Extracellular Mg$^{++}$ Manipulation Prevents the Proarrhythmic Activity of Cromakalim in Ischemic/Reperfused Diabetic Hearts

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ABSTRACT

Cromakalim, an adenosine triphosphate-sensitive potassium channel opener, shows proarrhythmic activity at moderate doses (1–10 μmol/liter) in the ischemic and reperfused myocardium. We studied the effects of extracellular Mg$^{++}$ ([Mg$^{++}$]) on the incidence of reperfusion-induced ventricular fibrillation and ventricular tachycardia in isolated working hearts (n = 12 in each group) subjected to 20 min of global ischemia followed by 30 min of reperfusion, a model eliciting a low incidence of reperfusion arrhythmias, obtained from 8-wk streptozotocin-induced diabetic rats. Cromakalim, at a concentration of 3 μmol/liter, perfused 5 min before the induction of ischemia and throughout reperfusion increased the incidence of ventricular fibrillation and ventricular tachycardia from their drug-free diabetic control values of 25 and 42% ([Mg$^{++}$] = 1.2 mmol/liter) to 92% (P < .05) and 100% (P < .05), respectively. Glibenclamide at a concentration of 3 μmol/liter prevented the proarrhythmic activity of cromakalim. Increasing concentration of [Mg$^{++}$], to 2.4, 3.6 and 4.8 mmol/liter in the perfusion buffer, the arrhythmogenic effect of cromakalim was also abolished. Thus, with 2.4, 3.6 and 4.8 mmol/liter of [Mg$^{++}$], perfused before the administration of cromakalim and the onset of ischemia, the incidence of reperfusion-induced ventricular tachycardia was reduced from 92% (in cromakalim treated group) to 67%, 42% (P < .05), and 25% (P < .05), respectively. The incidence of reperfusion-induced ventricular tachycardia showed the same pattern. Elevated [Mg$^{++}$], prevented the cromakalim-induced cellular Na$^{+}$ gain and K$^{+}$ loss, measured by atomic absorption spectrophotometer. [Mg$^{++}$] could prevent the proarrhythmic activity of cromakalim, and the use of cromakalim as an antihypertensive or antiischemic agent may be of particular concern in the population of postischemic diabetic subjects who are known to be at high risk of sudden coronary death.

Animal models (Feuvray et al., 1979; Lopaschuk et al., 1983; Bakth et al., 1986; Liu et al., 1993) provide an opportunity for the detailed study of the multitude of interacting factors contributing to the syndrome of insulin- and noninsulin-dependent diabetes that is not feasible in humans. It is reasonable to expect that studies in chemically induced diabetic animals will lead to a more complete understanding of the etiology, pathogenesis and treatment, and may be applicable to human subjects. Alterations in myocardial metabolism and function as a result of experimentally induced diabetes mellitus has been extensively investigated (Schaffer et al., 1993; Norton et al., 1996). VF has been identified as a leading cause of sudden death during episodes of myocardial ischemia/reperfusion in animal and human subjects. The acute phase cardiac ischemia is associated with a high incidence of life threatening ventricular arrhythmias (Pogwizd and Corr, 1987; Misier et al., 1995), and the subsequent reperfusion can intensify the incidence of ventricular arrhythmias including VF, VT and premature ventricular beats (Soloman et al., 1993; Boissel et al., 1996).

VF was first described by visual observation some 100 yr ago (McWilliam, 1887), and since 1887 the armamentarium of antiarrhythmic drugs has been widely extended to clinical studies. Recently, the use of $K_{ATP}$ channel openers has been introduced for the prevention of ischemia/reperfusion-induced damage (Grover et al., 1995; Mizumura et al., 1995). To date, $K_{ATP}$ channel openers have been extensively studied in hearts obtained from intact animals, and very few data are available in diseased (e.g., diabetic) myocardium (Tosaki et al., 1995). Clinical studies have shown that diabetic patients have been an elevated mortality rate after acute myocardial infarction. Both experimental and clinical evidence indicate that chronic diabetes leads to myocardial dysfunction and...
cardiomyopathy (Jackson et al., 1985; Afzal et al., 1989). K<sub>ATP</sub> channel openers could increase the intracellular potassium loss via the K<sub>ATP</sub> channels during/or after an ischemic period leading to an increased incidence of arrhythmias because the potassium status of the myocardium plays a crucial role in arrhythmogenesis (Coronel et al., 1995). Our previous studies show (Tosaki et al., 1993; Tosaki and Hellegouarch, 1994) that K<sub>ATP</sub> channel blockers protected the myocardium against ischemia/reperfusion-induced damage, and K<sub>ATP</sub> channel openers aggravated postischemic cardiac damage. We hypothesized that the electrophysiological and functional changes, in cromakalim-treated diabetic groups, are related to the cellular potassium status of the myocardium. Therefore, in our work, we studied the role and proarrhythmic effect of cromakalim, a K<sub>ATP</sub> channel opener, and glibenclamide, a K<sub>ATP</sub> channel blocker, in ischemic/reperfused diabetic hearts. Furthermore, we investigated whether the manipulation of extracellular magnesium, a potent antiarrhythmic agent (Schneider et al., 1995; Fiset et al., 1996), could prevent the proarrhythmic activity of cromakalim. To test these hypotheses we used isolated working hearts obtained from 8-wk streptozotocin-induced diabetic rats. The incidence of reperfusion-induced arrhythmias, cardiac function and the maldistribution of cellular ions (Na, K, Ca and Mg) induced by ischemia/reperfusion were studied in drug-treated and drug-free hearts.

**Methods**

**Animals.** Male, Sprague-Dawley (320–350 g body weight, 19-wk-old at the moment streptozotocin injection), streptozotocin-induced diabetic and age-matched nondiabetic control rats were used for all studies. All animals received humane care in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Sciences and published by the National Institute of Health (NIH Publication no. 85-23, revised 1985).

**Induction of diabetes.** Diabetes was induced by an i.v. injection (tail vein) of streptozotocin (55 mg/kg) dissolved in 0.1 M citrate buffer (pH 4.5). Nondiabetic control animals (age-matched controls) received injections with an equivalent volume of the vehicle (citrate buffer) only. All rats were allowed to drink a 10% glucose solution for the first 24 hr after the injection of streptozotocin. Diabetes mellitus was confirmed by the presence of hyperglycemia. Serum thyroxine (T<sub>4</sub>) and serum triiodothyronine (T<sub>3</sub>) were analyzed by radioimmunoassay (Weeke and Orskov, 1975; Gotzsche, 1983). Animals in the diabetic groups were excluded from the study if blood glucose was less than 250 mg/dl. In some animals (approximately 10–15%), despite the streptozotocin injection, diabetes was not developed. Therefore, these animals were excluded from the study and were immediately replaced.

**Isolated working heart preparation.** Rats were anesthetized with i.p. pentobarbital sodium (60 mg/kg body weight) and then given i.v. heparin (500 IU/kg). After thoracotomy, the heart was excised and placed in ice-cold perfusion buffer. Immediately after preparation, the aorta was cannulated, and the heart was perfused according to the Langendorff method (buffer was oxygenated with the mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub> at 37°C) for a 5-min washout period at a constant perfusion pressure equivalent to 100 cm of water (10 kPa). The perfusion medium consisted of a modified Krebs-Henseleit bicarbonate buffer ([millimolar concentration] sodium chloride 118, potassium chloride 4.7, calcium chloride 1.7, sodium bicarbonate 25, potassium biphosphate 0.36, magnesium sulfate 1.2 and glucose 10). After the washout period, the Langendorff preparation was switched to the working mode with a left atrial filling pressure of 1.7 kPa (17 cm H<sub>2</sub>O) and aortic afterload pressure of 10.0 kPa (100 cm H<sub>2</sub>O) as previously described (Tosaki and Hellegouarch, 1994). Aortic flow was measured by an in-line calibrated rotometer. Coronary flow rate was estimated by timed collection of the coronary perfusate that dripped from the heart.

**Induction of ischemia and reperfusion.** After a 10-min aerobic perfusion of the heart, the atrial inflow and aortic outflow lines were clamped at a point close to the origin of the aortic cannula. Reperfusion was initiated by unclamping the atrial inflow and aortic outflow lines. To prevent the myocardium from drying out during normothermic global ischemia, the thermostated glassware (in which hearts were suspended) was covered and the humidity was kept at a constant level (95–98%) and controlled by a hydrometer.

**Measurement of cellular Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> electrolytes in the diabetic and nondiabetic myocardium were measured as described previously (Tosaki et al., 1993). Before ischemia, after ischemia and reperfusion, the hearts were rapidly cooled to 0 to 5°C by submersion in, and perfused for 5 min with, an ice-cold ion-free buffer solution containing 100 mmol/liter of trishydroxy-methyl-amino-methane and 220 mmol/liter of sucrose (pH adjusted to 7.4 with HCl; PO<sub>2</sub> and osmolality were 0.0–4.0 kPa and 300–330 mosmol/g, respectively) to washout ions from the extracellular space (vascular) and to stop, or at least reduce, the activity of membrane enzymes responsible for membrane ion transports. Five minutes of cold washing of the myocardium washes out >90% of the ions from the extracellular space (Pridjian et al., 1987). After the washout, hearts (left and right ventricles) were dried for 48 hr at 100°C and ashed at 550°C for 20 hr. The ash was dissolved in 5 ml of 3 M nitric acid and diluted 10-fold with deionized water. Myocardial Na<sup>+</sup> was measured at a wavelength of 330.3 nm, K<sup>+</sup> was measured at 404.4 nm, Ca<sup>2+</sup> at 422.7 and Mg<sup>2+</sup> at 286.0 nm in air-acetylene flame using an atomic absorption spectrophotometer [Perkin-Elmer (Norwalk, CT) 1100-B]. The washout perfusion method and the method of determination of myocardial or intracellular ion contents have been described previously by different laboratories in normoxic, anoxic and ischemic/reperfused hearts (Alto and Dhalla, 1979; Pridjian et al., 1987). Because a small amount (<10%) of extracellular ions can contaminate the samples after washing out of the extracellular space (Alto and Dhalla, 1979), the data obtained in our study are termed myocardial rather than intracellular ion contents. Different methods (microelectrode, nuclear magnetic resonance, aequorin-loading) have been used to measure myocardial Na<sup>+</sup>, K<sup>+</sup> or Ca<sup>2+</sup> contents, but atomic absorption spectroscopy is the most sensitive and quantitative method available for measuring net Mg<sup>2+</sup> transport (Romani and Scarpa, 1990). Although the microelectrode or aequorin-loading could be more sensitive and specific than atomic absorption spectroscopy (measuring Na<sup>+</sup>, K<sup>+</sup> or Ca<sup>2+</sup>) atomic absorption spectroscopy makes it possible to measure myocardial Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> all from the same sample. The washout method for measurement of ions has been successfully used in experimental eye research (Szabo et al., 1993).

**Indices measured.** Blood samples were obtained from all rats before excision of the heart, serum glucose was measured by a spectrophotometer at a wavelength of 340 nm using standard assay kits (Sigma Chemical Co., St. Louis, MO), T<sub>4</sub> and T<sub>3</sub> were measured by radioimmunoassay. An epicardial ECG was recorded by a polygraph throughout the experimental period by two silver electrodes attached directly to the myocardium. The ECGs were analyzed to determine the incidence of VF and VT. After 3 min of VF (sustained VF), if there was any, hearts were defibrillated and myocardial function was recorded. The heart was considered to be in VF if an irregular undulating baseline was apparent on the ECG. VT was defined as five or more consecutive premature ventricular complexes, and this classification included repetitive monomorphic VT which is difficult to dissociate from rapid VT. Before ischemia and during reperfusion, HR, CF and AF rates were registered. LVPD, which was defined as the difference between left ventricular systolic
and end-diastolic pressure, and the LVdp/dt, were also recorded by the insert of a Millar catheter (Millar Instruments, Houston, TX) into the left ventricle via the left atria and mitral valve. In additional studies, hearts were blotted dry and weighed. The ratio between the heart weight and body weight was also calculated. The myocardial ion contents were analyzed as described in the previous section.

**Experimental time course.** A basic requirement for our studies was that untreated diabetic hearts exhibit a low vulnerability to reperfusion-induced arrhythmias. This gave a maximum scope for the demonstration of any proarrrhythmic activities of cromakalim, and to study the abolition of its proarrrhythmic effect by glibenclamide, a $K_{ATP}$ channel blocker, and magnesium in diabetic subjects. Previous studies show (Tosaki et al., 1990) a bell-shaped vulnerability characterstic for isolated hearts subjected to various ischemic/reperfusion periods. Because our experiments were designed to examine the proarrrhythmic action of cromakalim, a global normothermic ischemia/reperfusion protocol had to be used that gave a low incidence of reperfusion-induced arrhythmias in the drug-free diabetic group. This was achieved using a 20-min period of normothermic global ischemia followed by 30-min reperfusion.

In the cromakalim-treated group (first protocol), after the washout of blood from the myocardium, the perfusion buffer was switched to a buffer containing various doses of cromakalim. Hearts were perfused for 5 min before the initiation of the ischemic period, and each concentration was maintained throughout the experiment. In additional experiments (second protocol), to study the interaction between cromakalim and glibenclamide on the incidence of reperfusion-induced arrhythmias, 1 or 3 μmol/liter glibenclamide were perfused for 5 min before the administration of cromakalim, and each concentration was maintained through the experiment.

In the third protocol, before the onset of ischemia various extracellular Mg$^{2+}$ concentrations (1.2 as control, 2.4, 3.6 and 4.8 mmol/liter) were perfused for 5 min followed by the perfusion of 3 μmol/liter of cromakalim in the presence of elevated extracellular Mg$^{2+}$ for another 5 min. During reperfusion, various concentrations of extracellular magnesium and cromakalim were coadministered. To keep the osmolality of the perfusion buffer within 290 and 330 msmol/liter, the extracellular Na$^+$ was reduced by 15% in the elevated Mg$^{2+}$-treated groups. This reduction in extracellular Na$^+$ content does not result in a change in the incidence of reperfusion-induced arrhythmias (Tosaki et al., 1989).

**Statistics.** Cardiac function, body weight, heart weight, heart/body weight ratio, $T_4$, $T_3$ and myocardial ion were expressed as the mean ± S.E.M. Two-way analysis of variance was first carried out to test for any differences between the mean values of all groups. If differences were established, the values of the nondiabetic and diabetic groups were compared to those of the drug-treated groups by Dunnett’s test. An analog procedure was followed for distribution of discrete variables such as the incidence of VF and VT. An overall $χ^2$ tests to compare individual groups. A change of $P < .05$ was considered significant.

**Results**

**Arrhythmias in diabetic hearts.** The results demonstrate (fig. 1A) that in rats subjected to 8 wk of diabetes and isolated hearts perfused with 1, 3 and 10 μmol/liter of cromakalim, the incidence of reperfusion-induced VF was increased from its diabetic drug-free control value of 25 to 42% (NS), 92% (P < .05) and 100% (P < .05), respectively. The incidence of reperfusion-induced VT showed the same pattern (fig. 1B).

In additional studies, the perfusion of the heart with 3 μmol/liter of glibenclamide prevented the proarrrhythmic effect of cromakalim (fig. 2). In other words, the difference in the incidence of reperfusion-induced VF (fig. 2A) and VT (fig. 2B) between the diabetic drug-free group and glibenclamide/
The manipulation of extracellular Mg, comparison with the 8-wk diabetic drug-free control group. Contractility (AF, LVDP and LVdp/dt) of the myocardium in vasodilation effect, attenuated the recovery of postischemic sion-induced injury. Cromakalim (table 3), in addition to its reduced resistance of diabetic hearts to ischemia/reperfu-

Table 3 shows the postischemic recovery of cardiac function after 20-min ischemia followed by 30-min reperfusion in age-matched nondiabetic and 8-wk diabetic drug-free control groups (table 2). Magnesium, at concentrations of 3.6 and 4.8 mmol/liter, completely abolished the cromakalim-induced cardiac disfunction (AF, LVDP and LVdp/dt) in diabetics (table 2). An elevation in extracellular Mg did not change significantly the cromakalim-induced vasodilation (table 2).

Table 3 shows the postischemic recovery of cardiac function after 20-min ischemia followed by 30-min reperfusion in age-matched nondiabetic, and hearts subjected to 8-week diabetes. Eight-wk diabetes significantly reduced the recovery of myocardial function upon reperfusion compared to the age-matched nondiabetic control values (table 3), indicating a reduced resistance of diabetic hearts to ischemia/reperfusion-induced injury. Cromakalim (table 3), in addition to its vasodilation effect, attenuated the recovery of postischemic contractility (AF, LVDP and LVdp/dt) of the myocardium in comparison with the 8-wk diabetic drug-free control group. The manipulation in extracellular Mg content prevented the cromakalim-induced cardiac abnormalities in ischemic/reperfused diabetic myocardium (table 3).

Cellular Na⁺, K⁺, Ca⁺⁺ and Mg⁺⁺ in diabetic hearts. The electrophysiological activity of mammalian hearts is largely governed by the activity of ion channels. These in turn regulate transmembrane ion fluxes underlying several components of the membrane current, which are particularly important for production of cellular action potentials. Therefore, it is reasonable to assume that diseases alter either the ionic compositions of the interstitial and intracellular milieu, the characters of the ion channels themselves, or both. Table 4 shows that 8-wk diabetics, before the induction of ischemia, have an increase in myocardial Na⁺ and Ca⁺⁺ contents in comparison with the nondiabetic age-matched controls. Thus, myocardial Na⁺ and Ca⁺⁺ were increased in comparison with their 8-wk age-matched nondiabetic control values (table 4). A loss in myocardial K⁺ content was also observed in diabetic subjects (table 4). Cromakalim treatment further increased myocardial K⁺ loss and Ca⁺⁺ accumulation before the induction of normothermic ischemia in diabetic hearts (table 4). Before the onset of an ischemic period, the coadministration of magnesium with cromakalim resulted in a significant improvement in myocardial Na⁺, K⁺, and Ca⁺⁺ status in diabetics. Myocardial Mg⁺⁺ did not show any significant fluctuation in treated or untreated hearts.

Twenty min of normothermic global ischemia (table 4) increased myocardial Na⁺ accumulation and K⁺ loss in both nondiabetic and 8-wk diabetic hearts. However, after 20-min ischemia, the relative changes in myocardial Na⁺ gain and K⁺ loss in the 8-wk diabetic group and nondiabetic age-matched control group were very similar. The absolute values in myocardial Na⁺ gain and K⁺ loss were higher, as well as before ischemia, in the diabetic group compared to the age-matched nondiabetic group. After 20-min ischemia, myocardial Ca⁺⁺ and Mg⁺⁺ were not significantly changed in comparison with the preischemic values. Cromakalim (table
4), after 20-min ischemia, further increased tissue Na$^{+}$ gain and K$^{-}$ loss in comparison with their diabetic and non-diabetic ischemic control values ($P < .05$). After this 20-min ischemic period, the drug did not increase myocardial Ca$^{2+}$ gain and Mg$^{2+}$ loss in this group in comparison with the diabetic control. However, a recovery of Na$^{+}$ gain and K$^{-}$ loss was noticed in diabetic subjects (Stone et al., 1989). The effects of K$_{ATP}$ channel openers have been extensively investigated in myocardial ischemia/reperfusion (Grover et al., 1990; Gross and Auchampach, 1992; Ferdinandy et al., 1995), and to our knowledge very few data are available to give some additional information about the cardiovascular effects of these agents in disease, e.g., diabetic subjects (Tosaki et al., 1996; Wilde, 1996). The recent explosive growth in the studies of K$_{ATP}$ channel openers in ischemic/reperfused myocardium has led to some rather extravagant claims and some equally optimistic projections as to their impact on the management of cardiovascular diseases. It has been suggested that the shortening of action potential duration is an important mechanism, preventing intracellular Ca$^{2+}$ overload, of arrhythmic agents (Savitt and Feuvray, 1996). K$_{ATP}$ channel openers are able to shorten the action potential duration (Sanguinetti et al., 1988; Findlay et al., 1989; Lathrop et al., 1990; Spinelli et al., 1991), therefore it was believed that these agents may reduce the arrhythmogenic activity of the ischemic/reperfused myocardium. However, clinical evalua-

Discussion

Diabetes is often associated with cardiovascular complications including coronary artery lesions and diabetic cardiomyopathy resulting in an increased risk of myocardial infarction and congestive heart failure (Stone et al., 1989; Norton et al., 1996). Postmortem evaluation of myocardial infarction also appears to be worse in diabetics, who exhibit a higher incidence of congestive cardiac failure and death compared to nondiabetic subjects (Stone et al., 1989). The effects of K$_{ATP}$ channel openers have been extensively investigated in myocardial ischemia/reperfusion (Grover et al., 1990; Gross and Auchampach, 1992; Ferdinandy et al., 1995), and to our knowledge very few data are available to give some additional information about the cardiovascular effects of these agents in disease, e.g., diabetic subjects (Tosaki et al., 1996; Wilde, 1996). The recent explosive growth in the studies of K$_{ATP}$ channel openers in ischemic/reperfused myocardium has led to some rather extravagant claims and some equally optimistic projections as to their impact on the management of cardiovascular diseases. It has been suggested that the shortening of action potential duration is an important mechanism, preventing intracellular Ca$^{2+}$ overload, of arrhythmic agents (Savitt and Feuvray, 1996). K$_{ATP}$ channel openers are able to shorten the action potential duration (Sanguinetti et al., 1988; Findlay et al., 1989; Lathrop et al., 1990; Spinelli et al., 1991), therefore it was believed that these agents may reduce the arrhythmogenic activity of the ischemic/reperfused myocardium. However, clinical evalua-

TABLE 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight (g)</th>
<th>Wet Heart Weight (g)</th>
<th>Heart/Body Weight (mg/g)</th>
<th>Glucose (mg/dl)</th>
<th>$T_4$ (µg/dl)</th>
<th>$T_3$ (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nondiabetic controls</td>
<td>370 ± 7</td>
<td>1.52 ± 0.05</td>
<td>4.11 ± 0.14</td>
<td>128 ± 15</td>
<td>3.4 ± 0.4</td>
<td>0.79 ± 0.11</td>
</tr>
<tr>
<td>8-Wk Diabetics</td>
<td>265 ± 8</td>
<td>0.95 ± 0.08</td>
<td>3.58 ± 0.16</td>
<td>498 ± 25</td>
<td>2.2 ± 0.3</td>
<td>0.28 ± 0.12</td>
</tr>
</tbody>
</table>

$N = 12$ in each group, Mean ± S.E.M., comparisons were made to the non-diabetic age-matched control group.

* $P < .05$.

TABLE 2

<table>
<thead>
<tr>
<th>Function</th>
<th>Nondiabetic</th>
<th>8-Wk Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>CR</td>
</tr>
<tr>
<td>HR</td>
<td>320 ± 7</td>
<td>298 ± 6</td>
</tr>
<tr>
<td>CF</td>
<td>26.9 ± 0.8</td>
<td>26.2 ± 0.8</td>
</tr>
<tr>
<td>AF</td>
<td>49.6 ± 1.5</td>
<td>36.2 ± 1.7</td>
</tr>
<tr>
<td>LVDP</td>
<td>17.8 ± 0.2</td>
<td>15.1 ± 0.4</td>
</tr>
<tr>
<td>LVdp/dt</td>
<td>800 ± 27</td>
<td>682 ± 23</td>
</tr>
</tbody>
</table>

$N = 12$ in each group, mean ± S.E.M. Heart rate (HR, beats/min); coronary flow (CF, ml/min); aortic flow (AF, ml/min); left ventricular developed pressure (LVDP, kPa); the first derivative of LVDP (LVdp/dt, kPa/s); Cromakalim (CR) 3 µmol/liter; extracellular magnesium (Mg) 3.6 or 4.8 mmol/liter. Note: Extracellular Mg$^{2+}$ was 1.2 mmol/liter in the age-matched nondiabetic, 8-wk diabetic control and 8-wk diabetic plus CR groups.

* * $P < .05$; Comparison were made to the * nondiabetic and the * 8-wk diabetic control groups.

TABLE 3

<table>
<thead>
<tr>
<th>Function</th>
<th>Non-diabetic</th>
<th>8-Wk Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>CR</td>
</tr>
<tr>
<td>HR</td>
<td>291 ± 6</td>
<td>271 ± 7</td>
</tr>
<tr>
<td>CF</td>
<td>25.6 ± 0.6</td>
<td>26.6 ± 0.7</td>
</tr>
<tr>
<td>AF</td>
<td>35.1 ± 0.8</td>
<td>14.9 ± 0.5</td>
</tr>
<tr>
<td>LVDP</td>
<td>15.2 ± 0.3</td>
<td>11.6 ± 0.3</td>
</tr>
<tr>
<td>LVdp/dt</td>
<td>689 ± 20</td>
<td>497 ± 19</td>
</tr>
</tbody>
</table>

$N = 12$ in each group, mean ± S.E.M. Heart rate (HR, beats/min); coronary flow (CF, ml/min); aortic flow (AF, ml/min); left ventricular developed pressure (LVDP, kPa); the first derivative of LVDP (LVdp/dt, kPa/s); Cromakalim (CR) 3 µmol/liter; extracellular magnesium (Mg) 3.6 or 4.8 mmol/liter. Note: Extracellular Mg$^{2+}$ was 1.2 mmol/liter in the age-matched nondiabetic, 8-wk diabetic control and 8-wk diabetic plus CR groups.

* * $P < .05$; Comparison were made to the * nondiabetic and the * 8-wk diabetic control groups.
TABLE 4

<table>
<thead>
<tr>
<th>Group</th>
<th>Non-DIAB</th>
<th>8-Wk DIAB</th>
<th>8-Wk DIAB + CR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before ischemia</td>
<td>Na</td>
<td>K</td>
<td>Ca</td>
</tr>
<tr>
<td></td>
<td>29 ± 3</td>
<td>302 ± 6</td>
<td>302 ± 8</td>
</tr>
<tr>
<td>After 20-min ischemia</td>
<td>Na</td>
<td>K</td>
<td>Ca</td>
</tr>
<tr>
<td></td>
<td>29 ± 3</td>
<td>267 ± 6</td>
<td>267 ± 8</td>
</tr>
<tr>
<td>After 20 min Reperfusion</td>
<td>Na</td>
<td>K</td>
<td>Ca</td>
</tr>
<tr>
<td></td>
<td>35 ± 5</td>
<td>267 ± 6</td>
<td>267 ± 8</td>
</tr>
<tr>
<td>8-Wk DIAB</td>
<td>Mg</td>
<td>Mg</td>
<td>Mg</td>
</tr>
<tr>
<td></td>
<td>43 ± 4a</td>
<td>265 ± 7a</td>
<td>265 ± 9a</td>
</tr>
<tr>
<td>8-Wk DIAB + CR</td>
<td>Mg</td>
<td>Mg</td>
<td>Mg</td>
</tr>
<tr>
<td></td>
<td>44 ± 4a</td>
<td>265 ± 6a</td>
<td>265 ± 9a</td>
</tr>
<tr>
<td>8-Wk DIAB + CR + Mg (6 mM)</td>
<td>Mg</td>
<td>Mg</td>
<td>Mg</td>
</tr>
<tr>
<td></td>
<td>35 ± 4</td>
<td>267 ± 6b</td>
<td>267 ± 8b</td>
</tr>
</tbody>
</table>
| n = 12 in each group. mean ± S.E.M. Comparison were made by the *non-diabetic age-matched control and 8-wk diabetic drug-free groups at fixed time points. Cromakalin (CR), Diabetic (DIAB) **P < 0.05. Anchor 

Today, when so many advances are being made in molecular biology and cell physiology, we tend to lose sight of the potential importance of basic ions (e.g., Na⁺, K⁺, Ca²⁺ and Mg²⁺) in both experimental and clinical medicine. Our study emphasizes the importance of Na⁺, K⁺, Ca²⁺ and Mg²⁺ in the maintenance of ionic balance across the cardiac membrane, because various clinical conditions are frequently complicated by tachyarrhythmias that originate from the ionic imbalance of the myocardium. Our findings provide a basis for inquiry but do not dissociate the different routes and pathways involved in the postischemic ion accumulation or loss in ischemic/reperfused diabetic hearts. Diabetes induces a variety of abnormalities in sarcosomal, ion transport including depression of Na⁺-H⁺ and Na⁺-Ca²⁺ exchanges (Makino et al., 1987; Pierce et al., 1990) and a reduction in the activity of Ca²⁺ and Na⁺-K⁺ ATPase (Yu et al., 1994). It is of interest to note that a HCO₃⁻-dependent mechanism could contribute to intracellular pH recovery and arrhythmogenesis when Na⁺/H⁺ exchange is blocked by amiloride in diabetic ischemic/reperfused hearts (Khandoudi et al., 1995). Although such changes induced by diabetes might be expected to modify the arrhythmogenic consequences of myocardial ischemia and reperfusion, few experimental studies have addressed this issue. For instance, Kusama et al. (1992) observed a reduced susceptibility to ischemia/reperfusion-induced arrhythmias in diabetic hearts, but Beatch and McNeil (1988) found a similar susceptibility to ischemia-induced arrhythmias in diabetic and nondiabetic control rats, which is in contrast to the increased susceptibility in diabetic dogs reported by Bakth et al. (1986). An increase in cell Na⁺ can stimulate the Na⁺-Ca²⁺ exchanger leading to a calcium overloading in the myocardium (Khandoudi et al., 1990). In the diabetic rat heart, sarcolemmal Na⁺/H⁺ exchange (Khandoudi et al., 1990; Pierce et al., 1990) and Na⁺/Ca²⁺ exchange activities are both reduced and these may result in a reduced susceptibility to reperfusion-induced dysfunction. Data from diabetic rat hearts with reduced activity of the Na⁺/H⁺ exchange mechanism or blocking the Na⁺/H⁺ exchange by amiloride provide support for the observation of K⁺ channel openers in patients with essential hypertension suggests therapeutic efficacy of these agents with an incidence of dose-related side effects of edema formation, palpitation, and ventricular tachycardia (Ahnfelt-Ronne, 1988; Goldberg et al., 1988). The increased incidence of VT, after the administration of cromakalin, has been reported by Fox et al. (1991) in healthy volunteers. In addition to the previously mentioned reasons, the K⁺ channel openers as antiischemic agents might be useful tools for the treatment of an ischemic myocardium inasmuch as the dose required for cardiac effects are less, without the manifestation of side effects, to those required for antihypertensive therapy. It is not the intention of our studies to denigrate in any way the current high level of interest or activity in the field of K⁺ channel openers, because both authors of our work have published studies in the area and continue to do so. We emphasize that the treatment of the ischemic myocardium with a K⁺ channel opener is not always beneficial, and may have some detrimental effects especially in the reperfused as opposed to the ischemic period. Thus, thee should be due care in extrapolating from experiments to any actual clinical situation.
for a critical role of this ionic exchange particularly in the initial phase of reperfusion (Khandoudi et al., 1990).

Our purpose, in part, was to evaluate the electrophysiologic characteristics, the proarrhythmic risk of cromakalim, in a model of sudden cardiac death in diabetic subjects. Cromakalim did not exhibit antiarrhythmic activity after 30 min of ischemia followed by reperfusion (Tosaki et al., 1995), although a wide pharmacologic range of interventions reduced the vulnerability of hearts to reperfusion-induced arrhythmias and improved cardiac function in such a model. In experiments in which the duration of ischemia was reduced from 30 to 20 min, the incidence of reperfusion-induced arrhythmias was approximately decreased by 70% in diabetic subjects (from 100% after 30-min ischemia). After 20-min ischemia, cromakalim, in a dose-related manner, significantly increased the incidence of reperfusion-induced arrhythmias up to 100% in diabetic hearts. These results, we believe, are consistent with the known ability of K<sub>ATP</sub> channel openers to enhance potassium efflux. In the presence of a superimposed acute ischemic event followed by reperfusion, cromakalim increased the potential for the development of VF and VT in posts ischemic diabetic hearts. Glibenclamide, a K<sub>ATP</sub> channel blocker, prevented the proarrhythmic effect of cromakalim indicating the role of K<sub>ATP</sub> channels in arrhythmogenesis in the diabetic myocardium. Furthermore, the cromakalim-induced vasodilation could not be considered as an effective therapeutic approach to reduce the incidence of reperfusion-induced arrhythmias because, despite the high coronary flow rate during reperfusion, the incidence of arrhythmias was significantly higher in the cromakalim-treated diabetic group than those of the drug-free diabetic control group. This aggravation in cromakalim-induced arrhythmias in diabetics was reflected in the cardiac function of the myocardium. Although the duration of diabetes on cardiac function was not specifically studied in our investigation, it is of interest to note that (1) a decreased susceptibility to reperfusion-induced arrhythmias was observed in the early stage of diabetes, (2) the duration of ischemia could alter the susceptibility of the diabetic myocardium, (3) and cromakalim does not reduce, but enhances the incidence of reperfusion-induced arrhythmias irrespective of the duration of diabetes or ischemia in both nondiabetic and diabetic subjects in our model (Tosaki et al., 1995; Tosaki et al., 1996).

In our study, we directly measured myocardial Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> contents to test the hypothesis that a K<sub>ATP</sub> channel opener, cromakalim, may increase cellular K<sup>+</sup> efflux from myocardial cells, which could not be beneficial to the diabetic heart after an ischemic episode. Furthermore, we hypothesized that an elevation in extracellular Mg<sup>2+</sup> might antagonize, via the stabilization of cell membranes, the cromakalim-induced cardiac malfunction and ion metabolism. Our results show that cromakalim increased Na<sup>+</sup> gain and K<sup>+</sup> loss in cardiac cells leading to the development of cardiac arrhythmias in our diabetic model. Elevated [Mg<sup>2+</sup>], attenuated the cromakalim-induced ischemia- and reperfusion-induced Na<sup>+</sup> gain and K<sup>+</sup> loss that can be responsible for the regulation of cardiac function and arrhythmias. The net cellular K<sup>+</sup> loss may result from at least four different mechanisms: 1 a decrease in K<sup>+</sup> influx as a result of the reduced Na/K pump activity (Khandoudi et al., 1990), 2 an increase in K<sup>+</sup> efflux as a consequence of intracellular acidosis (Khandoudi et al., 1995), 3 an increase in K<sup>+</sup> efflux through Ca<sup>2+</sup> activated K<sup>+</sup> channels (Coronel et al., 1992; Venkatesh et al., 1992) and 4 an increase in K<sup>+</sup> efflux as a result of the opening of K<sub>ATP</sub> channels (Billman et al., 1993; Friedrichs et al., 1993).

An important finding of our study was that elevated [Mg<sup>2+</sup>], can protect the ischemic/reperfused myocardium against the cromakalim-induced damage in diabetics. Because Mg<sup>2+</sup> is essential for stabilizing cellular K<sup>+</sup> and its transport across cell membranes (Altura and Altura, 1984), reperfusion in the presence of elevated [Mg<sup>2+</sup>], could improve the recovery of myocardial K<sup>+</sup> content, cardiac function and could prevent the cromakalim-induced K<sup>+</sup> loss. Furthermore, low [Mg<sup>2+</sup>], can aggravate tissue K<sup>+</sup> loss and cardiac function (Herzog et al., 1994) possibly by an effect of Mg<sup>2+</sup> on membrane Na-K-ATPase (Borchgrevink et al., 1989). Such an effect may also be important in the antiarrhythmic effects of Mg<sup>2+</sup> in ischemic/reperfused diabetic hearts. Furthermore, low Mg<sup>2+</sup> concentration is likely to enhance Ca<sup>2+</sup>-induced Ca<sup>2+</sup> release from sarcoplasmic reticulum (Fabio and Fabiato, 1975) and to produce an increase in myofibrillar Ca<sup>2+</sup> sensitivity (Donaldson and Kerrick, 1978). Both of these latter processes tend to exacerbate ischemia/reperfusion-induced injury. Our results show that the Mg<sup>2+</sup> effect is most likely to operate by competition with cromakalim preventing the cromakalim-induced K<sup>+</sup> loss in ischemic/reperfused diabetic hearts.

In our studies, hearts were subjected to a period of normothermic global ischemia. Ischemia-induced arrhythmias have been reported to be related to the square root of the occluded zone size in rats in vivo (Johnston et al., 1983). This relationship has been suggested to imply that the transmural interface between the ischemic and nonischemic regions governs arrhythmogenesis in ischemia (Curtis et al., 1987). However, Curtis and Hearse (1989) suggested that an equivalent hypothesis is untenable in the case of reperfusion-induced arrhythmias. The relationship between occluded zone size and reperfusion-induced VF saturated with increasing occluded zone size (Curtis and Hearse, 1989). Thus, 100% incidence of VF was reached with mean occluded zone sizes of 45% of the total ventricular weight. With global ischemia (100% occluded zone), the incidence of reperfusion-induced VF remained at nearly 100% (Curtis and Hearse, 1989). This has important implications for the mechanism of arrhythmogenesis during reperfusion. When regionally ischemic myocardium is reperfused, the nonischemic tissue interfaces with the ischemic-reperfused tissue. However, when globally ischemic tissue is reperfused there is no such interface, because there is no nonischemic tissue. Because susceptibility to reperfusion-induced VF was identical after both regional and global ischemia (Curtis and Hearse, 1989), it follows that an electrophysiological interaction between ischemic-reperfused tissue and nonischemic tissue is not involved in arrhythmogenesis during reperfusion in the rat heart. If such an interaction were important, the incidence of reperfusion-induced VF in hearts subjected to global ischemia and reperfused would have been zero. This finding may be important in those species, e.g., rats, where the collateral circulation is negligible.

In conclusion, to our knowledge, our study is the first attempt to examine the effect of cromakalim in relation to myocardial ion contents and cardiac function in diabetic hearts, but does not differentiate the routes involved in isch-
emic and postischemic ion accumulation or loss. However, in the presence of an acute ischemic/reperfusion event, cromakalim increases the potential for the development of ventricular fibrillation and tachycardia in postischemic diabetic hearts, and this proarrhythmic effect could be antagonized by the elevation of extracellular magnesium. Therefore, the role of KATP channel activators as antihypertensive or cardioprotective agents may be of particular concern in that population of postinfarction diabetic patients who are known to be at high risk of sudden coronary death.

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