The ATP-sensitive potassium channel in the heart. Functional, electrophysiological and molecular aspects
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K\textsubscript{ATP} channel opening during ischemia: effects on myocardial noradrenaline release and ventricular arrhythmias


Abstract

**Purpose.** Cardioprotection by K<sub>ATP</sub> channel openers during ischemia is well documented although ill understood. Pro-arrhythmic effects may be an important drawback. K<sub>ATP</sub> channel modulation influences neurotransmitter release during ischemia in brain synaptosomes. Therefore, we studied the effects of K<sub>ATP</sub> channel modulation on myocardial noradrenaline release and arrhythmias in ischemic rabbit hearts.

**Methods.** Isolated rabbit hearts were perfused according to Langendorff and stimulated. Local electrograms were recorded and K<sup>+</sup>-selective electrodes were inserted in the left ventricular free wall. Cromakalim (3 μM) or glibenclamide (3 μM) was added 20 min prior to induction of global ischemia. After 15, 20 or 30 min of ischemia, hearts were reperfused and noradrenaline (NA) content of the first 100 ml reperfusate was measured.

**Results.** Cromakalim (n=16) prevented the second rise of extracellular [K<sup>+</sup>] in accordance with its cardioprotective effect. Cromakalim significantly reduced NA release after 15 min (mean 169 ± SEM 97 pmol/gr dry weight vs. control 941 ± 278; p<0.05) and 20 min of ischemia (230 ± 125 pmol/gr dry wt vs. control 1460 ± 433; p<0.05), but after 30 min of ischemia, the difference in NA release was no longer significant (cromakalim 2703 ±1195 pmol/gr dry wt vs. control 5413 ±1310; p=0.08). Ventricular fibrillation (VF) or ventricular tachycardia (VT) occurred in 10 out of 13 (77%) control hearts (n=19), in 6 out of 10 (60%) glibenclamide treated hearts (n=15) and in 6 out of 14 (43%) cromakalim treated hearts (p=NS). Cromakalim significantly accelerated onset of VT or VF (means ± SEM onset after 12.5 ± 1.6 min ischemia vs. control 16.2 ± 0.7 min; p<0.05). NA release only occurred in cromakalim treated hearts with early onset arrhythmias while no NA release was observed in cromakalim treated hearts without VT or VF.

**Conclusion.** Our results show that activation of the K<sub>ATP</sub> channel by cromakalim during ischemia reduces myocardial noradrenaline release and postpones the onset of irreversible damage, contributing to the cardioprotective potential of K<sub>ATP</sub> openers during myocardial ischemia.
**Introduction**

Myocardial ATP-sensitive potassium (K\textsubscript{ATP}) channels are closed during physiological conditions but open during the course of ischemia concomitant with a decrease of intracellular ATP-concentration (Noma 1983). Addition of K\textsubscript{ATP} channel opening drugs before onset of ischemia results in a delayed time of onset of irreversible damage during ischemia (Tan et al. 1993), reduction of infarct size and enhanced recovery of post-ischemic, reperfused myocardium (Yao et al. 1994, Grover et al. 1990, Auchampbach et al. 1993). However, K\textsubscript{ATP} channel openers may have pro-arrhythmic effects during ischemia through their action potential shortening property (Wilde and Janse 1994). Blockade of K\textsubscript{ATP} channels by sulfonylurea antagonists and sodium 5-hydroxydecaonate reverses the beneficial effects of K\textsubscript{ATP} channel opening (McCullough et al. 1991, Grover et al. 1989).

Although the exact mechanism of cardioprotection by K\textsubscript{ATP} activation has not yet been unequivocally established, it has been shown that action potential shortening is not a prerequisite for cardioprotection to occur (Yao et al. 1994). A role for mitochondrial K\textsubscript{ATP} channels has been suggested, but further studies are needed for clarification (Szewczyk 1997).

K\textsubscript{ATP} channels are present throughout the brain and are located on both pre- and postsynaptic neurons (Mourre et al. 1990). Recent studies have focused on the effects of K\textsubscript{ATP} channel modulation on the release of certain potentially toxic neurotransmitters in the brain. Indeed, during simulated brain ischemia, K\textsubscript{ATP} channel opening reduced, whereas K\textsubscript{ATP} channel blockade increased the release of the neurotransmitter GABA in brain synaptosomes (Amoroso et al. 1990, Zini et al. 1993).

During myocardial infarction, noradrenaline release from adrenergic nerve-endings in the heart is observed after 10-15 minutes of ischemia and progressively increases with duration of ischemia (Wilde et al. 1988, Schömig et al. 1984). Reduction or prevention of noradrenaline release during ischemia delays the onset of irreversible myocardial damage and improves myocardial function (Culling et al. 1984, Schömig 1990). Furthermore, noradrenaline release may be associated with the occurrence of ventricular arrhythmias (Kurz et al. 1995). Therefore, we sought to investigate whether modulation of K\textsubscript{ATP} channels in the heart during myocardial ischemia influences endogenous noradrenaline release, thus contributing to the cardioprotective effect conferred by K\textsubscript{ATP} channel opening. Our results show that opening of K\textsubscript{ATP} channels reduces endogenous myocardial noradrenaline release during global ischemia and delays the onset of irreversible damage in the myocardium.
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Methods

Experimental set-up and protocol
Hearts of New Zealand White rabbits of either sex (2.0-3.0 kg) were retrogradely perfused according to Langendorff with modified Tyrode’s solution (37-38°C), as described in the Materials and Methods section. Perfusion pressure was maintained at 70 mmHg. A bipolar stimulus electrode was inserted in the right ventricular outflow tract and the hearts were stimulated with a basic cycle length of 280 ms (210 beats/min). After 50 minutes of equilibration, global ischemia was induced by complete interruption of flow. Either the K<sub>ATP</sub> blocking agent glibenclamide (3μmol/l, Sigma) or the K<sub>ATP</sub> opener cromakalim (3μmol/l, Smith, Kline and Beecham) (both dissolved in dimethyl sulfoxide, DMSO) was added to the perfusate 20 minutes before onset of ischemia. After 10, 15, 20 or 30 minutes of ischemia, pacing was stopped (to prevent reperfusion arrhythmias) and hearts were reperfused. The first 100 ml of reperfusate was collected and the time required for this amount to be obtained was measured (t<sub>rep</sub>). After the experiment, hearts were dry frozen and their dry weights were measured.

Extracellular electrograms and measurement of extracellular potassium
Extracellular electrograms were recorded with two bipolar electrodes on the left ventricular free wall. The occurrence of ventricular arrhythmias was recorded and QT-intervals were measured as an indication of action potential duration (APD) variation (see Materials and Methods). To prevent reperfusion arrhythmias, pacing was stopped during the first minutes of reperfusion. Extracellular potassium concentration ([K<sup>+</sup>]<sub>i</sub>) was measured using potassium selective electrodes inserted in the left ventricular free wall. For information on construction and calibration, see the Materials and Methods section.

Noradrenaline measurements
Noradrenaline concentration was measured in one-minute control samples collected from the coronary venous effluent before addition of glibenclamide, in the last minute before ischemia in all hearts, and in the first 100 ml of coronary venous effluent after reperfusion. Reduced gluthatione (200 μl) was immediately added to the catecholamine samples as antioxidant and the samples were put on ice. Noradrenaline content was measured using a Radio Immune Assay Technique (Wilde et al. 1988). Total release of noradrenaline after reperfusion was corrected for basal release rate to obtain net ischemia-induced noradrenaline release.
**Table 1. Baseline characteristics and drug effects**

<table>
<thead>
<tr>
<th></th>
<th>Coronary flow (ml/min)</th>
<th>QT-interval (msec)</th>
<th>Noradrenaline release (pmol/min/gr dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control (n=19):</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline (t=0 min)</td>
<td>45.8 ± 2.1</td>
<td>161.5 ± 1.8</td>
<td>-</td>
</tr>
<tr>
<td>prior to ischemia</td>
<td>43.7 ± 1.9</td>
<td>163.1 ± 1.6</td>
<td></td>
</tr>
<tr>
<td><strong>Cromakalim (n=16):</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline (t=0 min)</td>
<td>50.9 ± 2.0</td>
<td>162.7 ± 2.3</td>
<td>-</td>
</tr>
<tr>
<td>prior to ischemia</td>
<td>70.3 ± 2.7*</td>
<td>134.2 ± 4.1*</td>
<td></td>
</tr>
<tr>
<td><strong>Glibenclamide (n=15):</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline (t=0 min)</td>
<td>43.5 ± 4.6</td>
<td>163.6 ± 1.3</td>
<td>9.4 ± 2.4</td>
</tr>
<tr>
<td>prior to ischemia</td>
<td>28.1 ± 4.4*</td>
<td>165.5 ± 1.8</td>
<td>11.1 ± 2.0</td>
</tr>
</tbody>
</table>

Data presented as mean ± SEM; # p<0.0001 vs. baseline; * p<0.01 vs. baseline

**Table 2. Noradrenaline (NA) release (pmol/gr dry weight) measured in reperfusate after global ischemia**

<table>
<thead>
<tr>
<th></th>
<th>10 min ischemia:</th>
<th>15 min ischemia:</th>
<th>20 min ischemia:</th>
<th>30 min ischemia:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control:</strong></td>
<td>34 ± 23</td>
<td>941 ± 278</td>
<td>1460 ± 433</td>
<td>5413 ± 1310</td>
</tr>
<tr>
<td>n=3</td>
<td>n=5</td>
<td>n=6</td>
<td>n=5</td>
<td></td>
</tr>
<tr>
<td><strong>Cromakalim:</strong></td>
<td>-</td>
<td>169 ± 97 #</td>
<td>230 ± 125 #</td>
<td>2703 ± 1195 *</td>
</tr>
<tr>
<td>n=5</td>
<td>n=5</td>
<td>n=5</td>
<td>n=5</td>
<td></td>
</tr>
<tr>
<td><strong>Glibenclamide:</strong></td>
<td>65 ± 18</td>
<td>607 ± 184</td>
<td>1709 ± 501</td>
<td>-</td>
</tr>
<tr>
<td>n=5</td>
<td>n=5</td>
<td>n=5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data presented as mean ± SEM; # p<0.05 vs. control; * p=0.08 vs. control

**Statistics**

Data are presented as mean ± standard error of the mean (SEM), unless otherwise specified. Comparison of means was performed using Student’s t-test. Differences between groups were analyzed using Fisher’s exact test. Analysis of variance (ANOVA) was applied to compare multiple sets of data. Pearson’s correlation coefficient was calculated to test for association between variables. A p-value of <0.05 was considered significant.
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Results

Baseline parameters and drug effects on coronary flow, noradrenaline release and ECG characteristics.

Baseline coronary flow and noradrenaline release and QT-interval duration in control, glibenclamide and cromakalim hearts are shown in Table 1. Perfusion with cromakalim before onset of ischemia resulted in a significant increase in coronary flow (p<0.0001), a significant shortening of the QT-interval on the extracellular electrogram (p<0.01), but had no effect on noradrenaline release. Perfusion with glibenclamide before onset of ischemia significantly reduced coronary flow (p<0.0001) but had no effect on noradrenaline release and QT duration (Table 1).

Potassium accumulation and noradrenaline release during ischemia.

During the first minutes of ischemia, the extracellular potassium concentration ([K+]o) increased to a "plateau" after 8.3 ± 0.5 minutes (mean ± SEM) of ischemia in control hearts and after 7.9 ± 0.4 minutes in cromakalim hearts (p=NS) (Figure 1A). There was no significant difference in "plateau" [K+]o between control and cromakalim hearts, but in glibenclamide treated hearts the "plateau" [K+]o was significantly lower compared to control. However, this difference gradually disappeared with increasing duration of ischemia (Figure 1B). After approximately 20 minutes of ischemia, a second phase of increase in [K+]o started in control hearts, indicating the onset of irreversible damage. Cromakalim prevented this second phase of increase of [K+]o, in accordance with its cardioprotective effect (Figure 1A). Glibenclamide did not have a significant effect on the time of onset of the second rise of [K+]o. However, there was a large variation in [K+]o between 15 and 20 minutes of ischemia in glibenclamide treated hearts (Figure 1B).

No net ischemia-induced noradrenaline release occurred after 10 minutes of ischemia, but after 15, 20 and 30 minutes of ischemia net noradrenaline release markedly increased (Table 2, Figure 2). There were no differences in t_rep between the different treatment and ischemia groups. Pre-treatment with cromakalim significantly reduced noradrenaline release after both 15 minutes (mean 169 ± 97 pmol/gr dry weight vs. control 941 ± 278; p<0.05), and 20 minutes of ischemia (mean 230 ± 125 pmol/gr dry weight vs. control 1460 ± 433; p<0.05) (Table 2, Figure 2). After 30 minutes of ischemia reduction in noradrenaline release was still observed, although not statistically significant compared to control. Treatment with glibenclamide did not affect noradrenaline release during 15 and 20 minutes of ischemia compared to control, neither did it accelerate the onset of noradrenaline release. Therefore, we did not investigate the effects of glibenclamide on hearts with 30 minutes of ischemia and thus no potassium data are available after 20 minutes of ischemia. If the cardioprotective effects of cromakalim are, in least in part,
Figure 1. Extracellular potassium concentration during ischemia in control and cromakalim treated hearts (A), and control and glibenclamide treated hearts (# p<0.05) (B).
due to a reduction in noradrenaline release, then a comparable result may be expected with a beta-adrenoceptor antagonist. Therefore, we assessed the effects of the beta-adrenoceptor antagonist propranolol (1 µM) during 30 minutes of ischemia. Propranolol significantly reduced noradrenaline release at 30 minutes of ischemia compared to control (mean 1373 ± SEM 699 pmol/gr dry weight vs. control 5413 ± 1195; p<0.05). Furthermore, the onset of the second rise in [K⁺]₀ was delayed until 26 minutes of ischemia or later; the mean “plateau” [K⁺]₀ was not significantly different from control (data not shown). None of the propranolol treated hearts showed sustained ventricular tachycardia (VT) or ventricular fibrillation (VF), although nonsustained VT was observed in all of them.

**Ventricular arrhythmias during ischemia and reperfusion**

None of the hearts of either group showed arrhythmias during control perfusion. Ventricular tachycardia (VT) or ventricular fibrillation (VF) was not observed in control and glibenclamide treated hearts during the first 10 minutes of ischemia. In order to analyse overall incidence of ventricular arrhythmias during ischemia, all hearts from the ischemia groups 15, 20 and 30 minutes were pooled. In the inset of Fig. 3, the onset of VT/VF in each heart in the various ischemia groups is represented. In hearts with 15, 20 or 30 minutes of ischemia, sustained VT or VF occurred in 10 out of 13 control hearts (77%); mean time of onset of sustained VT or VF was 16.2 ± SEM 0.7 minutes of ischemia. In cromakalim treated hearts the incidence of VT/VF was 6 out of 14 hearts (43%) (NS vs. to control), but arrhythmias occurred earlier during the ischemic episode (mean onset 12.5 ± 1.6 minutes; p<0.05 vs. control) (Figure 3). In hearts treated with glibenclamide, VT/VF was observed in 6 out of 10 (60%, NS vs. control), with a mean onset after 15.8 ± 0.9 minutes of ischemia (NS vs. control). It is possible that some hearts in the group with 15 minutes ischemia would have also experienced VT/VF if the ischemic period was prolonged. Therefore, the reported incidence may in fact be an underestimation of the true incidence of VT/VF in these hearts. QT-intervals showed a progressive shortening during the first 10 minutes of ischemia in control hearts (Figure 4a). Glibenclamide had no influence on the QT-interval during control perfusion, but attenuated shortening of the QT-interval during ischemia (Table 1). Cromakalim treatment significantly shortened the QT-interval during control perfusion and QT-intervals in cromakalim treated hearts shortened significantly more during ischemia than in control hearts (Figure 4a). Figure 4b compares QT-interval durations in cromakalim treated hearts that exhibited an early onset of VT or VF (i.e. before 15 minutes of ischemia) with cromakalim treated hearts with “late” arrhythmias (after 15 minutes of ischemia) or without arrhythmias. The QT-interval shortened more in cromakalim
Figure 2. Noradrenaline release (pmol/gr dry weight) as a function of duration of ischemia in all hearts (A) and in hearts without VT/VF or with onset of VT/VF after 15 minutes of ischemia ("late" arrhythmias) (B).
treated hearts with "early" arrhythmias, which became significant after 2-3 minutes of ischemia (p<0.05).

**Relation between the occurrence of arrhythmias and NA release during ischemia**

Figure 2b shows noradrenaline release from hearts without arrhythmias or with "late" onset VT/VF (after more than 15 minutes of ischemia). Both in control and in glibenclamide hearts, a substantial amount of noradrenaline was released. In contrast, only limited noradrenaline release was observed in any of the remaining cromakalim treated hearts. In other words, cromakalim greatly reduced noradrenaline release with the exception of those hearts in which it induced "early" onset arrhythmias, whereas control and glibenclamide treated hearts showed progressive noradrenaline release with increasing duration of ischemia, irrespective of the presence of arrhythmias. Of all the cromakalim treated hearts, only three hearts showed substantial noradrenaline release (Figure 2a), which however occurred after long duration (30 minutes) of ischemia. In these three hearts, "early" onset arrhythmias (before 15 minutes of ischemia) occurred and thus these hearts suffered from a long period of arrhythmias. In fact, an earlier onset of arrhythmias (i.e. longer duration of arrhythmias) was associated with a progressively larger noradrenaline release in cromakalim hearts (r=0.92; Figure 5).

**Discussion**

The results described in this study show that the \( K_{ATP} \) channel opener cromakalim reduces myocardial noradrenaline (NA) release during global ischemia. In general, \( K_{ATP} \) channel activation during ischemia postpones the onset of irreversible damage and reduces infarct size (Tan et al. 1993, Yao et al. 1994, Grover et al. 1990, Auchampbach et al. 1993). Accordingly, in this study cromakalim prevented the second rise of extracellular potassium, considered to represent the time of onset of irreversible damage (Cascio et al. 1990). However, the exact mechanism underlying the cardioprotective effects conferred by \( K_{ATP} \) opening is not yet fully understood. The original hypothesis that action potential shortening and decreased calcium influx into the cell causes a more favorable metabolic state, has been proven at least partly incorrect, since cardioprotection was also observed with \( K_{ATP} \) channel openers at doses with negligible effect on action potential duration (Yao et al. 1994). Our present results indicate that \( K_{ATP} \) channel openers strongly affect endogenous myocardial NA release during ischemia. This may provide an additional mechanism to explain cardioprotection by \( K_{ATP} \) channel opening. During ischemia, local noradrenaline (NA) accumulation in the
Figure 4. (A) Change in QT-interval during the first 10 minutes of ischemia. Data are presented as percentages (normalized to QT-interval at start of ischemia; $*p<0.05$, $#p<0.005$, $fp<0.0001$ vs. control); (B) Change in QT-interval duration in cromakalim treated hearts with onset of arrhythmias before 15 minutes of ischemia ("early") compared to cromakalim treated hearts with no arrhythmias or onset after 15 minutes of ischemia ("late"). Data are presented as percentages (normalized to QT-interval at start of ischemia; $p<0.05$).
myocardium occurs. Increased re-uptake of NA into the nerve-ending (uptake-1) by a carrier-mediated, sodium-dependent transport process prevents local NA accumulation during early ischemia (Paton 1976). However, as ischemia progresses, the sodium gradient across the neuronal membrane necessary for neuronal re-uptake gradually collapses, and NA starts to accumulate (Schömig et al. 1984). Local non-exocytotic NA release replaces exocytotic ATP-dependent NA release (Schömig 1990), causing extracellular NA accumulation in the micromolar range (1000-fold increase) (Wilde et al. 1988, Schömig et al. 1985). This release process is independent of extracellular calcium and is counteracted by glucose (Datt et al. 1987). The nerve-ending membrane potential also plays a role; ischemia-induced depolarization may facilitate NA release (Amoroso et al. 1990). Accumulation of NA in the ischemic myocardium is generally considered harmful, causing increased myocardial damage (calcium overload) and increased propensity to ventricular arrhythmias (Culing et al. 1984, Schömig et al. 1990). Therefore, any intervention capable of reducing or preventing NA release during myocardial ischemia is of potential benefit.

The presence of ATP-sensitive potassium (K_{ATP}) channels in the central nervous system, both on pre- and postsynaptic neurons, has been described (Mourre et al. 1990). It has been shown that release of neurotransmitters in the brain can be influenced by neuronal K_{ATP} modulation, both under normoxic and ischemia-like conditions (Amoroso et al. 1990, Zini et al. 1993). Blockade of K_{ATP} increased the release of y-aminobutyric acid (GABA) from the substantia nigra during anoxia, as well as glutamate release from rat hippocampal slices during simulated ischemia (Amoroso et al. 1990, Zini et al. 1993), whereas opening of K_{ATP} channels in these conditions reduced the release of neurotransmitters (Zini et al. 1993, Ye et al. 1997). Opening of neuronal K_{ATP} channels may at least partly prevent the ischemia-related neuronal depolarization and/or postpone the onset of irreversible damage secondary to energy deprivation and consequently delay massive release from the nerve terminal. Extrapolating from the beneficial effects of K_{ATP} channel openers in reducing cerebral injury, they may from a mechanistic point of view be equally effective in myocardial ischemia (Remme and Wilde 1999). Indeed, Oe and colleagues described inhibition of stimulation-evoked NA release by cromakalim in isolated normoxic guinea pig, but not human, atrium (Oe et al. 1999). Our study extends these observations to conditions of myocardial ischemia; cromakalim significantly decreased NA release during global ischemia in the rabbit heart. We have used cromakalim in a known effective concentration which was previously shown by our group to cause K_{ATP} channel opening in the myocardium, resulting in action potential shortening and delay in onset of the second rise in [K^+]_o until more than 30 minutes of ischemia (Wilde 1993). In the present study we investigated whether the cardioprotective effect of cromakalim may (partly) be due to a reduction of NA release in the ischemic
Figure 3. Percentage and mean time of onset of VT/VF. Time of onset is presented as minutes of ischemia (mean ± SEM; # p<0.05 time of onset of VT/VF for cromakalim vs. control). Inset: time of onset (min) of VT/VF in hearts of each ischemia and treatment group.

Figure 5. Noradrenaline release (pmol/gr dry weight) as a function of duration of VT/VF in cromakalim treated hearts. Duration of VT/VF is time (minutes) between onset of VT/VF and start of reprefusion. A longer duration of VT/VF (i.e. earlier onset of VT/VF) is associated with a progressively larger amount of noradrenaline release (r=0.92).
myocardium, in addition to its known effects on $K_{ATP}$ channels in the myocyte. We also analysed whether the $K_{ATP}$ channel blocker glibenclamide, which is widely used by patients with coronary artery disease, either increased or accelerated the onset of NA release during the ischemic episode. Neither of these effects of glibenclamide were observed, which may be explained by the reported loss of efficacy of $K_{ATP}$ channel blockade during ongoing metabolic inhibition (Findlay 1993).

If the cardioprotective effects of cromakalim are, in least in part, due to a reduction in NA release, then a comparable result may be expected with a beta-adrenoceptor antagonist. However, since we observed that propranolol inhibited NA release itself, as was previously reported (Du et al. 1993), it is difficult to interpret its inhibitory effect on the myocyte and compare the outcome to cromakalim. In any case, cromakalim has additional beneficial effects mediated through $K_{ATP}$ channels on the myocyte, which may explain the more pronounced effect on the onset of irreversible damage (i.e. second rise in $[K^+]_o$) compared to propranolol.

Although cardioprotective effects of $K_{ATP}$ openers are well documented, an important drawback is their putative pro-arrhythmic potential (Wilde and Jansc 1994). During early ischemia, ventricular arrhythmias occur in two distinct phases: phase 1a occurs between 2-10 minutes of ischemia and are considered to be caused by re-entry, while phase 1b arrhythmias start after 15-20 minutes and may be related to the onset of irreversible damage (Janse and Wit 1989). Due to enhanced action potential shortening, $K_{ATP}$ openers may promote re-entrant ventricular arrhythmias. In this study, cromakalim indeed significantly shortened the QT-interval, suggesting a shortening of action potential duration, which was associated with significant acceleration of onset of ventricular arrhythmias in comparison with untreated hearts. In addition, QT-interval shortening was more pronounced in hearts with “early” arrhythmias. $K_{ATP}$ channel openers may on the other hand delay or decrease 1b arrhythmias by postponing the onset of irreversible myocardial damage. In fact, although “early” arrhythmias were increased by cromakalim in our study, the incidence of VT/VF between 15-20 minutes was clearly reduced, concomitant with a delayed onset of irreversible damage. Depending on the experimental model used, the effect of $K_{ATP}$ channel openers on either 1a and/or 1b arrhythmias may vary, resulting in different overall arrhythmia incidences. In a previous study by Chi et al. (1993), the effect of pinacidil was investigated in a rabbit heart model of global hypoxia and reoxygenation. The incidence of VF during the 12 minute hypoxic period was 10% in control versus 50% in pinacidil treated hearts. It is conceivable that during a more prolonged period of hypoxia the incidence in control hearts would increase and therefore show a similar pattern compared to our results. Nevertheless, our results show a similar increase in arrhythmias due to cromakalim during the first 12 minutes of ischemia. In another study by Wolleben et
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al.(1989), the high incidence of VF during low-flow ischemia in rat hearts was not decreased by either BRL34915 (cromakalim) or pinacidil, although both drugs shortened the time required for the hearts to develop VF, as shown for cromakalim in our study. Low-flow ischemia induces more heterogeneous ischemia compared to global ischemia and is considered more arrhythmogenic, explaining the higher incidence compared to our results. Nevertheless, in cromakalim treated hearts with “early” arrhythmias, NA release of similar magnitude as control hearts was observed after 30 minutes of ischemia. In these hearts, the beneficial effect of cromakalim regarding NA release was abolished by its electrophysiological effect, causing early arrhythmias and concomitant metabolic stress. In contrast, hearts without accelerated onset of arrhythmias released very little NA even after 30 minutes of ischemia, which is of potential benefit to the metabolically compromised heart.

In conclusion, our results show that opening of ATP-sensitive potassium channels during myocardial ischemia significantly decreases endogenous myocardial noradrenaline release and postpones the onset of irreversible damage. This mechanism contributes to the cardioprotective potential of K\textsubscript{ATP} openers during myocardial ischemia.

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