ELSEVIER

European Journal of Pharmacology 333 (1997) 33–38

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Does the opening of ATP-sensitive K^+ channels modify ischaemia-induced ventricular arrhythmias in anaesthetised dogs?

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Received 6 February 1997; revised 10 June 1997; accepted 13 June 1997

Abstract

These experiments were designed to determine whether there is any change in the severity of ventricular arrhythmias resulting from coronary artery occlusion in anaesthetised mongrel dogs if ATP-sensitive potassium channels were already open at the time of coronary occlusion. To achieve this we locally infused the K_{ATP} channel opener levcromakalim, in a total dose of 3 μg/kg, and given by slow infusion over a 30 min period directly into a side branch of the left anterior descending coronary artery. This dose increased blood flow in that main artery by 30% (and by 7% in the adjacent left circumflex artery). The degree of inhomogeneity of electrical activation, measured from the left ventricular wall distal to the occlusion site, was unaffected by levcromakalim administration but there was significant epicardial ST-elevation, perhaps indicating K^+ egression from cells. Following coronary artery occlusion there was no marked difference in the severity of arrhythmias between control and levcromakalim-treated dogs, except for an increased number of episodes of ventricular tachycardia due entirely to effects in two of the nine treated dogs. We conclude that opening cardiac K_{ATP} channels with levcromakalim, at this one dose level, and administered directly to the left ventricular wall, does not significantly modify arrhythmia severity during ischaemia. These results cannot be extrapolated to studies in which such drugs markedly reduce coronary perfusion pressure. © 1997 Elsevier Science B.V.

Keywords: Arrhythmia; Ischaemia; K^+ channel activator; Levcromakalim; Reperfusion

1. Introduction

The debate has been recently renewed as to whether openers of adenosine triphosphate (ATP) sensitive potassium channels (K_{ATP}) have potential for enhancing propensity for ventricular arrhythmias during ischaemia and reperfusion (Chi et al., 1990; Coetzee, 1992; Gross and Auchampach, 1992; Black and Lucchesi, 1994; D'Alonzo and Grover, 1994; Wilde and Janse, 1994; Friedrichs et al., 1996; Vegh et al., 1996). In some models (Grover et al., 1990, 1995) an increased incidence of ventricular fibrillation has been reported during ischaemia, and one suggested mechanism for this was a reflex increase in cardiac sympathetic nervous activity as a result of systemic hypotension (D'Alonzo and Grover, 1994). One way of obviating the confounding effect of systemic hypotension would be to administer an opener of ATP-sensitive potassium channels directly into a coronary artery and, indeed, this was attempted in an early study with cromakalim (Grover et al., 1990). Grover and his colleagues used pentobarbitone anaesthetised dogs, and infused cromakalim, commencing 10 min before occlusion of the left circumflex coronary artery dogs, in order to determine the effect of this treatment on myocardial infarct size. Although they did not evaluate ischaemia-induced arrhythmias they did show (Grover et al., 1990) that at the end of a 90 min occlusion period, the incidence of ventricular fibrillation on reperfusion was significantly reduced by local cromakalim administration. This may simply have been a reflection of the marked reduction in myocardial infarct size (about 50%). In a more recent paper the same authors have attempted to evaluate the correlation between shortening of action potential duration by cromakalim and cardioprotection (Grover et al., 1995). They showed that there was no significant correlation between action poten-

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tial shortening and the reduction in infarct size. Arrhythmias during ischaemia reperfusion were again not evaluated in this study.

In the present study we have attempted to evaluate whether the opening of ATP-sensitive potassium channels prior to the onset of ischaemia modifies the severity of ischaemia (and reperfusion) induced ventricular arrhythmias. As in the Grover studies (Grover et al., 1990, 1995) we chose a dose of levcromakalim which moderately increased coronary blood flow, but which had no marked systemic haemodynamic effects. The increase in coronary blood flow was similar to that observed when nicorandil was infused by the same intracoronary route. This drug, which is also a K_{ATP} channel opener but with an additional ability to 'donate' nitric oxide, has been recently shown to protect dogs against ischaemia and reperfusion-induced arrhythmias (Vegh et al., 1996).

2. Materials and methods

2.1. Animals and the measurement of haemodynamic variables

We used mongrel dogs, of either sex, with a body weight in excess of 17 kg (mean 26.7 ± 1.1 kg). They were anaesthetised with a mixture of chloralose and urethane (60 and 200 mg/kg i.v., respectively), ventilated with room air using a Harvard Respirator at a rate and volume sufficient to maintain arterial blood gases and pH within normal limits (Vegh et al., 1992). They were then thoracotomised at the fifth intercostal space and the anterior descending branch of the left coronary artery prepared for occlusion just proximal to the first main diagonal branch. A side branch of this artery, proximal to the occlusion, was catheterised for the intracoronary administration of levcromakalim, which was administered in a total dose of 3 μg/kg given by slow infusion over a 30 min period in nine dogs. The coronary artery was then occluded for 25 min. Twenty dogs, which were not given levcromakalim, acted as controls. Blood flow was measured on both the left circumflex coronary artery (with an electromagnetic flow probe coupled to a Statham SP 2202 flow meter) and on the left anterior descending artery with a Doppler flow probe (Triton). Epicardial ST-segment changes and the degree of inhomogeneity of electrical activation were measured from the left ventricular wall distal to the occlusion site using a composite electrode described previously (Vegh et al., 1992). This gives a summarised recording of R-waves from 30 epicardial measuring points. In the adequately perfused and oxygenated myocardium these sites are activated virtually simultaneously, resulting in a single spike. Following occlusion, widening and fractionation of the summarised R-waves occurs indicating that adjacent fibres are not simultaneously activated because of inhomogeneity of conduction. We expressed this as the greatest delay in activation (ms) within the ischaemic area.

Recordings from these electrodes, together with a limb lead electrocardiogram, systemic arterial, left ventricular systolic and end-diastolic pressures (Statham P23 Dp transducers) and left ventricular dP/dt were recorded on an 8 channel Medicor R81 recorder.

2.2. Assessment of arrhythmias

Ventricular arrhythmias during a 25 min period of coronary occlusion (ischaemia) and reperfusion were assessed and analysed as outlined previously (Vegh et al., 1992). We measured the total number of ventricular premature complexes over the occlusion period, the incidence and number of episodes of ventricular tachycardia and the incidence of ventricular fibrillation both during occlusion and reperfusion. Survival indicates those dogs that were in sinus rhythm following the 25 min occlusion and reperfusion insult. The area at risk assessed (by infusing patent blue V dye into the occluded artery) at the end of the experiment, is consistently between 35 and 47% of the free

Table 1
Haemodynamic effects of levcromakalim (3 μg/kg by intracoronary administration) and of subsequent coronary artery occlusion

	Pre-drug	Post-cromakalim (pre-occlusion)	5 min post-occlusion
Arterial blood pressure			
Systolic (mmHg)	113 ± 7	101 ± 9	96 ± 9
Diastolic (mmHg)	74 ± 5	63 ± 4 [a]	61 ± 4
Mean (mmHg)	87 ± 5	76 ± 5 [a]	73 ± 5
Left ventricular end-diastolic Pressure (mmHg)	7 ± 1	9 ± 1	28 ± 3 [a]
LV$dP/dt_{max}$			
(+ve; mmHg/s)	2667 ± 203	2789 ± 189	2232 ± 249 [a]
(−ve; mmHg/s)	2403 ± 128	2744 ± 156	1512 ± 164 [a]
Heart rate (beats/min)	134 ± 7	137 ± 8	136 ± 10

[a] $P < 0.05$.

left ventricular wall and septum; in this study the mean was 38.5 ± 2.0%.

2.3. Data analysis

Data was analysed statistically as previously described (Vegh et al., 1992), i.e., data are expressed as means ± S.E.M. and differences between means were compared by Student's *t*-test corrected for multiple comparisons (two way ANOVA), or, for arrhythmias, by the Mann-Whitney *U* test. For comparison of incidences of arrhythmias (ventricular tachycardia, ventricular fibrillation and survival from the combined occlusion–reperfusion insult) the χ^2-test for independence in a 2×2 table was used. Differences between groups were considered significant when P was < 0.05.

Although all the experiments were carried out at Szeged the protocol complies with UK Home Office regulations (project licence number 60/00307).

3. Results

3.1. Haemodynamic effects of levcromakalim

The haemodynamic effects of intracoronary levcromakalim are summarised in Table 1. At the end of the 30 min infusion period there were slight reductions in arterial pressure (−11 ± 2 mmHg mean and diastolic), but no significant change in left ventricular dP/dt or heart rate. The most pronounced effect of levcromakalim was an increase in blood flow in both the anterior descending and circumflex branches of the left coronary artery (Fig. 1). This was more pronounced in the anterior descending branch (which was the artery into which cromakalim was infused) with an increase in flow of around 30%, compared with only 7% in the circumflex artery. With the reduction in diastolic perfusion pressure calculated coronary vascular resistance was reduced in both vascular beds (Fig. 1). There was also a consistent, and significant, increase in ST-segment elevation as recorded from the epicardial electrocardiogram, which began almost immediately after the commencement of the levcromakalim infusion (Fig. 2).

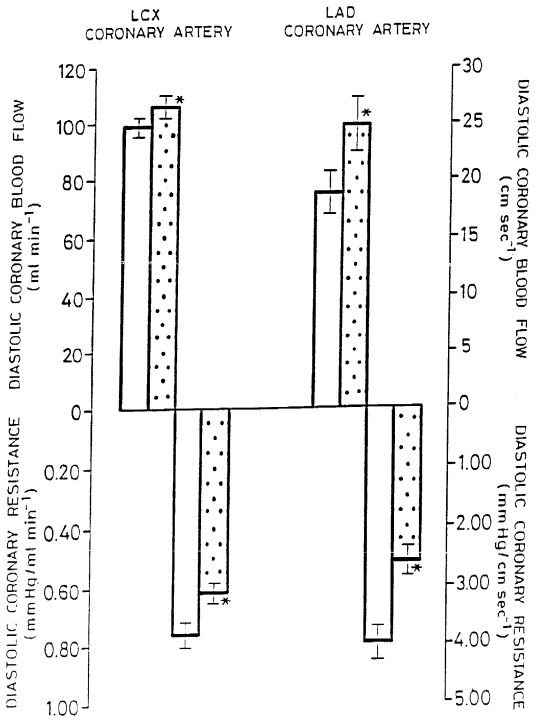

Fig. 1. Changes in diastolic coronary blood flow in the circumflex (LCX) and anterior descending (LAD) branches of the left coronary artery before and after the intracoronary administration of levcromakalim. Levcromakalim increases blood flow in both vascular beds, but more particularly in that supplied by the anterior descending coronary artery, and decreases coronary vascular resistance.

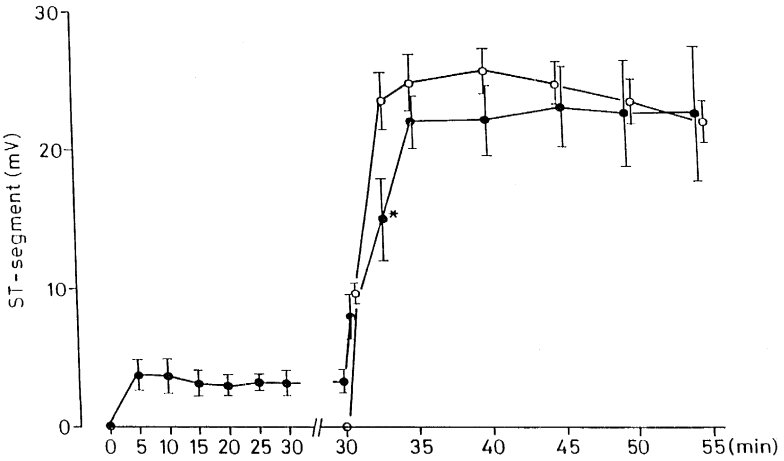

Fig. 2. Changes in ST-segment elevation, recorded from epicardial electrodes, during the administration of levcromakalim (●) and following coronary artery occlusion (at time 30 min). In control animals there is a marked increase in ST-segment elevation (○) after coronary artery occlusion which is not significantly different (except after 3 min) in those dogs treated with levcromakalim. Note, however, that the drug itself induces some ST-segment elevation, probably as a result of K^+ egress.

3.2. The effects of coronary artery occlusion

In control dogs subjected to coronary artery occlusion there was a reduction in mean arterial pressure (from 99 ± 5 to 84 ± 4 mmHg; $P < 0.05$), an increase in left ventricular end-diastolic pressure (from 5.6 ± 0.4 to 22.2 ± 1.9 mmHg; $P < 0.01$) and reductions in both positive left ventricular dP/dt_{max} (3027 ± 355 to 2089 ± 319 mmHg/s; $P < 0.05$) and left ventricular dP/dt_{max} negative (2269 ± 271 to 1689 ± 266; $P < 0.05$). Heart rate was unchanged (146 ± 5 beats/min). These changes were somewhat similar in those dogs administered levcromakalim (Table 1) with significant increases in left ventricular end-diastolic pressure and in blood flow in the circumflex artery ('compensatory vasodilatation'). This amounted to a mean increase of 7 ± 3 ml/min in diastolic flow and a reduction in end-diastolic coronary vascular resistance from 0.63 ± 0.03 to 0.51 ± 0.03 mmHg/ml per min ($P < 0.05$).

In control dogs coronary artery occlusion resulted in significant elevation of the ST-segment recorded from epicardial electrodes (Fig. 2) and a marked increase in the degree of inhomogeneity of electrical activation within the ischaemic area (Fig. 3). These changes were similar in those dogs administered levcromakalim indicating that, despite an increase in coronary flow, changes in the severity of ischaemia following coronary artery occlusion were uninfluenced by the prior administration of levcromakalim.

3.3. Levcromakalim and arrhythmias resulting from ischaemia and reperfusion

The main interest in the study was in the severity of ventricular arrhythmias that occurred during ischaemia and reperfusion. The results are illustrated in Fig. 4. It is clear that the marked increase in ventricular ectopic activity that occurs during ischaemia was largely unaltered by the prior

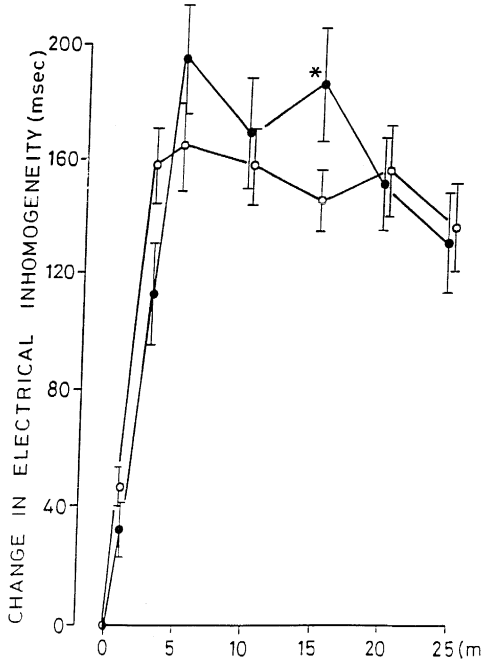

Fig. 3. Changes in the inhomogeneity of electrical activation (ms) following coronary artery occlusion (at time 0) in control dogs and in dogs previously administered levcromakalim (●). Except at the 15 min time point, there is no significant difference in the degree of inhomogeneity within the ischaemic area between the two groups.

administration of levcromakalim. The only index of arrhythmia severity that was significantly modified was the number of episodes of ventricular tachycardia. However, this increase largely resulted from an abnormally high number (60) of episodes in one of the nine dogs. The incidences of ventricular tachycardia (67%) and of ventricular fibrillation during occlusion (56%) were similar to those in control dogs. In contrast to the controls (survival 0%) two of the four dogs given levcromakalim that were

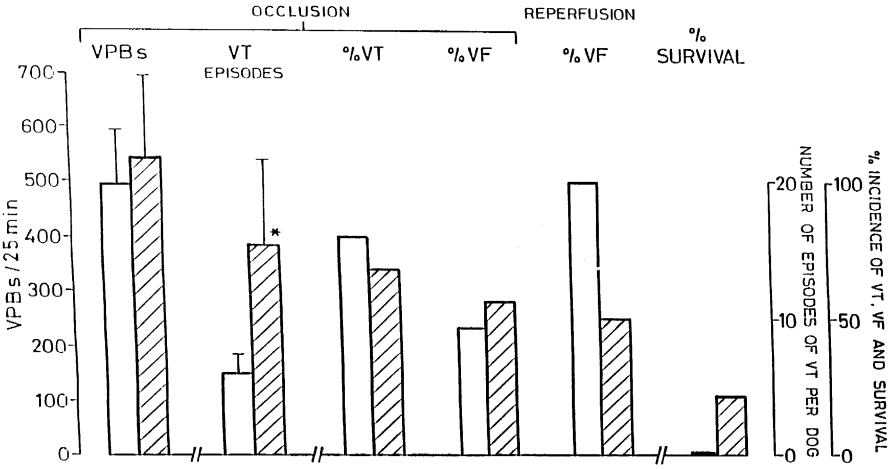

Fig. 4. Ventricular arrhythmias during occlusion and reperfusion in control dogs (open histograms) and in dogs administered levcromakalim (shaded histograms). Shown are the number of ventricular premature beats (VPBs), the number of animals in which ventricular tachycardia (VT) and ventricular fibrillation (VF) occurred, and the number of episodes of ventricular tachycardia per dog. Also shown is the percentage incidence of ventricular fibrillation following reperfusion, and the survival from the combined ischaemia-reperfusion insult. Except for the number of episodes of ventricular tachycardia which were elevated in dogs given levcromakalim, there is no significant difference in arrhythmia severity between the two groups.

alive at the end of the occlusion period survived reperfusion.

4. Discussion

The question as to whether potassium channel openers are likely to be profibrillatory in the diseased human heart is an important one, particularly in view of the possible widespread use of this class of pharmacological agents in patients with underlying ischaemic heart disease. Because of their well documented cardioprotective effects (reducing myocardial ischaemic damage following a period of coronary artery occlusion and reperfusion; enhanced recovery of contractile functioning following a similar myocardial insult) one might expect that these agents would display indirect antiarrhythmic efficacy through such cardioprotective mechanisms. On the other hand, these agents shorten action potential duration (Black and Lucchesi, 1994; Grover et al., 1995) and, as with class 1C antiarrhythmics such as in encainide and flecainide (Campbell et al., 1991) the possibility remains that this might increase the severity of ventricular arrhythmias under conditions of myocardial ischaemia and in patients following myocardial infarction. A further possible profibrillatory effect of such drugs would be if marked systemic hypotension occurred, with activation of the cardiac sympathetic nerves, the release of noradrenaline and the induction of potentially life-threatening ventricular arrhythmias.

The present studies were an attempt to discern whether local administration of a potent potassium opener (levcromakalim) increases the severity of ischaemia-induced arrhythmias when a coronary artery was occluded following drug administration by this route. We know from recent work (Grover et al., 1995) that cromakalim reduces action potential duration when given by this route, although in a much larger dose than that used in the present studies. Unfortunately, in the present experiments assessment of action potential duration was not possible from the epicardial electrograms. Grover et al. (1995) found that a dose of 10 μg/kg (loading dose) starting 10 min before ischaemia and followed by a slow infusion (0.3 μg/kg per min for 90 min) reduced action potential duration at 90% repolarisation by up to 20% during the occlusion period. This was significantly more marked than the effect of ischaemia itself (reduction in action potential duration of around 10%).

The dose of cromakalim that we used was chosen to limit systemic effects, although a slight reduction in blood pressure still occurred. We suggest that the dose of cromakalim used was effective in opening potassium channels for two reasons. First, because of the marked increase in blood flow that occurred in the coronary vascular bed supplied by the small artery in which the drug was administered (Fig. 1). Second, although there was no effect of the drug on electrical activation within the potentially ischaemic area (49 ± 4 ms both before levcromakalim administration and after administration and immediately preceding coronary artery occlusion) ST-segment elevation recorded from epicardial electrodes was increased following cromakalim administration (by 3.2 ± 0.7 mV), and this despite a substantial increase in coronary blood flow. There are two possible explanations for this. First, it could result from K^+ egress from cardiac myocytes because K_{ATP} channels have been opened by the drug; the local intracoronary administration of potassium itself causes similar changes in ST-segment elevation, measured from epicardial electrodes (Marshall and Parratt, 1979). Second, but less likely, it could result from a blood flow steal from epicardial to endocardial regions of the left ventricular wall.

When the coronary artery was occluded following levcromakalim administration the usual haemodynamic changes were as marked in the presence of the drug as they were in control dogs. For example, there were slight reductions in arterial pressure, a marked elevation in left ventricular end-diastolic pressure and evidence of reduced myocardial contractility (the reductions in LV dP/dt_{max} were more marked than one would expect from the changes in perfusion pressure). ST-segment changes, recorded from epicardial electrodes, were as marked in the treated animals as they were in the controls (Fig. 2). Further, there was no modification of the marked inhomogeneity of electrical activation that occurs in the ischaemic myocardium following coronary artery occlusion (Fig. 3); this is probably a better index of ischaemia severity (Vegh et al., 1992), especially under conditions where drugs may modify the ST-segment for reasons other than through ischaemia.

We evaluated ischaemia-induced ventricular arrhythmias in a number of ways (Vegh et al., 1992). The only index of arrhythmia severity that was modified by levcromakalim administration was a substantial increase in the number of episodes of ventricular tachycardia. Nearly all dogs have brief periods of ventricular tachycardia following coronary occlusion (the incidence in this control group was 80%) and the mean number of episodes per dog, in this particular group of 20 controls, was less than 5. In contrast, there were three times as many episodes in those dogs given levcromakalim. This was almost entirely accounted for by two dogs which had a very large number of episodes of ventricular tachycardia (60 and 43, respectively); the mean in the remaining 7 dogs was similar to that in the controls. Despite this, there was no evidence for an increased incidence of ventricular fibrillation during occlusion (Fig. 4) and indeed, in this particular model, there is no relationship between the number of episodes of ventricular tachycardia (or even indeed the number of ventricular premature complexes) and the likelihood of a dog fibrillating during ischaemia. We conclude from these results that there is no greatly increased likelihood of ventricular fibrillation when levcromakalim is given in this dose, and by this route. However, we cannot draw the

conclusion that susceptibility to fibrillation would not be increased following the intravenous (or oral) administration of considerably larger doses of the drug, especially if they substantially reduced arterial blood pressure. One point of interest, also shown previously (Grover et al., 1995), was that following reperfusion there was an increased survival in those dogs administered cromakalim. This is a particularly severe ischaemia-reperfusion model in which survival following reperfusion is extremely rare (there were no survivors among the 20 dogs in the present studies); this might indicate that cromakalim has some antiarrhythmic effect following reperfusion either after 25 min of ischaemia, as in the present study, or after 90 min (as in the Grover study; Grover et al., 1995). Based on the present study, limited to one particular K_{ATP} channel opener at one dose level and administered by a somewhat unusual route, we might conclude that potassium channel openers are rather unlikely to increase the possibility of ventricular fibrillation under conditions of myocardial ischaemia (or reperfusion) unless marked systemic hypotension occurred.

It is of some interest to compare the present results with those recently published in the journal with another K_{ATP} channel opener nicorandil (Vegh et al., 1996). As in the present study, nicorandil was infused by the intracoronary route and gave an increase in blood flow similar to that achieved in the present study with levcromakalim. However, there were marked differences in the responses to coronary artery occlusion between dogs given nicorandil and those given levcromakalim. Nicorandil reduced ST-segment elevation during occlusion and also decreased electrical inhomogeneity within the ischaemic area. In addition, there was a decrease in arrhythmia severity and an increase in survival from the combined ischaemia-reperfusion insult. Three possible explanations for this protective effect were discussed; the opening of K_{ATP}-sensitive channels, an increase in coronary blood flow and the ability of nicorandil to 'donate' nitric oxide to the ischaemic myocardium. The failure of levcromakalim to modify arrhythmia severity in the acutely ischaemic myocardium in the present study suggests that the major explanation for the beneficial effects of nicorandil on arrhythmia severity in this model are probably dependent upon its ability to 'donate' nitric oxide to the ischaemic heart.

The present results may also contribute to the debate as to whether the cardioprotective effects of ischaemic preconditioning, for example the reduction in cellular necrosis, are due to receptor-stimulated protein kinase C translocation from the cytosol to the cell membrane and a subsequent opening of ATP-sensitive potassium channels prior to the prolonged period of ischaemia (recently reviewed by Grover, 1996). This is clearly not the explanation for the powerful antiarrhythmic effects of preconditioning in this species (e.g., Vegh et al., 1992); the present study, using a potent activator of these channels, shows that opening them prior to the prolonged ischaemic episode fails to influence arrhythmia severity. A similar conclusion regarding the role of ATP-sensitive potassium channels in preconditioning was reached using the approach of blocking such channels with glibenclamide (Vegh et al., 1993).

Acknowledgements

We are grateful for the support of the British Council (in collaboration with the Hungarian Committee for Technical Development), the European Economic Community (grant numbers BMHI-CT-92-1893 and ERC-CIPA-CT094-409) and the Hungarian State Government (OTKA).

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