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Anti-arrhythmic effects of levcromakalim in the ischaemic rat heart: a dual mechanism of action?

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Abstract

The action of pharmacological openers of K_{ATP} channels depends on the availability and levels of various intracellular nucleotides. Since these are subject to change during myocardial ischaemia, K_{ATP} channel openers may affect ischaemic and non-ischaemic tissue differentially. Using a recently developed dual coronary perfusion method, we investigated the effects on arrhythmias of the prototypical K_{ATP} channel opener levcromakalim when applied selectively to ischaemic and/or non-ischaemic tissue. A novel perfusion cannula was used to independently perfuse the left and right coronary beds of hearts isolated from rats. Selective infusion of levcromakalim (3, 10 or 30 μM) into the left coronary bed in the absence of ischaemia did not induce ventricular arrhythmias. Regional zero-flow ischaemia was induced by cessation of flow to the left coronary bed and hearts received levcromakalim selectively into either the left, right, or both coronary beds. When applied selectively to the ischaemic left coronary bed, levcromakalim (3, 10 or 30 μM; $n = 10$/group) delayed the onset of ventricular tachycardia in a dose-dependent manner (by 21*, 43* and 112%* at 3, 10 and 30 μM; *$P < 0.05$ vs. control). When applied only to the non-ischaemic right coronary bed, levcromakalim reduced the incidence of ventricular tachycardia during later phases of ischaemia (from 100% in controls to 30%*). When present in both coronary beds, levcromakalim had a striking anti-arrhythmic effect — the overall incidence of ventricular tachycardia being reduced from 100% in controls to 20%*. We conclude that levcromakalim may have an anti-arrhythmic effect when applied either to ischaemic or non-ischaemic tissue but that the mechanisms may differ depending on the metabolic state of the heart.

Keywords: Dual coronary perfusion method; Coronary bed; Perfusion cannula

1. Introduction

Plasmalemmal K^+ channels that are opened by low cytosolic ATP levels (K_{ATP} channels) (Noma, 1983; Trube and Hescheler, 1984) are fundamentally involved in a variety of physiological responses, including pancreatic insulin secretion (Ashcroft, 1988). Although they are abundantly expressed in the sarcolemma of cardiac myocytes, the physiological function of K_{ATP} channels has yet to be completely defined under normal conditions in the myocardium. In contrast, accumulating evidence demonstrate a crucial role for K_{ATP} channels during myocardial ischaemia, reperfusion following an ischaemic event (Coetzee, 1992) and phenomena such as ischaemic preconditioning (Hearse, 1995). Much of our current understanding of the role of K_{ATP} channels in ischaemia has been derived from studies using compounds that block K_{ATP} channels (e.g. sulfonylureas such as glibenclamide) or increase their open probability (K^+ channel openers). The use of "organelle specific" K_{ATP} channel modulators (such as diazoxide and 5-hydroxydecanoate) led to the concept that mitochondrial K_{ATP} channels are the end-effectors in mediating ischaemic preconditioning (Gross and Fryer, 1999). However, the extent of K_{ATP} channel block/opening by these compounds depends on the metabolic state of the cell. The actions of both blockers and openers of K_{ATP} channels are strongly dependent on the complement of intracellular nucleotides. For example, early experiments revealed an apparent competition between intracellular ATP and K_{ATP} channel openers in determining the level of channel activity (Thuringer and Escande, 1989). Also, alterations in intracellular ADP inhibits glibenclamide

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binding (Bernardi et al., 1992) whilst its presence is essential for the activity of some K_{ATP} channel openers, including nicorandil and diazoxide (Jahangir et al., 1994; D'hahan et al., 1999). Thus, the action of K_{ATP} channel openers on cardiac K_{ATP} channels is determined by the metabolic state of the myocyte. Since intracellular nucleotide concentrations are subject to change during ischaemia, K_{ATP} channel modulators may exert differential effects on normoxic and ischaemic tissue. It is therefore likely that the response to K_{ATP} channel modulators will depend on whether the predominant site of exposure to the compounds is non-ischaemic or ischaemic tissue. This issue is particularly relevant to regional myocardial ischaemia (as occurs during coronary artery obstruction), since under-perfused ischaemic tissue may not be exposed fully to the K_{ATP} channel modulator. However, until now, this concept has not been tested, given the limitations of available experimental models of regional ischaemia.

To address this issue, we used the recently developed dual coronary artery perfusion technique, in which the left and right coronary beds of isolated hearts from rats can be independently perfused with experimental solutions through their respective coronary ostia (Avkiran and Curtis, 1991). Given that collateral flow is low in the rat heart (Hale and Kloner, 1987), this novel technique has the advantage that the left or right coronary beds can selectively be treated with experimental compounds. By cessation of coronary flow to either coronary bed, compounds can thus be applied selectively to non-ischaemic or ischaemic zones. We used levcromakalim, a prototypical K_{ATP} channel openers, and designed the present study with the following primary objectives: (i) to determine (in the absence of ischaemia) whether regional infusion of levcromakalim induces ventricular arrhythmias, (ii) to assess the pro- or anti-arrhythmic potential of levcromakalim during regional ischaemia, and (iii) to identify the site of action of any pro- or anti-arrhythmic effect of levcromakalim during regional ischaemia. The use of isolated hearts also precluded any contribution to the arrhythmia profile from peripheral haemodynamic effects of K_{ATP} channel opening. Our results indicate that, in this model, levcromakalim does not possess significant pro-arrhythmic action in either the presence or the absence of regional ischaemia. To the contrary, levcromakalim was found to exhibit anti-arrhythmic properties during regional ischaemia, through a combination of actions in both the ischaemic and the non-ischaemic tissue.

2. Materials and methods

2.1. Animals and perfusion technique

All experiments were conducted in accordance with institutional guidelines and the Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986. Male Wistar rats (200–330 g in body weight), obtained from B&K Universal (Hull, UK), were anaesthetised with diethylether and injected with heparin sodium (100 I.U. iv). The heart was then rapidly removed, immersed in cold (4°C) perfusion solution and the aorta attached to a dual-perfusion cannula, to enable independent perfusion of the left and right coronary beds (Avkiran and Curtis, 1991). Both coronary beds were initially perfused at a constant pressure of 75 mm Hg, with Krebs–Henseleit solution at 37°C. When required, perfusion of one or both coronary beds was switched to a constant flow system supplied by a precalibrated roller pump (Gilson Minipuls), as previously described (Yasutake et al., 1994). A unipolar electrogram was obtained through a silver electrode attached to the epicardial surface of the left ventricular free wall and a reference electrode connected to the stainless steel outer casing of the dual perfusion cannula. Throughout the experiment, the heart was kept in a temperature-regulated chamber (at 37°C) and the right atrium and sino-atrial node were superfused (at 37°C) at a flow rate of 6–8 ml/min, to maintain a constant sinus rate (Avkiran and Curtis, 1991).

2.2. Solutions and drugs

All hearts were perfused with modified Krebs–Henseleit solution (composition in mM: 118.5 NaCl, 25.0 $NaHCO_3$, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 1.2 $CaCl_2$ and 11.0 glucose). The solution was continuously gassed with 95% O_2 plus 5% CO_2 (pH 7.4 at 37°C). A 10-mM stock solution of levcromakalim ((−)6-cyano-3,4-dihydro-2,2-dimethyl-trans-4-(2-oxo-1-pyrrolidyl)-2*H*-1-benzopyran-3-ol; donated by SmithKline Beecham) was prepared by dissolving the drug in 70% ethanol. This was serially diluted to obtain final drug concentrations of 3, 10 and 30 μM in the perfusion solution, with a final vehicle (ethanol) concentration of 0.7% in all cases. In all protocols, control hearts received vehicle-containing perfusion solution in the relevant coronary bed(s) during the appropriate period.

2.3. Study protocols

All studies were carried out in a prospectively randomized and blinded manner. Following a 15-min washout period, during which basal flow rates to the left and right coronary beds were noted, perfusion of one or both coronary beds was switched to a constant flow system, which supplied perfusion solution containing drug or vehicle at the relevant basal flow rate. When drug was given only to one coronary bed, the contralateral bed continued to receive normal perfusion solution at constant pressure. The protocols employed are described in Section 3.

2.4. Measured parameters

2.4.1. Coronary flow

Flow rates to the left and right coronary beds were continuously monitored using in-line flow meters (Avkiran

and Curtis, 1991) and noted at 2-min intervals during the experimental time course.

2.4.2. Heart rate and arrhythmias

Heart rate and arrhythmias were assessed from the unipolar electrogram. The signal was amplified and continuously monitored on a digital oscilloscope (Gould type 1421) and recorded on a chart recorder (Gould 2200S). The electrogram was analyzed for the incidence of ventricular premature beats, occurring singly or as bigeminy or salvos, ventricular tachycardia and ventricular fibrillation, in accordance with the Lambeth Conventions (Walker et al., 1988).

2.4.3. Size of coronary beds

In the first two experimental protocols, the mass of tissue supplied by each coronary bed was determined at the end of the experiments through the unilateral infusion of disulfine blue dye (0.016% wt/vol), as previously described (Avkiran and Curtis, 1991). The atria and mediastinal tissue were removed and the dyed tissue was carefully dissected, following which the dyed and non-dyed of tissues were lightly blotted and weighed. This enabled the calculation of flow in each coronary bed relative to the mass of tissue supplied by that bed (i.e. in units of ml/min/g). For inter-group comparisons, the mass of tissue supplied by the left coronary bed (representing the ischaemic zone where appropriate) was expressed as a percentage of total ventricular wet weight (Avkiran and Curtis, 1991).

2.4.4. Tissue high-energy phosphate content

Samples of left and right ventricular free wall (supplied by the left and right coronary beds, respectively) were obtained using tongs pre-cooled in liquid nitrogen. The samples were stored in liquid nitrogen and were lyophilized prior to analysis. Tissue levels of adenosine triphosphate (ATP) and creatine phosphate were determined through the fluorimetric detection of NADPH. In brief, lyophilized tissue samples (50 mg) were homogenized in perchloric acid (6%) and centrifuged at 2000 rpm for 10 min (4°C). The supernatant (2 ml) was removed and neutralized with 330 μl of saturated K_2CO_3 (5 M). Following mixing and centrifugation, 200 μl aliquots of the supernatant were stored at −80°C until analysis. The standard reaction mixture contained (in mM) 45.7 Tris-aminomethane, 0.05 NADP, 0.09 ADP, 0.09 glucose, 0.5 dithiothreitol and 4.6 $MgCl_2$, plus 0.046 I.U. glucose-6-phosphate-dehydrogenase (pH = 8.1). The reaction was started by adding 2 ml of reaction mixture containing hexokinase (to determine ATP) or hexokinase plus creatine kinase (to determine creatine phosphate, by subtraction) to cuvettes containing 20 μl of sample or standard solution. Following 30 min of incubation at room temperature, NADPH fluorescence was measured using an excitation wavelength of 365 nm and an emission wavelength of 460 nm. Calibration was through the use of standard ATP solutions (0–1.0 mM) and ATP and creatine phosphate contents were expressed in μmol/g dry weight.

2.5. Data analysis

Values are expressed as means ± S.E.M., where appropriate. One-way analysis of variance (ANOVA) was used to make inter-group comparisons. If significance was reached, Dunnet's test was used to compare individual groups against the control group. The paired *t*-test was used assess temporal changes within each group (e.g. comparison of post-drug values with pre-drug values). Chi-square test was used to analyze binomially distributed variables, such as the incidences of various arrhythmias. If a significant value was found, the Fisher's exact test was used for individual comparisons with control. $P < 0.05$ was considered significant.

3. Results

3.1. Effects of levcromakalim in the absence of ischaemia

3.1.1. Levcromakalim applied selectively to the left coronary bed

Vehicle or levcromakalim (3, 10 or 30 μM; $n = 4$/group) was administered selectively into the left coronary bed for a 30-min period (in the absence of any ischaemia) at a constant flow rate. Although a few isolated incidences of ventricular premature beats were observed in some of the hearts, their incidence or frequency of occurrence were unrelated to the administration of levcromakalim into the left coronary bed. We observed no single episode of ventricular tachycardia or ventricular fibrillation throughout the experimental protocol in any of the experimental groups. Thus, the selective application of levcromakalim to a region of the heart did not lead to the onset of severe ventricular arrhythmias in this experimental model. Basal heart rate (before the onset of vehicle or drug administration) ranged from 263 ± 11 to 283 ± 14 beats/min (NS) in these experimental groups. Heart rate was not significantly affected by vehicle or drug infusion and ranged from 260 ± 10 to 293 ± 14 beats/min (NS) at the end of the experimental protocol.

At the end of the experimental protocol, the mass of tissue supplied by each coronary bed was determined. There were no inter-group differences in basal flow rates of the two coronary beds, expressed either in absolute units (left coronary bed flow range: 4.5 ± 0.5 to 5.8 ± 0.3 ml/min, right coronary bed flow range: 3.3 ± 0.5 to 3.6 ± 0.5 ml/min) or relative to the ventricular mass supplied by each bed (left coronary bed flow range: 10.4 ± 0.7 to 13.1 ± 0.9 ml/min/g, right coronary bed flow range: 11.3 ± 0.8 to 16.8 ± 2.3 ml/min/g). The constant-flow infusion of levcromakalim selectively into the left coro-

nary bed caused a dose-dependent increase in flow to the right coronary bed (Fig. 1). This increase in flow to the right coronary bed was reversible upon cessation of drug infusion into the left coronary bed. Since application of levcromakalim to the left coronary bed led to an increase in flow to the right coronary bed, there might be a degree of cross-flow between the two coronary beds. However, the extent of cross-flow may be very small, since the presence of very low concentrations of levcromakalim can cause significant increases in coronary blood flow (Sato et al., 1994). Furthermore, using the dye exclusion technique, we observed no significant differences in the ventricular mass supplied by the left coronary bed in the absence or presence of levcromakalim. Thus, we determined that the left coronary bed supplied 65 ± 2% of the ventricular mass in the control group and 62 ± 3%, 68 ± 8% and 61 ± 4% of the ventricular mass in the groups that received 3, 10 or 30 μM levcromakalim (NS). These data support the concept that very little cross-flow occurs between the left and right coronary beds in rat myocardium.

The increase in left coronary bed flow upon cessation of drug infusion was a consequence of the switch from a

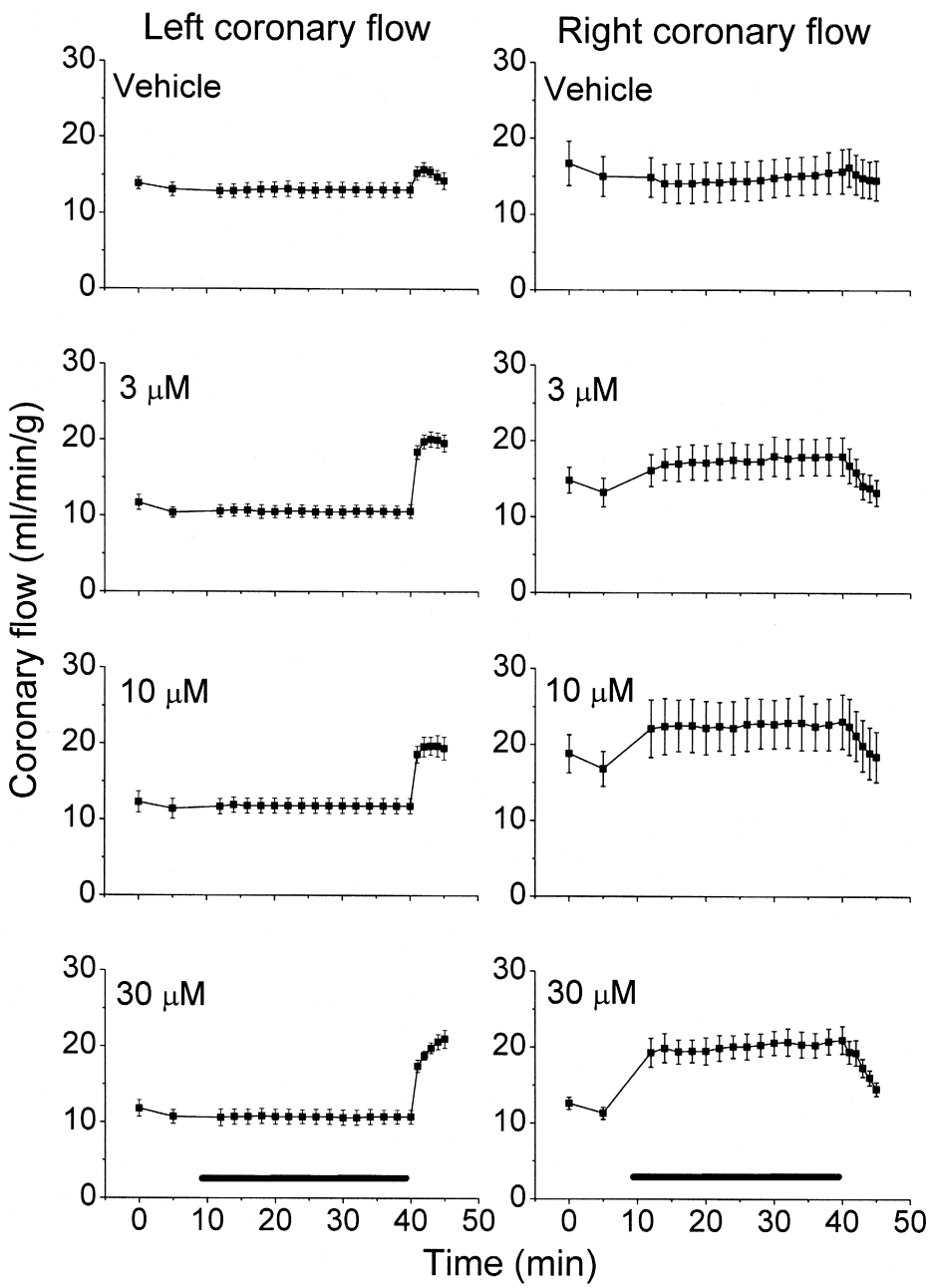

Fig. 1. The effect of levcromakalim on coronary flow in the absence of ischaemia. Vehicle or levcromakalim (3, 10 or 30 μM) was infused selectively into the left coronary bed at constant flow for a 30-min period, while the right coronary bed continued to receive drug-free solution at constant pressure. Horizontal bars indicate period of drug/vehicle infusion into the left coronary bed.

constant flow to a constant pressure (unregulated flow) perfusion system. It appears that all concentrations of drug produced maximal vasodilation in the left coronary bed, and this was manifest as a large increase in left coronary bed flow following the switch to constant pressure (and hence variable flow) perfusion. It is expected that if the drug-free perfusion period had been extended, left coronary bed flow would have returned to baseline. Only a limited amount of drug had access to the right coronary bed during drug infusion into the left coronary bed, and this was readily washed out following cessation of drug infusion; hence, the rapid normalization of right coronary bed flow.

3.2. Effects of levcromakalim in the presence of left ventricular ischaemia

3.2.1. Levcromakalim applied selectively to the left coronary bed

Vehicle or levcromakalim (3, 10 or 30 μM) was administered selectively into the left coronary bed for 5 min, after which flow to this bed was stopped to induce regional ischaemia for 30 min ($n = 10$/group). The drug was thus "trapped" within the ischaemic zone while the non-ischaemic right ventricle was perfused in the absence of levcromakalim. At the end of the ischaemic period, the mass of tissue supplied by each coronary bed (i.e. ischaemic zone size; measured in the first five hearts in each group) and the tissue content of high-energy phosphates (the last five hearts of each group) were determined.

None of the hearts in any experimental group exhibited ventricular fibrillation in this study protocol, regardless of the concentration of levcromakalim used. The incidence of ventricular premature beats was high in the control group (9/10). Levcromakalim did not reduce the incidence of ventricular premature beats in this study protocol, which occurred in 100%, 100% and 90% of hearts that received 3, 10 or 30 μM levcromakalim. Similarly, in this study protocol, the incidence of ventricular tachycardia was unaffected by levcromakalim, which occurred in 80% in controls and 80%, 90% and 50% in the levcromakalim groups, respectively (NS).

Despite a lack of effect of levcromakalim on the overall incidence of ventricular premature beats or ventricular tachycardia, the onset of arrhythmias was significantly delayed in a dose-dependent manner. Thus, ventricular premature beats occurred after 7.6 ± 2.0 min in the control group, but the time to the onset of ventricular premature beats was increased to 12.3 ± 2.1, 14.0 ± 2.8 and 19.0 ± 4.1 ($P < 0.05$) min by 3, 10 or 30 μM levcromakalim. Similarly, the time to the onset of ventricular tachycardia was increased from 12.6 ± 0.5 min in the control group to 15.2 ± 0.8 ($P < 0.05$), 18.0 ± 1.7 ($P < 0.05$) and 26.7 ± 2.0 ($P < 0.05$) min in the respective levcromakalim groups.

Fig. 2 shows the time course of ventricular tachycardia occurrence in the various study groups during the 30-min ischaemic period. The delay in the onset of ventricular tachycardia was manifested by a suppression of ventricular tachycardia during the early period of ischaemia. When the ischaemic period was divided arbitrarily into early (0–20 min) and delayed (20–30 min) phases, the incidence of ventricular tachycardia during the early phase was 80% in the control group and 80%, 60% and 0%* in the groups that received 3, 10 or 30 μM levcromakalim (*$P < 0.05$

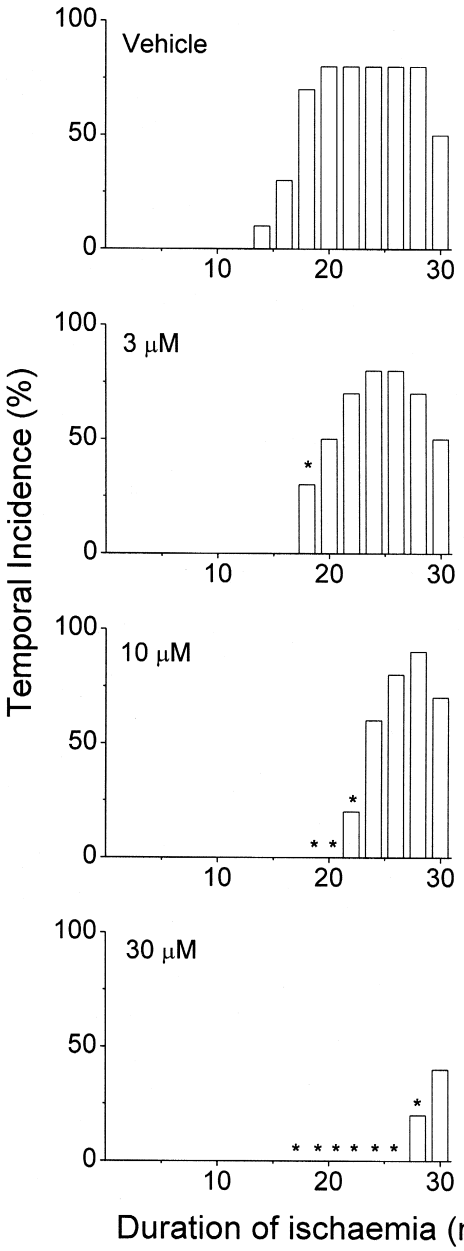

Fig. 2. Effect of levcromakalim on the incidence of ventricular tachycardia in hearts isolated from rats and subjected to regional ischaemia. Vehicle or levcromakalim (3, 10 or 30 μM) was applied selectively to the left coronary bed for 5 min, prior to the induction of zero-flow ischaemia in this bed. During 2-min intervals throughout the 30-min period of ischaemia, the number of hearts that exhibited ventricular tachycardia was determined and expressed as a percentage of the total number of hearts ($n = 10$ in each group). *$P < 0.05$ vs. vehicle.

vs. control). In contrast, the incidence of ventricular tachycardia during the late phase was not significantly affected by drug treatment (80% in controls vs. 50–90% in the levcromakalim groups).

Before the onset of drug treatment, there were no differences in coronary flow rates to the two coronary beds between the experimental groups, irrespective of whether flows are expressed in absolute units (left coronary bed: flow range: 4.6 ± 0.2 to 5.4 ± 0.3 ml/min, right coronary bed flow range: 2.9 ± 0.1 to 3.2 ± 0.3 ml/min) or relative to the ventricular mass supplied by each bed (left coronary bed: flow range: 8.9 ± 0.7 to 13.6 ± 1.0 ml/min/g, right coronary bed flow range: 10.5 ± 1.3 to 12.0 ± 1.2 ml/min/g). A small increase in coronary flow to the right coronary bed occurred when levcromakalim was selectively infused into the left coronary bed at constant flow (Fig. 3), once again indicating that some drug had access to the right coronary bed. However, this increase was

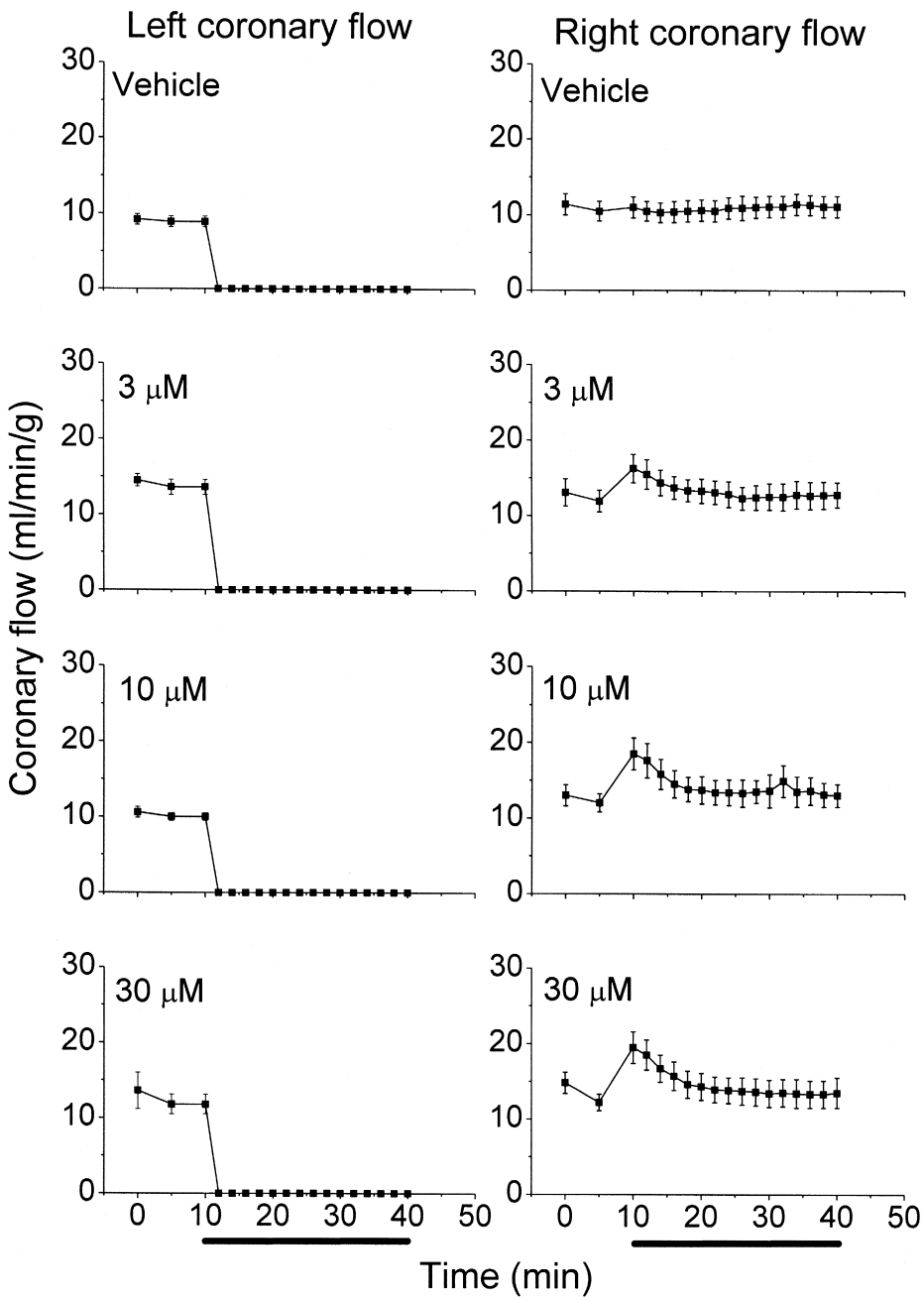

Fig. 3. The effect of levcromakalim on coronary flow in the presence of ischaemia. Vehicle or levcromakalim (3, 10 or 30 μM) was infused selectively into the left coronary bed at constant flow for 5 min prior to the induction of a 30-min period (starting at the 10-min time point) of zero-flow ischaemia in this bed. Throughout the experiment, the right coronary bed continued to receive drug-free solution at constant perfusion pressure. Data are shown only for the hearts that were not freeze-clamped for analysis of high-energy phosphates, in which the tissue masses supplied by each coronary bed could be determined ($n = 5$ in each group). Horizontal bars indicate period of drug/vehicle infusion into the left coronary bed.

temporary and flow to the right coronary bed returned towards basal levels shortly after the cessation of flow to the left coronary bed, illustrating the success of the experimental design in selectively applying drug to the ischaemic zone only. There were no significant inter-group differences in heart rate before (range: 277 ± 6 to 284 ± 8 beats/min) or after (range: 285 ± 6 to 292 ± 8 beats/min) drug infusion, or at the end of the ischaemic period (range: 270 ± 10 to 286 ± 4 beats/min). The ischaemic zone sizes were also similar, at $64 \pm 3\%$ in controls and $62 \pm 5\%$, $69 \pm 5\%$ and $63 \pm 5\%$ in the groups that received 3, 10 or 30 μM levcromakalim (NS).

Fig. 4 illustrates ATP levels in left (ischaemic) and right (non-ischaemic) ventricular tissue, measured at the end of the 30-min ischaemic period. The linear model test (Graybill, 1976) was used to test for overall differences between lines (linear data) as well as for differences between slopes and *y*-intercepts. This analysis shows that levcromakalim resulted in a dose-dependent preservation of tissue ATP in the left coronary bed during ischaemia. The creatine phosphate level was unaltered (3.5 ± 0.1, $n = 4$ without drug vs. 4.4 ± 0.47, $n = 5$ μmol/g dry weight in the 30 μM levcromakalim group, p = NS). The complement of high-energy nucleotides in the non-ischaemic right coronary bed was unaltered by infusion of levcromakalim into the left coronary bed. The ATP levels at different concentrations of levcromakalim are shown in upper panel in Fig. 4. Creatine phosphate levels were similarly unchanged (18.6 ± 1.11 without drug and 20.3 ± 1.00 μmol/g dry weight in the 30-μM levcromakalim group, p = NS). These data illustrate that levcromakalim preserved the ATP content in the ischaemic tissue whilst not affecting levels of high-energy phosphates in the non-ischaemic tissue.

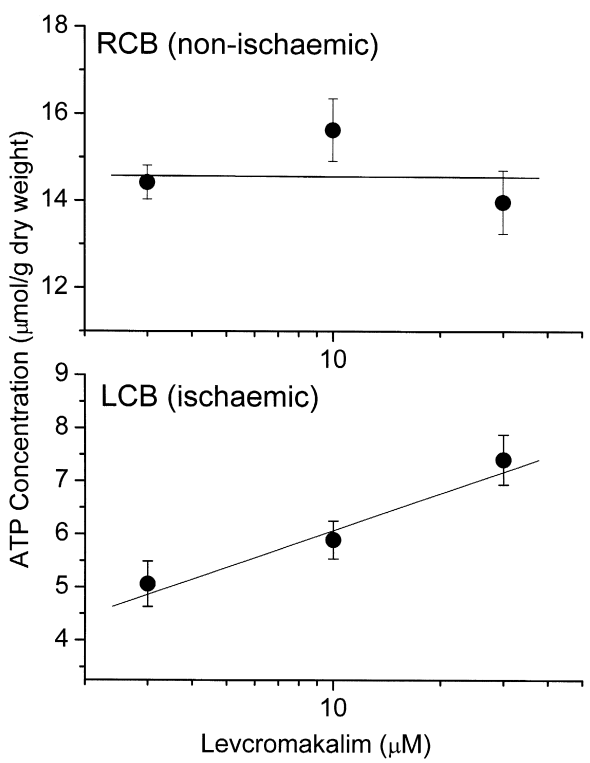

Fig. 4. ATP levels in ventricular tissue supplied by the right coronary bed (upper panel) and left coronary bed (lower panel), at the end of the 30-min period of zero-flow ischaemia in this bed. Vehicle or levcromakalim (3, 10 or 30 μM) was infused into the left coronary bed for 5 min prior to the induction of zero-flow ischaemia. The lines through the data points were drawn by linear regression. The slopes of the two lines in the lower panel were significantly different from each other ($p < 0.05$).

3.2.2. *Levcromakalim applied selectively to the non-ischaemic right coronary bed*

For these experiments, we focused on the concentration of levcromakalim that had the largest effect in the preceding experimental regimens. Consequently, vehicle or levcromakalim (30 μM) was administered selectively into the right coronary bed for 5 min, after which flow to the left coronary bed was stopped to induce regional ischaemia for 30 min ($n = 10$/group). The right coronary bed continued to receive vehicle or drug throughout the period of regional ischaemia.

As in the preceding study groups, none of the control hearts exhibited ventricular fibrillation during 30 min of regional ischaemia induced by cessation of flow to the left coronary bed. Similarly, no ventricular fibrillation episodes occurred during regional ischaemia when levcromakalim was infused selectively into the right coronary bed. The overall incidences of ventricular premature beats and ventricular tachycardia were not reduced by levcromakalim. The incidence of ventricular premature beats was 100% in the control group and 80% in the group that received 30 μM levcromakalim (NS) and the corresponding values for ventricular tachycardia were 100% and 70% (NS). The time to onset of ventricular premature beats was not altered (7.3 ± 1.7 in control vs. 9.9 ± 2.3 min with levcromakalim) while there was a small but significant increase in the time to onset of ventricular tachycardia (from 11.2 ± 0.3 in control to 12.5 ± 0.5 min in the levcromakalim group ($P < 0.05$)). Fig. 5 shows the time course of ventricular tachycardia occurrence in the two study groups during the 30-min ischaemic period. Contrary to the observation made when levcromakalim was given selectively to the ischaemic left coronary bed, infusion of the drug selectively into the non-ischaemic right coronary bed did not affect the incidence of ventricular tachycardia during the early phase of ischaemia (100% vs. 70%, NS) but reduced it significantly during the late phase of ischaemia (from 80% to 30%, $P < 0.05$).

There were no significant inter-group differences in basal flow to the left coronary bed (6.7 ± 0.8 and 5.2 ± 0.3 ml/min) or right coronary bed (3.1 ± 0.2 and 3.0 ± 0.2 ml/min), measured before the onset of drug/vehicle infusion. In the 5 min before the onset of ischaemia, during which time the drug was infused into the right coronary

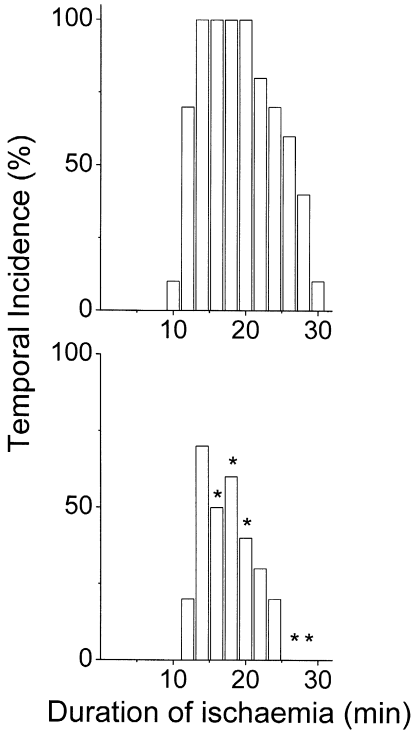

Fig. 5. Effect of levcromakalim on the incidence of ventricular tachycardia in isolated hearts subjected to regional ischaemia. Vehicle (top panel) or levcromakalim (30 μM, bottom panel) was applied only to the right coronary bed from 5 min before the induction of zero-flow ischaemia in the left coronary bed. During 2-min intervals throughout the course of the 30-min period of ischaemia, the number of hearts that exhibited ventricular tachycardia was determined and expressed as a percentage of the total number of hearts ($n = 10$ in each group). $^{*}P < 0.05$ vs. no-drug.

bed at its basal flow rate, there was a 33% increase in flow to the left coronary bed. Taken together with earlier observations, this suggests that, regardless of which bed the drug was infused into, limited access to the contralateral bed inevitably occurred. There were no significant differences between the two groups in heart rate before (305 ± 11 and 293 ± 10 beats/min) or after (310 ± 8 and 304 ± 9 beats/min) drug infusion, or at the end of the ischaemic period (300 ± 6 and 288 ± 10 beats/min).

3.2.3. Levcromakalim applied to both the left and right coronary beds

Vehicle or levcromakalim (30 μM) was administered into both coronary beds for 5 min, after which flow to the left coronary bed was stopped to induce regional ischaemia for 30 min ($n = 10$/group). The right coronary bed continued to receive vehicle or drug during the period of regional ischaemia.

Ventricular fibrillation was not observed in either group during 30 min of regional ischaemia by cessation of flow to the left coronary bed. The overall incidence of ventricular premature beats was 100% in controls but was reduced to 20% ($P < 0.05$) in the levcromakalim group. The overall incidence of ventricular tachycardia was also reduced, from 90% in controls to 20% in the levcromakalim group ($P < 0.05$). Although levcromakalim appeared to accelerate the onset of both ventricular premature beats (from 13.0 ± 0.4 in controls to 8.5 ± 1.9 min) and ventricular tachycardia (from 13.2 ± 0.5 in controls to 7.2 ± 2.8 min), neither effect was statistically significant. Fig. 6 illustrates the profiles of ventricular tachycardia occurrence during the period of regional ischaemia. In contrast to the observations made when levcromakalim was infused selectively into a single coronary bed, administration of the drug to both coronary beds reduced the incidence of ventricular tachycardia during both the early phase (from 90% to 20%; $P < 0.05$) and the late phase (from 90% to 10%; $P < 0.05$) of ischaemia.

There were no significant inter-group differences in basal flow to the left coronary bed (5.8 ± 0.4 and 5.2 ± 0.3 ml/min) or right coronary bed (2.9 ± 0.2 and 3.1 ± 0.2 ml/min), measured prior to vehicle/drug infusion. Perfusion of both coronary beds was then switched to a constant flow system, supplying flow at the total basal flow rate. After 5 min of drug infusion into both beds at constant flow, the left coronary bed was subjected to 30 min of zero-flow ischaemia while flow to the right coronary bed was maintained at its basal value. There were no signifi-

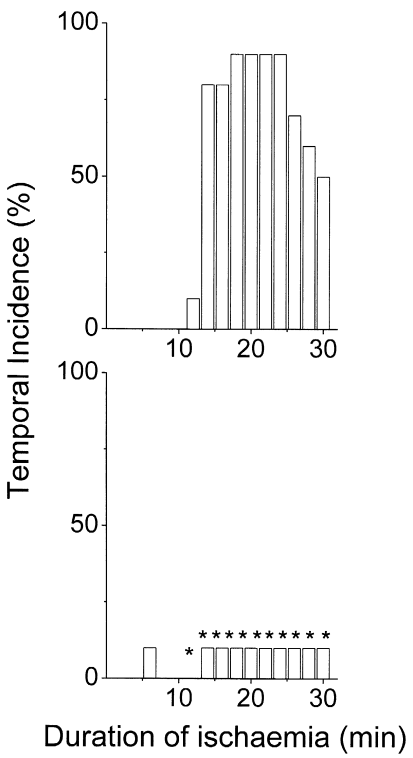

Fig. 6. Effect of levcromakalim on the incidence of ventricular tachycardia in isolated hearts subjected to regional ischaemia. Vehicle (top panel) or levcromakalim (30 μM, bottom panel) was applied to both the left and the right coronary beds from 5 min before the induction of zero-flow ischaemia in the left coronary bed. During 2-min intervals throughout the 30-min period of ischaemia, the number of hearts that exhibited ventricular tachycardia was determined and expressed as a percentage of the total number of hearts ($n = 10$ in each group). $^{*}P < 0.05$ vs. vehicle.

cant differences between the two groups in heart rate before (293 ± 13 and 278 ± 9 beats/min) or after (306 ± 10 and 298 ± 9 beats/min) drug infusion, or at the end of the ischaemic period (296 ± 13 and 301 ± 10 beats/min).

4. Discussion

4.1. Role of cardiac K_{ATP} channels in arrhythmias

When cardiac sarcolemmal K_{ATP} channels open during myocardial ischaemia, K^+ efflux from the cells contributes to extracellular K^+ accumulation (Kantor et al., 1990). The resulting net outward K^+ current can drastically shorten the cardiac action potential duration. The different properties of K_{ATP} channels in various regions of the heart, and their differential responses to ischaemia (Furukawa et al., 1991) makes it is very likely that their opening will cause large regional differences in action potential duration (and heterogeneity) during ischaemia. The combination of these events can set the stage for the generation of serious arrhythmias (Harris, 1966; Curtis et al., 1985). Consistent with the notion that K_{ATP} channels are involved in these processes, it has been found that glibenclamide diminishes, whereas K_{ATP} channel openers augment, action potential shortening and extracellular K^+ accumulation during ischaemia (Kantor et al., 1990; Venkatesh et al., 1991; Weiss and Venkatesh, 1993; Wilde et al., 1990; Hicks and Cobbe, 1991; Jaing et al., 1991; Mitani et al., 1991). As expected from the inhibition of these events by blocking K_{ATP} channels, most studies ascribe an anti-arrhythmic affect to glibenclamide under ischaemic situations (Wolleben et al., 1989; Kantor et al., 1990; Bril et al., 1992; Pasnani and Ferrier, 1992; Billman et al., 1993; Siegl, 1994; Xiao and Holley, 1997). From theoretical considerations, K_{ATP} channel openers might be expected to be pro-arrhythmic by exacerbating action potential shortening and K^+ loss during ischaemia. Although these expected effects of K_{ATP} channel openers on action potential duration and K^+ efflux during ischaemia have been noted (Cole et al., 1991; D'Alonzo et al., 1992; Mitani et al., 1991), there are no consistent reports on their effects on arrhythmias. Pinacidil, cromakalim and nicorandil can increase (Chi et al., 1990; De La Coussaye et al., 1993), decrease (Kerr et al., 1985; Vegh et al., 1996), or have no effect (Grover et al., 1990; Vegh et al., 1997; Miyazaki et al., 1995) on arrhythmias in dog models of ischaemia.

The results presented in our study show an anti-arrhythmic effect of levcromakalim at concentrations ranging between 3 and 30 μM. Ischaemia was produced regionally (only in the left coronary bed), which allows for comparison with other models of regional ischaemia in rat heart. In this setting (which mimics clinically relevant ischaemia occurring in humans), moderate concentrations of K_{ATP} channel openers have been described generally to cause a decreased incidence of ventricular tachycardia and ventricular fibrillation (D'Alonzo et al., 1994; Lepran et al., 1996) or have no effect on arrhythmias (Rees et al., 1993; Ferdinandy et al., 1995). Pro-arrhythmic effects of K_{ATP} channel openers were observed when they were applied in high (near toxic) concentrations (Robert et al., 1997; Ferdinandy et al., 1995) or under hypokalemic conditions (Kempsford and Hawgood, 1989). In a model of global (low-flow) ischaemia, with continuous ventricular pacing at 300 bpm, moderate concentrations of cromakalim did not alter the incidence of ventricular fibrillation (86% in control vs. 83% with cromakalim) but decreased the average time to ventricular fibrillation (from about 20 min after the onset of low-flow ischaemia to about 15 min).

4.2. Mechanism of action of levcromakalim?

Opening of K_{ATP} channels can protect the ischaemic myocardium by delaying ischaemic damage. Recent studies suggest an involvement of *mitochondrial* K_{ATP} channels in some forms of protection (such as ischaemic preconditioning). For example, it has been known for some time that 5-HD, now appreciated to block mitochondrial K_{ATP} channels (Liu et al., 1996, 1998), can prevent ischaemic preconditioning (Mccullough et al., 1991). Furthermore, other recent findings show that the mitochondrial K_{ATP} channel opener, diazoxide, can mimic protection afforded by preconditioning (Grover, 1997). When mitochondrial K_{ATP} channels open during ischaemia, ATP production might be enhanced, thus minimizing the ischaemic insult. Thus, the protective effect of K_{ATP} channel openers during ischaemia can be due partly to opening of mitochondrial K_{ATP} channels.

Cardioprotection might occur through opening of *sarcolemmal* K_{ATP} channels (which will shorten the action potential, diminish Ca^{2+} overload and "spare" ATP by reducing contraction strength). However, at least theoretically, it also is possible that the opening of sarcolemmal K_{ATP} channels may be detrimental during myocardial ischaemia. Their opening, with the consequential K^+ loss and action potential duration changes, may cause dispersion of refractoriness and the onset of life-threatening arrhythmias. Thus, the conflicting studies on the effects of K_{ATP} channel openers on ischaemia-induced arrhythmias might be due to two independent actions: a possible protective effect (via an action on both mitochondrial and sarcolemmal K_{ATP} channels, as described above) and a possible detrimental effect (via actions mainly on sarcolemmal K_{ATP} channels to alter the dispersion of refractoriness). The data shown in the present study illustrate that levcromakalim possesses anti-arrhythmic properties in our experimental model. It is not clear whether levcromakalim acted via mitochondrial or sarcolemmal K_{ATP}

channels, since (at the concentrations used in our study) levcromakalim has been reported to affect both sarcolemmal and mitochondrial K_{ATP} channels (Escande et al., 1988; Holmuhamedov et al., 1998). Since levcromakalim produced a clear ATP-sparing effect in these studies, it is tempting to speculate that mitochondrial K_{ATP} channels may have been involved in part of the anti-ischaemic (and anti-arrhythmic) effect of levcromakalim. However, to resolve this issue, further studies would be required utilizing compounds that more specifically target sarcolemmal or mitochondrial K_{ATP} channels.

We conclude therefore that opening of mitochondrial and/or sarcolemmal K_{ATP} channels can be anti-arrhythmic under some circumstances.

4.3. A dual mechanism of action of levcromakalim on ischaemia-induced arrhythmias?

We found levcromakalim to delay the onset of ischaemia-induced arrhythmias in a dose-dependent manner, when applied only to the ischaemic tissue. The most plausible explanation is that levcromakalim may have had an anti-ischaemic, or energy-sparing action. Other studies have also described such energy-sparing effects: pinacidil was found to preserve high-energy phosphates (ATP and creatine phosphate) during ischaemia of guinea pig right ventricular wall (Mcpherson et al., 1993). In our study, with levcromakalim treatment of the ischaemic tissue, a clear ATP-sparing effect was observed. In contrast, no statistically significant differences were observed in creatine phosphate levels after a 30-min ischaemic period. It is likely that the degree of ischaemia was too severe in our study and that statistically significant differences may have been observed if these measurements were taken at earlier time points during ischaemia. Indeed, it has been shown that the preservation of high-energy phosphates by treatment with K_{ATP} channel openers was much more profound during early ischaemia, with the effect waning with prolonged ischaemia (Mcpherson et al., 1993).

When applied only to the non-ischaemic area, levcromakalim also had an anti-arrhythmic effect. However, in contrast to the delay in onset of arrhythmias that was observed when the drug was applied selectively to the ischaemic area, with this protocol we found a decrease in arrhythmias during the late phase of ischaemia. Therefore, it is likely that a different anti-arrhythmic mechanism may apply under these conditions. At present, it is not clear what this mechanism may be. One possibility is that refractoriness in the non-ischaemic area may have been decreased (Di Diego and Antzelevitch, 1993; Spinelli et al., 1990), which would lead to less heterogeneity of refractoriness during ischaemia. Further experiments would be required to determine the exact nature of this phenomenon.

When levcromakalim was applied both to ischaemic and non-ischaemic tissues, a very profound anti-arrhythmic action was observed. This observation is consistent with the suggestion that levcromakalim may have a dual mechanism of action, mediated through both the ischaemic and non-ischaemic zones, with a synergistic effect observed with global drug administration.

4.4. Potential limitations of this study

Our studied was designed to produce a low incidence of ventricular fibrillation, with the aim of detecting any possible pro-arrhythmic action of levcromakalim (which was not observed). To this end, we used an extracellular K^+ concentration of 5.9 mM, which reduces the incidence of ischaemia-induced arrhythmias (Curtis et al., 1985). At this K^+ concentration, which is higher than the normal plasma K^+ concentration, we observed a profound anti-arrhythmic effect of levcromakalim. We cannot rule out, however, the possibility that levcromakalim may not have the same anti-arrhythmic action at lower extracellular K^+ concentrations.

In order to be able to administer levcromakalim regionally, we used the dual-perfusion cannula in rat hearts. However, the coronary flow changes observed in individual beds during selective drug infusion indicate that a degree of cross-flow inevitably occurred; thus, in terms of arrhythmias also, we cannot discount the possibility of some drug action in the contralateral bed. Furthermore, although it has been argued that the rat is a useful model for the study of arrhythmias (Curtis et al., 1987), it is possible that the unique electrophysiological characteristics of this species may have influenced our results.

Finally, all studies to date rely on pharmacological approaches and are hampered by possible non-specific effects of the drugs used. In future, studies in mice with targeted disruption of sarcolemmal and/or mitochondrial K_{ATP} channels may provide more conclusive evidence regarding the roles of these channels in ischaemic pathogenesis.

4.5. Conclusion

In hearts isolated from rats, we found that regional application of levcromakalim did not induce arrhythmias in the absence of ischaemia. Furthermore, regional administration of levcromakalim protected against arrhythmias induced by regional ischaemia. Our results from global application of levcromakalim in conjunction with regional ischaemia suggest that the protective effect of this K_{ATP} channel opener may result from the synergistic outcome of delayed ischaemic injury (by drug action in ischaemic tissue) and reduced regional heterogeneity (by drug action in non-ischaemic tissue). These data are consistent with the hypothesis that the mechanism of the anti-arrhythmic action of levcromakalim depends on the metabolic state of the myocardium.

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