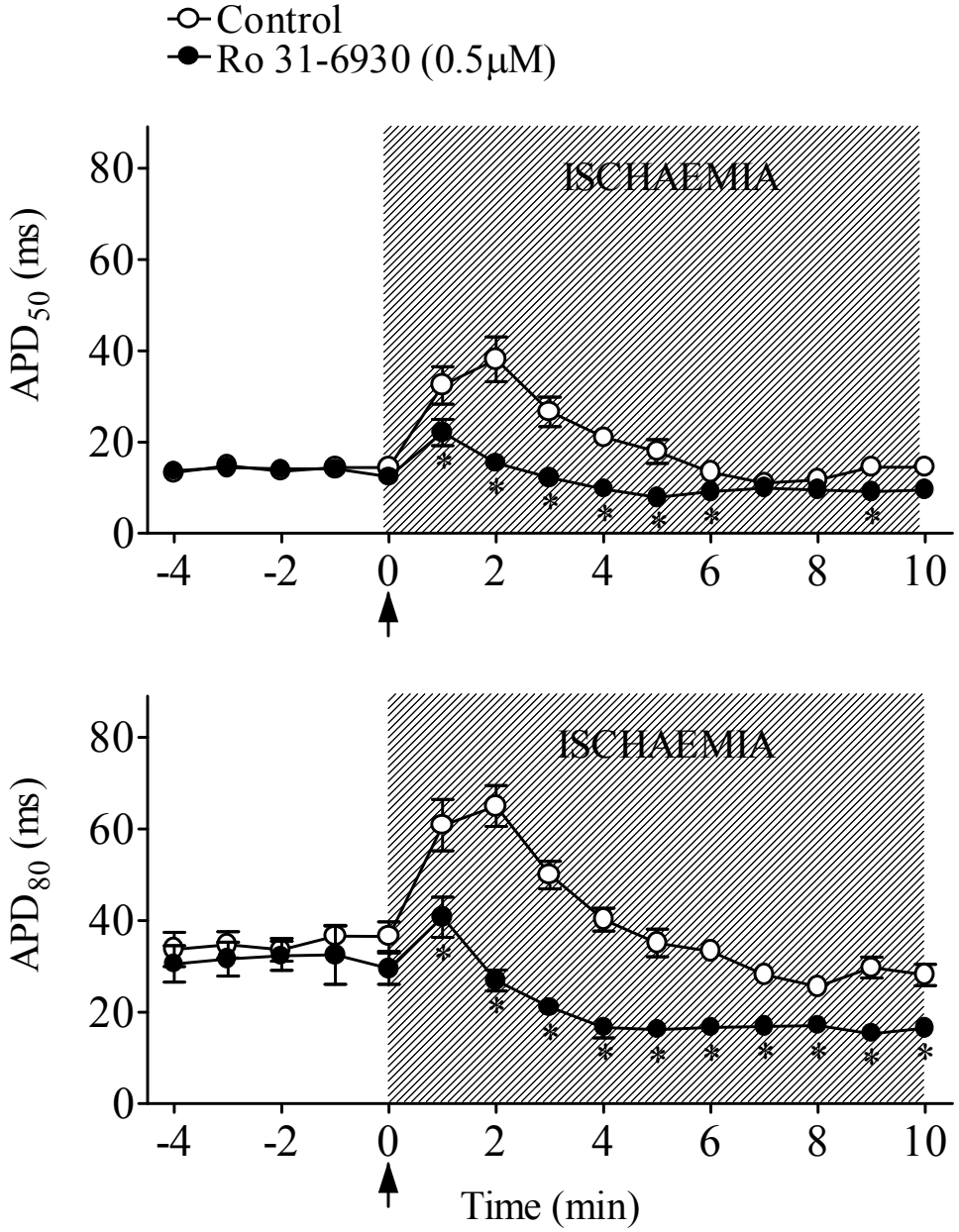

Control
Ro 31-6930 (0.5μM)
ISCHAEMIA
APD50 (ms)
APD80 (ms)
Time (min)
0
20
40
60
80
-4
-2
0
2
4
6
8
10

Fig. 5.

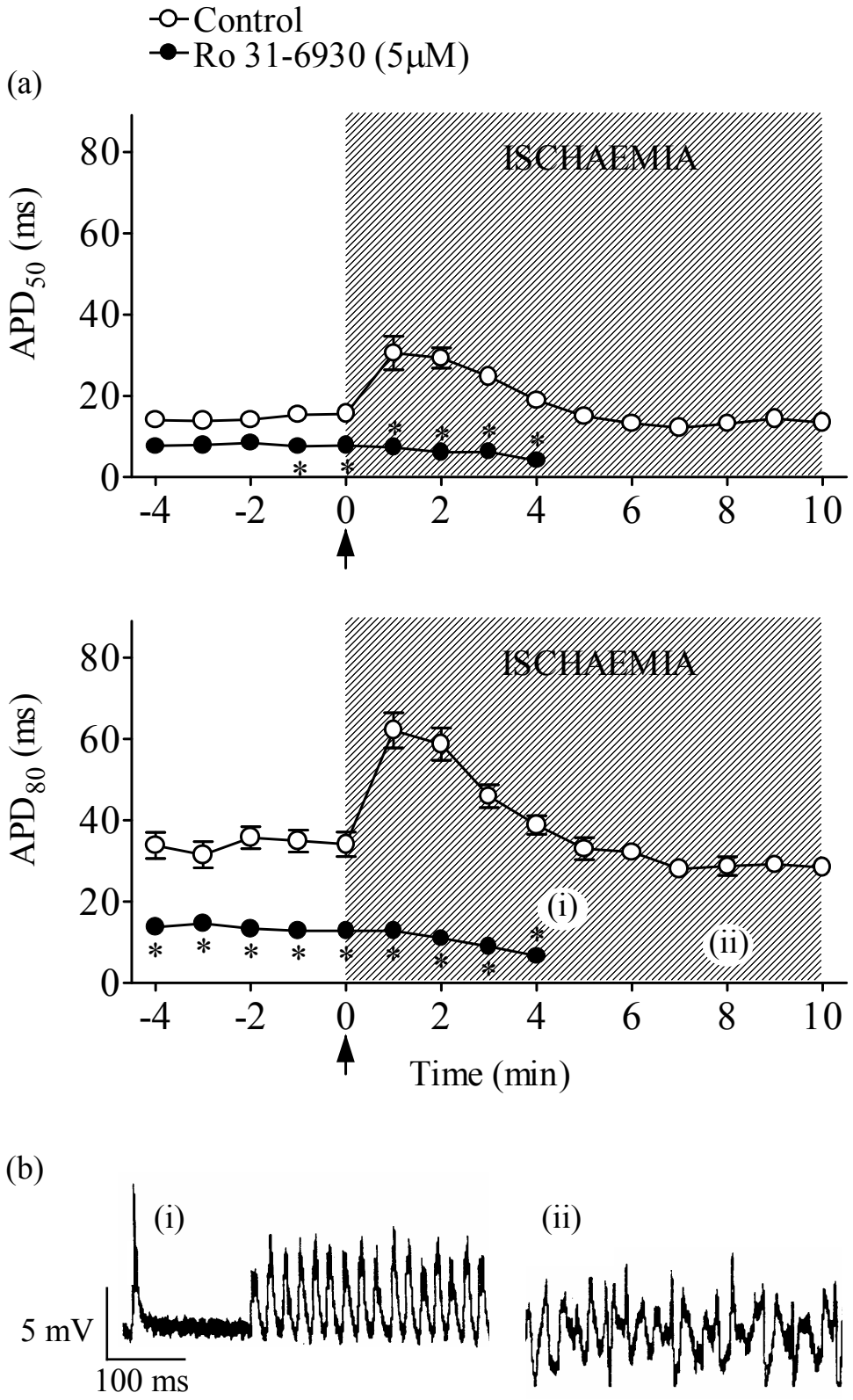

Control
Ro 31-6930 (5μM)
(a)
ISCHAEMIA
APD50 (ms)
APD80 (ms)
(i)
(ii)
Time (min)
(b)
5 mV
100 ms

Fig. 6.

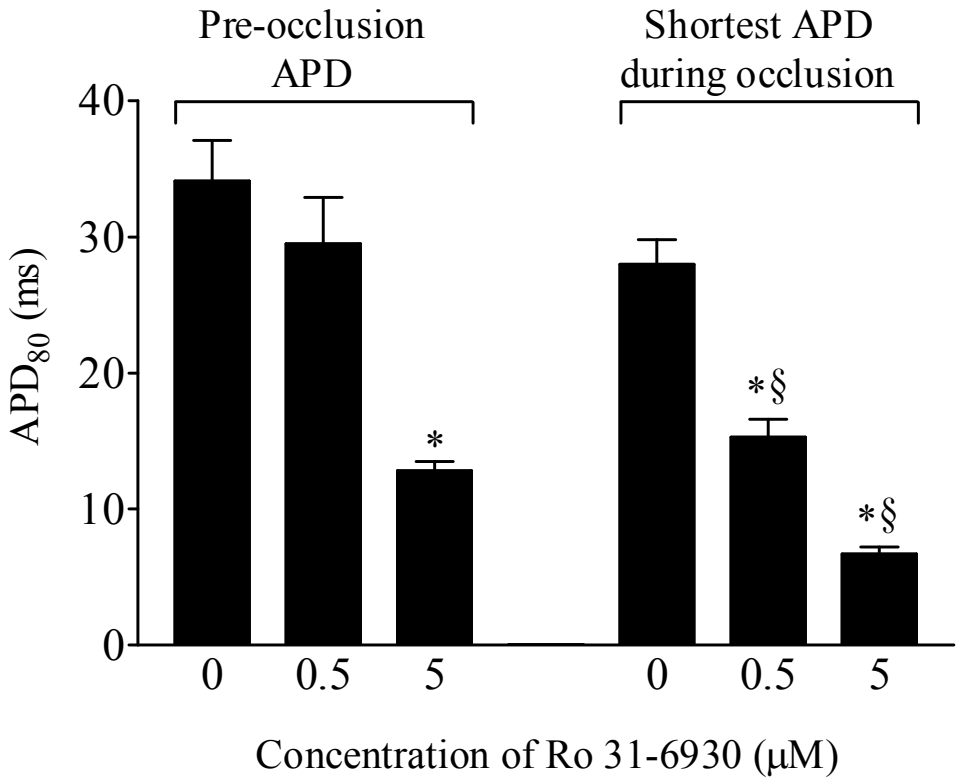
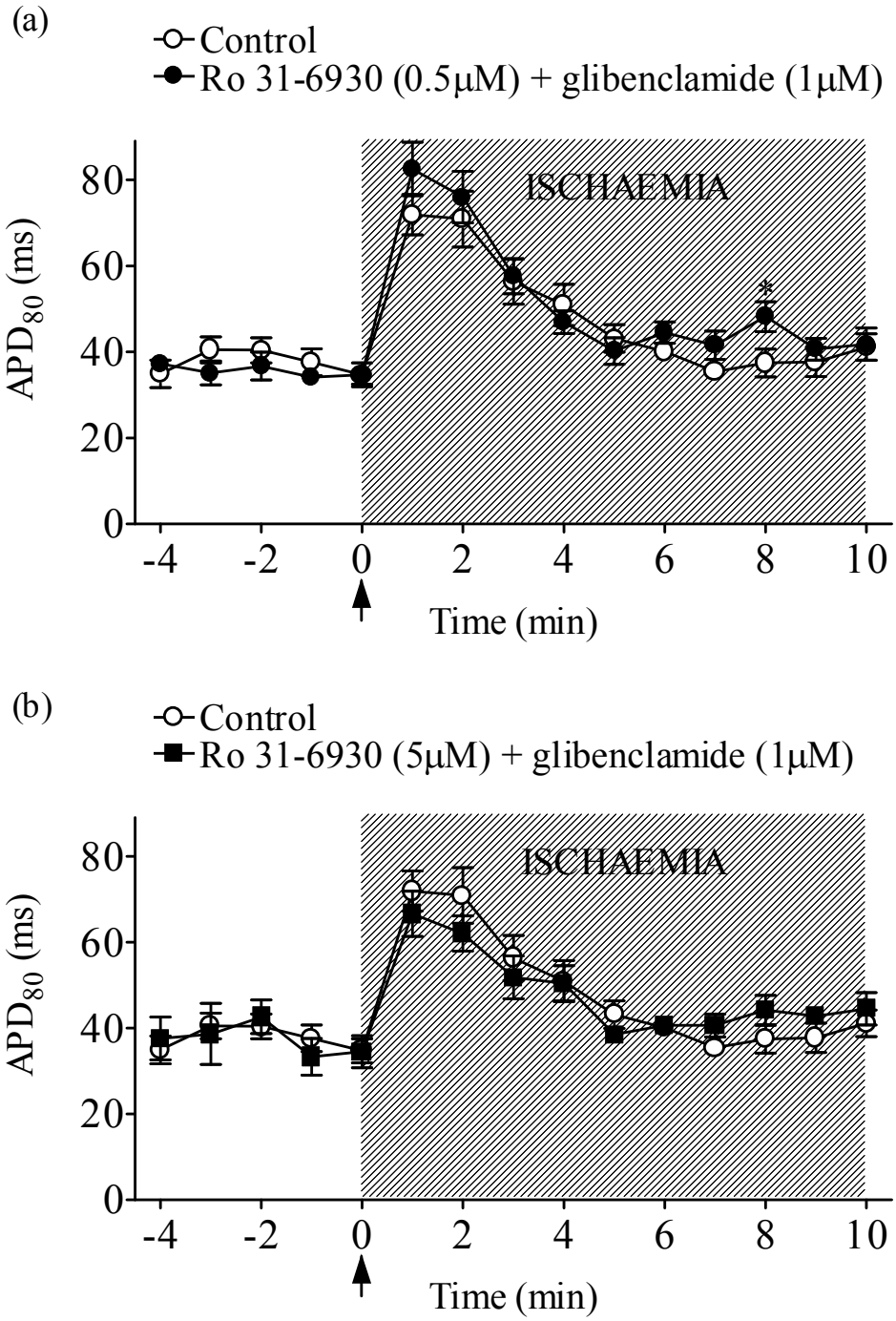

Pre-occlusion APD
Shortest APD during occlusion
40
30
20
10
0
APD80 (ms)
*
*§
*§
0
0.5
5
0
0.5
5
Concentration of Ro 31-6930 (μM)

Fig. 7.


(a)
Control
Ro 31-6930 (0.5μM) + glibenclamide (1μM)
ISCHAEMIA
APD80 (ms)
Time (min)
(b)
Control
Ro 31-6930 (5μM) + glibenclamide (1μM)
ISCHAEMIA
APD80 (ms)
Time (min)