Effects of the $K_{\text{ATP}}$ Channel Opener Bimakalim on Coronary Blood Flow, Monophasic Action Potential Duration, and Infarct Size in Dogs

Zhenhai Yao, MD, PhD; Garrett J. Gross, PhD

**Background** The major purpose of the present study was to determine the effect of the potassium channel opener bimakalim, administered intracoronary only during the initial 10 minutes of ischemia, on myocardial infarct size in anesthetized dogs. A second aim was to test the possibility that bimakalim mediates its cardioprotective effects by accelerating the rate of myocyte action potential shortening during early ischemia. A third aim was to determine the relative potency of bimakalim to open coronary vascular ATP-regulated potassium ($K_{\text{ATP}}$) channels versus myocyte $K_{\text{ATP}}$ channels.

**Methods and Results** Barbital-anesthetized open-chest dogs were used. In the initial studies, bimakalim (0.1 to 10 $\mu$g/min) was infused into the left anterior descending coronary artery (LAD), and changes in coronary blood flow and monophasic action potential duration (MAPD) were used as indexes of coronary vascular $K_{\text{ATP}}$ channel and myocyte $K_{\text{ATP}}$ channel activity, respectively. In subsequent infarct studies, dogs were subjected to 60 minutes of LAD occlusion followed by 4 hours of reperfusion. Based on preliminary studies, two doses of bimakalim that did not shorten MAPD during nonischemic conditions (0.1 and 0.3 $\mu$g/min) and one that markedly shortened MAPD during nonischemic conditions (3.0 $\mu$g/min) were used in equal volume of vehicle infused into the LAD during the initial 10 minutes of coronary artery occlusion. Transmural myocardial blood flow was measured at 5 and 30 minutes of occlusion by the radioactive microsphere technique, and infarct size was determined at the end of 4 hours of reperfusion by triphenyltetrazolium staining. The monophasic action potential duration at 50% repolarization (MAPD$_{50}$) was measured by an epicardial probe placed in the center of the ischemic area. Bimakalim had an approximately 10-fold greater affinity for the coronary vascular than the myocardial $K_{\text{ATP}}$ channel (ED$_{50}$ coronary, 0.3 $\mu$g/min; ED$_{50}$ myocyte, 3.0 $\mu$g/min). Three doses of bimakalim (0.1, 0.3, and 3.0 $\mu$g/min) all markedly reduced infarct size expressed as percent of the area at risk (12.6±3.3%, 14.5±2.2%, and 14.2±5.3%, respectively, versus 27.2±5.7% in controls) to nearly equal extents. Subsequently, we found that the two higher doses of bimakalim (0.3 and 3.0 $\mu$g/min) markedly accelerated yet the 0.1-$\mu$g/min dose of bimakalim did not significantly affect the ischemia-related shortening of the action potential during the initial 5 minutes of occlusion. In addition, 0.1 and 0.3 $\mu$g/min bimakalim did not increase the incidence of ventricular fibrillation during the 60 minutes of occlusion (0 of 7 and 0 of 8 dogs, respectively), whereas 3.0 $\mu$g/min bimakalim had a proarrhythmic effect (6 of 6) compared with the control group (1 of 8). There were no significant differences between groups in systemic hemodynamics, myocardial oxygen demand, ischemic bed size, or collateral blood flow to the ischemic region.

**Conclusions** The results of the present study clearly reveal that a small dose (0.1 or 0.3 $\mu$g/min) of the $K_{\text{ATP}}$ channel opener bimakalim administered only during the initial 10-minute period of ischemia markedly reduced myocardial infarct size to an extent equal to that of a higher proarrhythmic dose in barbital-anesthetized dogs. These data also suggest that bimakalim and other potassium channel openers may partially exert their cardioprotective effects by accelerating $K_{\text{ATP}}$ channel activation during early ischemia as evidenced by an enhanced rate of ischemic myocyte action potential shortening. However, the results also suggest that other cellular mechanisms may be involved in mediating the cardioprotection produced by a low dose of bimakalim (0.1 $\mu$g/min) because it did not significantly accelerate the shortening of the action potential duration, yet it had an efficacy to reduce myocardial infarct size similar to that of the two higher doses. (Circulation. 1994;89:1769-1775.)

**Key Words** • bimakalim • channels • potassium • action potentials • infarcts • EMD 52692

**ATP-dependent potassium ($K_{\text{ATP}}$) channels were originally discovered by Noma** in isolated membrane patches prepared from guinea pig ventricular myocytes. A number of studies have shown that potassium channel openers protect the ischemic myocardium against ischemia-reperfusion injury. However, studies on the effect of potassium channel openers on myocardial infarct size in vivo have yielded conflicting results. These compounds have been reported to decrease, increase, or have no effect on infarct size in different animal models. Bimakalim (EMD 52692), a derivative of the prototypic potassium channel opener cromakalim, has been demonstrated to produce potent systemic vasodilator effects and, at higher concentrations, to shorten myocyte action potential duration by activation of the $K_{\text{ATP}}$ channel. However, the relative selectivity of this compound for coronary vascular versus myocyte $K_{\text{ATP}}$ channels has not been rigorously examined. Therefore, one purpose of the present study was to determine the relative potency of bimakalim as a coronary vasodilator by opening coronary vascular $K_{\text{ATP}}$ channels versus its effect to shorten the myocardial monophasic action potential (MAP) by opening myocyte $K_{\text{ATP}}$ channels. Another major aim was to compare the effects on infarct size of two small doses of bimakalim that do not appear to open myocardial $K_{\text{ATP}}$ channels during nonischemic...
conditions and a higher dose of bimakalim that opens myocardial K_ATP channels under nonischemic conditions. Because K_ATP channel openers have been reported to open K_ATP channels more readily under conditions that simulate ischemia or hypoxia (ischemia selective)\(^1\) and might be expected to have a greater potency under actual ischemic conditions, the present experiments were designed to test this hypothesis.

Cole et al\(^1\) demonstrated in a buffer-perfused guinea pig right ventricular free wall preparation that activation of K_ATP channels with a resultant shortening of action potential duration preserved myocardial contractile function. Previous results obtained from this laboratory by Yao et al\(^2\) confirmed this finding in an in vivo canine model. Therefore, the effects of three doses of bimakalim on ischemia-induced myocardial MAP shortening were also investigated to test the possibility that bimakalim might exert its effect in reducing infarct size by accelerating the rate of action potential shortening during early ischemia.

Methods

General Preparation of Dogs

Adult mongrel dogs of either sex, weighing 19 to 25 kg, were fasted overnight, anesthetized with the combination of sodium barbital (200 mg/kg IV) and sodium pentobarbital (15 mg/kg IV), and ventilated by a respirator (model 607, Harvard Apparatus) with room air supplemented with 100% oxygen. Atelectasis was prevented by maintaining an expiratory pressure of 5 to 7 cm H\(_2\)O with a trap. Arterial blood pH, Po\(_2\), and PCO\(_2\) were monitored at selected intervals by an automatic blood gas system (AVL 995, AVL Scientific Corp) and maintained within a normal physiological range (pH 7.35 to 7.45; Po\(_2\), 80 to 120 mm Hg; and PCO\(_2\), 25 to 40 mm Hg) by adjusting the respiratory rate and oxygen flow and by intravenous infusion of 1.5% sodium bicarbonate when necessary. Body temperature was maintained at 38 ± 1°C by using a heating pad. Aortic and left ventricular systolic and end-diastolic pressures were measured by using a double pressure transducer-tipped catheter (Millar PC 771) inserted into the aorta and left ventricle via the left carotid artery. Left ventricular dP/dt was determined by electronic differentiation of the left ventricular pressure pulse. The right femoral vein and artery were cannulated for drug administration and for withdrawal of a reference blood flow sample used in the determination of myocardial tissue blood flow.

A left thoracotomy was performed at the fifth intercostal space, the lung was retracted, the pericardium was incised, and the heart was suspended in a cradle. A 1.0- to 1.5-cm segment of the left anterior descending coronary artery (LAD) was dissected from surrounding tissue, distal to the first diagonal branch, and a calibrated electromagnetic flow probe (Statham SP 7515) was placed around the vessel. A flowmeter (Statham 2202) was used to measure coronary blood flow, and a micrometer-driven mechanical occluder was placed distal to the flow probe. The occluder was used to zero the flow probe (LAD was occluded for 10 seconds) 20 minutes before the initial coronary occlusion and later to occlude the artery. If the basal heart rate was less than 150 beats per minute, the heart was paced at that rate with rectangular pulses of 4-millisecond duration and a voltage twice threshold level through bipolar electrodes sutured to the left atrial appendage. Pacing was not used in the few animals with initial rates of more than 150 beats per minute. Heart rate, hemodynamics, and LAD blood flow were monitored and recorded with a polygraph (model 7, Grass Instrument Co) throughout the experiment. The left atrial appendage was cannulated for radioactive microsphere injection. The right femoral vein was cannulated for drug administration. A small catheter with a needle (30 gauge) bent at 90° was inserted into the LAD distal to the flow probe and occluder for intracoronary drug infusions.

Chemicals

Bimakalim (EMD 52692) was generously supplied by Dr Pierre Schelling from E. Merck, Darmstadt, Germany. On the day of the experiments, 0.5 mg bimakalim was dissolved in 0.5 mL polyethylene glycol and then diluted with saline to appropriate concentrations.

Experimental Design

Animals were randomly assigned to one of four groups. In group 1, 0.3 μg/mL bimakalim solution (0.1 to 10 μg/min) was infused into the LAD at rates of 0.33 to 3.3 mL/min, and changes in coronary blood flow and MAP duration were used as indexes of coronary vascular and myocyte K_ATP channel activity, respectively. In four other groups, dogs were infused with either vehicle or 0.1, 0.3, or 3.0 μg/min bimakalim into the LAD at rates of 0.3 to 1.0 mL/min during the initial 10 minutes of occlusion to determine its effects on myocardial infarct size and MAP duration of the ischemic myocardium.

Infarct Size Determination

After 4 hours of reperfusion, the LAD was reoccluded and cannulated just distal to the occlusion site. Subsequently, 10 mL saline and 10 mL Patent blue dye were injected at equal pressures into the LAD and left atrium, respectively, to determine the anatomic area at risk (AAR) and the nonischemic area. The heart was then immediately fibrillated, removed, and sliced into serial transverse sections 6 to 7 mm in width. The nonstained ischemic area was separated from the blue-stained normal area, and the two regions were incubated at 37°C for 20 to 30 minutes in 1% 2,3,5-triphenyltetrazolium chloride (TTC) in 0.1 mol/L phosphate buffer adjusted to pH 7.4. The TTC stains the noninfarcted myocardium brick-red, indicating the presence of a formazin precipitate that results from the reduction of TTC by dehydrogenase enzymes present in viable tissue. After storage overnight in 10% formaldehyde, infarcted and noninfarcted tissues within the AAR were carefully separated and weighed. Infarct size (IS) was expressed as a percent of the AAR (IS/AAR).

Regional Myocardial Blood Flow

The radioactive microsphere technique was used to measure regional myocardial blood flow.\(^13\) Carbonized plastic microspheres (15-μm diameter, New England Nuclear) labeled with \(^{14}C\)Ce, \(^{109}Ru\), or \(^{99m}Nb\) and suspended in isotonic saline with 0.01% Tween 80 added to prevent aggregation were injected into the left atrium at 5 and 30 minutes of coronary artery occlusion, as well as at the end of the 4-hour reperfusion period. At the completion of experiments, regional myocardial blood flow in the subepicardium, midmyocardium, and subendocardium of ischemic and nonischemic areas was determined.

MAP

The epicardial MAP in the ischemic region was measured by a method previously described.\(^12,14\) Before and during the first 5 minutes of ischemia, the MAP was continuously monitored. The duration at 50% repolarization (MAPD\(_{50}\)) was measured immediately before LAD occlusion (baseline value) and at 1, 3, and 5 minutes after the start of occlusion. Shortening of the epicardial MAPD\(_{50}\) was used as an index of myocardial K_ATP channel activity in the ischemic region.

Criteria for Exclusion

To ensure that all animals included in data analysis were healthy and exposed to a similar extent of ischemia, the following criteria were used to exclude unsatisfactory dogs: (1) subendocardial collateral flow of more than 0.15 mL·min\(^{-1}\)·g\(^{-1}\); (2) heart rate of more than 170 beats per minute; and (3) more than
three consecutive attempts required to convert ventricular fibrillation with low-energy DC pulses applied directly to the heart.

Statistical Analysis

All values are expressed as mean±SEM. Differences between groups in hemodynamics, blood pH, myocardial collateral blood flow, and infarct size were compared using a two-factor ANOVA with repeated measures and Fisher’s least significant difference test. The incidence of ventricular fibrillation was analyzed by Fisher’s exact test. Linear regression analysis was also performed to determine the relation between transmural collateral blood flow and infarct size expressed as a percentage of the AAR. Differences in regression lines between groups were compared by ANCOVA. Differences between groups were considered significant if the P<.05.

Results

Effects of Bimakalim on Coronary Blood Flow and MAP

Six dogs were used to study the effects of intracoronary infusion with bimakalim (0.1 to 10 μg/min) on coronary blood flow and MAP duration. Fig 1 demonstrates that bimakalim produced dose-dependent effects on increasing LAD coronary blood flow and shortening myocardial MAP and suggests that bimakalim has an approximately 10-fold greater affinity for coronary vascular than the myocardial βAR channel (ED50 coronary, ~0.3 μg/min; ED50 myocyte, ~3.0 μg/min). Based on these results, infusion of 0.3- and 3.0-μg/min doses were chosen for the following infarct size studies.

Mortality and Exclusions

Thirty-two dogs were used in the infarct size study: 9 in the vehicle control group, 7 in the group treated with 0.1 μg/min bimakalim, and 8 each in groups treated with either 0.3 or 3.0 μg/min bimakalim. Two dogs in the group pretreated with 3.0 μg/min bimakalim and 1 in the vehicle control group were excluded because they did not recover after three attempts to defibrillate with DC countershock. Therefore, data were statistically analyzed for 8 vehicle-treated control dogs, 7 dogs treated with 0.1 μg/min bimakalim, 8 treated with 0.3 μg/min bimakalim, and 6 treated with 3.0 μg/min bimakalim.

Hemodynamics, pH, and Blood Gases

Hemodynamics and coronary blood flow data are summarized in Table 1. Compared with the vehicle-treated control group, bimakalim had no effect on heart rate, mean aortic blood pressure, left ventricular dP/dt, the rate-pressure product, and coronary blood flow at any dose throughout the experiments. Similarly, there were no differences in arterial pH or blood gases between groups (Table 2).

Regional Myocardial Blood Flow

Regional myocardial blood flow data in the ischemic (LAD) and nonischemic (left circumflex) regions at 5 and 30 minutes after occlusion and at 4 hours of reperfusion in the four series are summarized in Table 3. No significant differences were found between groups.

Table 2. Arterial Blood Gases and pH: Values Before Vehicle or Bimakalim

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>P&lt;sub&gt;Co2&lt;/sub&gt;, mm Hg</th>
<th>P&lt;sub&gt;O2&lt;/sub&gt;, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>103±6</td>
<td>36±2</td>
<td>7.42±0.01</td>
</tr>
<tr>
<td>Bimakalim 0.1 μg/min</td>
<td>101±9</td>
<td>30±3</td>
<td>7.40±0.02</td>
</tr>
<tr>
<td>Bimakalim 0.3 μg/min</td>
<td>98±8</td>
<td>34±2</td>
<td>7.43±0.01</td>
</tr>
<tr>
<td>Bimakalim 3.0 μg/min</td>
<td>108±5</td>
<td>32±2</td>
<td>7.42±0.01</td>
</tr>
</tbody>
</table>

Values are given as mean±SEM. There were no significant differences compared with the control.
TABLE 3. Transmural Myocardial Blood Flow (mL·min⁻¹·g⁻¹)

<table>
<thead>
<tr>
<th></th>
<th>Ischemic Region (LAD)</th>
<th>Nonischemic Region (LCx)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5-min Occ</td>
<td>30-min Occ</td>
</tr>
<tr>
<td>Vehicle control</td>
<td>0.07±0.01</td>
<td>0.10±0.02</td>
</tr>
<tr>
<td>Bimakalim 0.1 μg/min</td>
<td>0.09±0.03</td>
<td>0.07±0.02</td>
</tr>
<tr>
<td>Bimakalim 0.3 μg/min</td>
<td>0.08±0.01</td>
<td>0.10±0.01</td>
</tr>
<tr>
<td>Bimakalim 3.0 μg/min</td>
<td>0.10±0.02</td>
<td>0.14±0.05</td>
</tr>
</tbody>
</table>

Values are given as mean±SEM. There were no significant differences between the control and other groups. Occ indicates occlusion; Rep, reperfusion; LAD, left anterior descending coronary artery; and LCx, left circumflex coronary artery.

Myocardial Infarct Size

Left ventricular weight, AAR, and infarct size data are presented in Fig 2. The left ventricular weights and anatomic AAR as a percent of the left ventricle were not significantly different between groups. However, intracoronary infusion with bimakalim (0.1, 0.3, and 3.0 μg/min) produced a marked dose-independent reduction in infarct size expressed as percent of the ischemic area (12.6±3.3%, 14.5±2.2%, and 14.2±5.3% versus 27.2±5.7% in the vehicle control group, respectively; P<.05).

Fig 3 demonstrates the relation between infarct size as a percentage of the AAR (IF/AAR) and myocardial transmural blood flow of the ischemic region at 5 minutes of occlusion. An inverse relation was found between these two parameters, and the regression lines for the three bimakalim-treated groups were shifted downward and to the left compared with the control series by ANCOVA (P<.05). These results indicate that at any given transmural collateral flow, infarct size would be expected to be smaller in bimakalim-treated animals.

Incidence of Ventricular Fibrillation

All animals developed arrhythmias during the experiment, and 7 of the 29 dogs progressed to ventricular fibrillation at least once. The incidence of ventricular fibrillation during coronary occlusion was markedly increased (P<.05) in the group treated with 3.0 μg/min bimakalim (6 of 6) compared with that in the vehicle-treated group (1 of 8). There were no significant differences between the groups treated with either 0.1 or 0.3 μg/min bimakalim (0 of 7 and 0 of 8, respectively) and the vehicle-treated controls (1 of 8). In addition, there were no differences in the rate of ventricular fibrillation during reperfusion among the control group (6 of 8) and groups receiving 0.1 μg/min (4 of 7), 0.3 μg/min (4 of 8), or 3 μg/min (3 of 6) bimakalim.

MAP

MAP duration (MAPD₉₀) in the ischemic area was continuously monitored before and during the initial 5 minutes of occlusion (readings taken at 1, 3, and 5 minutes after the start of occlusion). Intracoronary infusion with 0.3 or 3.0 μg/min bimakalim markedly accelerated the rate of action potential shortening during the initial 5 minutes of the ischemic period to nearly equal extents. In contrast, infusion with 0.1 μg/min bimakalim did not significantly affect the ischemia-induced action potential shortening (Fig 4).
Discussion

Preliminary results showed that the novel K<sub>ATP</sub> channel opener bimakalim (0.1 to 10 µg/min by intracoronary infusion) dose-dependently increased coronary blood flow and shortened myocyte MAP duration (ED<sub>50</sub> coronary, =0.3 µg/min; ED<sub>50</sub> myocyte, =3.0 µg/min). Changes in coronary blood flow and MAP duration were used as indexes of coronary vascular and myocyte K<sub>ATP</sub> channel activity, respectively. These findings suggest that in vivo bimakalim possesses a 10-fold greater affinity for the coronary vascular than the myocyte K<sub>ATP</sub> channel. Based on these data, infusion doses of 0.1, 0.3, and 3.0 µg/min bimakalim were chosen for infarct size studies.

Subsequently, we found that (1) intracoronary infusion with three doses of bimakalim (0.1, 0.3, or 3.0 µg/min) for the initial 10 minutes of occlusion markedly reduced myocardial infarct size to similar degrees; (2) the effects of the two higher doses of bimakalim in reducing infarct size were positively related to their effects in accelerating the rate of MAP shortening during the initial brief period of occlusion; (3) although the 0.1-µg/min dose of bimakalim markedly reduced myocardial infarct size to a degree similar to that of the two higher doses, it did not affect ischemia-induced myocardial action potential shortening; and (4) the doses of 0.1 and 0.3 µg/min did not appear to have proarrhythmic effects, whereas the dose of 3.0 µg/min significantly increased the incidence of ventricular fibrillation during the ischemic period, although the three doses were equally efficacious in reducing infarct size.

Previous results obtained from our laboratory demonstrated that intravenous infusion of bimakalim before and throughout the occlusion period limited myocardial infarct size in canine hearts subjected to 90 minutes of coronary artery occlusion followed by reperfusion. However, intravenous administration of bimakalim was found to have marked effects on systemic hemodynamics, which might be a drawback for clinical application. To avoid systemic effects, the present study was designed to determine whether brief exposure of the heart to bimakalim by intracoronary infusion only during the initial 10 minutes of ischemia was sufficient to limit myocardial infarct size.

Surprisingly, all doses (0.1, 0.3, or 3.0 µg/min) of bimakalim administered only during the initial 10 minutes of ischemia markedly reduced infarct size to a similar extent compared with the control group. The reduction produced by the three doses of bimakalim occurred without significant effects on systemic hemody-
namics, coronary blood flow, arterial pH and blood gases, ischemic bed size, or collateral blood flow. These results suggest that bimakalim reduces infarct size through a direct mechanism on the ischemic myocardium during the initial 10 minutes of ischemia and that a small dose (0.3 μg/min) of bimakalim, which did not appear to open the myocyte \( K_{ATP} \) channel under nonischemic conditions, has a marked effect of accelerating the opening of this channel under ischemic conditions. Bimakalim has recently been shown to reduce infarct size in pigs when administered before occlusion but not during the reperfusion period. A number of studies have shown that other potassium openers—nicorandil, \(^{6}\) aprakalim, \(^{9}\) cromakalim, and pinacidil—also markedly limit infarct size. Collectively, these studies suggest that potassium channel openers reduce myocardial infarct size by a direct effect on the myocardium, probably through activating cardiac \( K_{ATP} \) channels. The results of this study further suggest that activation of myocardial \( K_{ATP} \) channels during the initial brief period of ischemia is a critical time for potassium channel openers to exert their beneficial effects against ischemia-reperfusion injury.

In contrast, several studies have yielded negative results concerning the effects of potassium channel openers on infarct size. Sakamoto et al.\(^{6}\) reported that pinacidil resulted in an increase in infarct size in dogs in which a decrease in coronary collateral flow known as “coronary steal” was observed. In one part of this study, \(^6\) pinacidil was administered to dogs with a coronary artery stenosis of the left circumflex coronary artery and total occlusion of the LAD at doses that produced significant hypotension, reflex tachycardia, and a reduction in myocardial blood flow so that any potential beneficial effects of pinacidil may have been masked by an increase in oxygen demand and/or by a decrease in collateral blood flow. Similar negative results with pinacidil, cromakalim, and celakalim have been found by Kitzben et al.\(^8\) In their study, \(^8\) the potassium channel openers were administered intracoronary to avoid systemic effects; however, collateral blood flow was not measured, thus it is difficult to determine if all groups of dogs were exposed to equal degrees of ischemia. Taken together, the present positive results and the previous negative results by others with potassium channel openers suggest that these agents are beneficial when administered directly into the ischemic myocardium (ischemia selective) but may be effective, ineffective, or even detrimental when administered systemically depending on the magnitude of the hemodynamic effects or other confounding factors. Thus, although there are conflicting reports on the effects of potassium channel openers on myocardial infarct size in dogs, it appears as though these agents exert direct cardioprotective actions that result in a reduction in myocardial infarct size if they are administered during or including the initial critical ischemic period and at doses that do not produce adverse hemodynamic effects.

The mechanism by which bimakalim and other potassium channel openers reduce infarct size is not fully understood. In the present study, we found that intracoronary infusion with bimakalim either at a dose of 3.0 μg/min, which markedly shortened action potential duration, or at a dose of 0.3 μg/min, which had no effect on MAP duration under nonischemic conditions, significantly accelerated the rate of MAP shortening of the ischemic myocardium during the initial 5 minutes of occlusion to nearly equal extents. This effect of bimakalim was parallel to its effects on reducing infarct size, which suggested that bimakalim may exert part of its cardioprotective effect to reduce infarct size through accelerating the activation of myocardial \( K_{ATP} \) channels and therefore the rate of MAP shortening during the initial brief period of ischemia. These results are in agreement with those of previous studies in which it has also been shown that \( K_{ATP} \) channel activation with the resultant shortening of MAP protects the myocardium against ischemia-reperfusion injury in vitro and in vivo.\(^{12}\)

It was surprising that 3.0 μg/min bimakalim did not shorten MAPD\(_{50}\) to a greater extent during coronary occlusion than the 0.3-μg/min dose of bimakalim and did not reduce infarct size more effectively. However, Tan et al.\(^{18}\) suggested that there may be a limit to the protection afforded by accelerating action potential shortening via \( K_{ATP} \) channels with ischemia or by \( K_{ATP} \) channel openers. In the present study, it appears that the combination of 0.3 μg/min bimakalim plus ischemia results in the maximal rate of shortening of MAPD\(_{50}\) and protection of the ischemic myocardium, whereas 3.0 μg/min bimakalim produces no more benefit and may actually result in an increased propensity for arrhythmia formation.

Shortening of action potential duration would be expected to attenuate intracellular calcium overload and preserve high-energy phosphates by rapidly decreasing contractile activity.\(^{19}\) Several studies have demonstrated that calcium antagonists exert salutary effects on myocardial ischemia-reperfusion injury.\(^{20,21}\) and provide evidence that intracellular calcium overload may be involved in the pathogenesis of ischemic and/or reperfusion injury. It is known that calcium enters myocytes via \( L \)-type calcium channels, mainly during the plateau phase of the cardiac action potential. A prolongation of action potential duration would be expected to exacerbate intracellular calcium overload, which has been postulated to produce injury of intracellular organelles.\(^{22}\) Thus, preventing or reducing calcium overload of the myocardium subjected to myocardial ischemia may be one important mechanism by which activation of \( K_{ATP} \) channels and a concomitant decrease in MAP duration preserve the myocardium during ischemia.

On the other hand, the findings that the 0.1-μg/min dose of bimakalim did not significantly accelerate the ischemia-induced shortening of action potential duration yet resulted in a reduction in infarct size equal to that observed with the two higher doses clearly suggest that other factors may be involved in the cardioprotective effects of these agents and also suggest that one may be able to dissociate the electrophysiological effects of potassium channel openers from their cardioprotective properties. Recently, Quast\(^{23}\) reviewed data that indicate that potassium channel openers reduce the production of IP\(_3\) and calcium release from the sarcoplasmic reticulum, decrease the sensitivity of the contractile elements to calcium, and reduce calcium influx via voltage-operated calcium channels in isolated porcine coronary arteries. Whether such changes also occur in the myocardium remains to be determined but would provide some alternative mechanisms to help explain
the cardioprotective effect of these compounds in the absence of enhanced action potential shortening.

$K_{\text{ATP}}$ channel activation has been proposed by some investigators to be proarrhythmic and to increase the incidence of ventricular fibrillation. In contrast, $K_{\text{ATP}}$ channel activation by pinacidil has also been reported to be antiarrhythmic. These conflicting results may be attributable to differences in the type of arrhythmias investigated, the conditions in which they occur, and species differences. The present results showed that 3.0 μg/min bimakalim significantly increased the incidence of ventricular fibrillation, whereas 0.1 or 0.3 μg/min bimakalim did not appear to have profibrillatory effects. Therefore, a 30-fold lower dose of bimakalim, which may lead to local concentrations during intracoronary infusion corresponding to those obtained in plasma after the intravenous application of bimakalim in therapeutic doses, was equipotent with the high, probably toxic dose in reducing infarct size but showed no arrhythmogenic tendency and no shortening of the action potential. These results indicate that therapeutic doses of bimakalim or perhaps other $K_{\text{ATP}}$ channel openers may be potential candidates for clinical trials, possibly on patients subjected to short periods of ischemia such as angioplasty.

In conclusion, the results of the present study clearly reveal that bimakalim administered only briefly during the initial period of ischemia markedly reduces myocardial infarct size in barbital-anesthetized dogs and suggest that bimakalim and other potassium channel openers may at least partially exert their cardioprotective effects in reducing infarct size by accelerating $K_{\text{ATP}}$ channel activation and the rate of ischemic myocyte action potential shortening. That the lowest dose of bimakalim reduced infarct size to a similar extent as two higher doses without accelerating the shortening of the action potential suggests that other mechanisms may also be involved in the cardioprotective actions of potassium channel openers. Because low doses of bimakalim reduced infarct size in the absence of proarrhythmic effects, brief exposure of the ischemic myocardium to a small dose of a potassium channel opener such as bimakalim may be a useful new approach for the treatment of certain clinical situations where acute myocardial ischemia is present.

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