Potassium Accumulation in the Globally Ischemic Mammalian Heart

A Role for the ATP-Sensitive Potassium Channel


We investigated the contribution of opening of the ATP-sensitive K+ channel to extracellular accumulation of K+ during ischemia with the use of glibenclamide, a specific blocker of this K+ channel. To characterize the electrophysiological effects of glibenclamide during metabolic inhibition (by either application of dinitrophenol or hypoxia) we performed patch-clamp studies in isolated membrane patches of guinea pig myocytes and in intact guinea pig myocytes and studied action potential parameters in isolated superfused guinea pig papillary muscle. We studied the effect of glibenclamide on extracellular accumulation of K+ and H+ in isolated retrogradely perfused globally ischemic hearts of rat, guinea pig, and rabbit. Experimental evidence is presented that supports the conclusions that glibenclamide 1) effectively blocks open K+ATP channels, 2) reverses the dinitrophenol-induced increase of the outward current and prevents the hypoxia-induced shortening of the action potential, 3) decreases the rate of K+ accumulation during the first minutes of ischemia in stimulated hearts, an effect which was entirely absent in quiescent hearts, and 4) does not influence the rate and extent of ischemia-induced extracellular acidification. (Circulation Research 1990;67:835–843)

Patch-clamp experiments on single ventricular and atrial cells have revealed a new class of K+-selective ion channels that open when the intracellular ATP concentration is abnormally low. Activation of these ATP-sensitive K+ channels (K+ATP channel) is very likely to be responsible for the action potential shortening observed in metabolically depressed cardiac cells whether intracellular ATP depletion is caused by hypoxia or by exposure to metabolic inhibitors. Recent experimental observations with glibenclamide, a specific blocker of K+ATP channels in cardiac cells, further emphasize the role of the channels during metabolic exhaustion.

During early ischemia, the action potential shortens and extracellular K+ accumulates as a result of a net K+ loss from the myocardial cells. This accumulation is important as a cause of slowing of conduction and altered refractoriness leading to reentrant arrhythmias. Several mechanisms to explain K+ accumulation have been proposed, including inhibition of the Na+-K+ pump, which resulted in a reduction of active inward transport of K+, an increased K+ efflux coupled to (pH-dependent?) influx of intracellularly generated anions, and an increase in sarcolemmal K+ conductance by modification of K+ channels (for discussion see References 14–18). In addition, [K+], may be modified by volume changes of intracellular and/or extracellular compartments due to transmembrane movement of water secondary to the production of osmotically active particles in the cell.

So far there is little evidence whether and to what extent extracellular K+ accumulation is associated with opening of K+ATP channels. The present study was addressed to evaluate the role of K+ATP channels in extracellular K+ accumulation during ischemia. Various techniques were employed from the single-channel level to the entire heart, with glibenclamide used as a tool to block K+ATP channels. The findings support the view that opening of K+ATP channels contributes significantly, albeit not exclusively, to extracellular K+ accumulation in the first few minutes of ischemia. A preliminary account of these data has been reported in abstract form.

Materials and Methods

Patch-Clamp Experiments

Ionic currents were recorded from single ventricular guinea pig myocytes in both the inside-out and...
the whole-cell configurations of the patch-clamp technique.\textsuperscript{20} Methods employed to disperse the cells and to record membrane currents were essentially the same as described elsewhere.\textsuperscript{11} In the inside-out configuration, the bath medium (intracellular solution) contained (mM) KCl 127, HEPES 10, KOH 13, EGTA 5, and glucose 11, pH 7.4, whereas the pipette medium (extracellular solution) contained (mM) KCl 140, CaCl\textsubscript{2} 2, MgCl\textsubscript{2} 1, HEPES 10, and glucose 11, pH adjusted with NaOH to 7.4. Single-channel current recordings were carried out at room temperature (19–20°C). In the whole-cell configuration, the extracellular solution contained (mM) NaCl 135, KCl 5.4, MgCl\textsubscript{2} 1.0, CaCl\textsubscript{2} 3, NaH\textsubscript{2}PO\textsubscript{4} 1.5, HEPES 10, glucose 10, and tetrodotoxin 50 \mu M, pH adjusted with NaOH to 7.4, whereas the pipette medium dialyzing the cell interior contained (mM) potassium aspartate 85, KCl 50, sodium pyruvate 5, MgCl\textsubscript{2} 1, EGTA 10, HEPES buffer 10, Mg-ATP 1, and glucose 11, pH adjusted with KOH to 7.4. These experiments were carried out at 34°C. Drugs were directly applied diluted at the desired final concentration in the extracellular (whole-cell configuration) or intracellular (inside-out configuration) solutions by means of a micropressure ejection system (Miniframe-PPS2, Medical System Corp., Greenvale, N.Y.) using multibarreled pipettes pulled with a vertical puller (Narishige U.S.A., Greenvale, N.Y.).

**Action Potential Recordings in Guinea Pig Papillary Muscles**

Isolated papillary muscles removed from guinea pig hearts (right ventricle) were superfused at 36°C with an extracellular solution containing (mM) NaCl 136, KCl 4, MgCl\textsubscript{2} 0.8, CaCl\textsubscript{2} 1.8, NaHCO\textsubscript{3} 24, NaH\textsubscript{2}PO\textsubscript{4} 0.35, and glucose 11.1 and stimulated at a frequency of 1 Hz (through extracellular bipolar electrodes using 1-msec rectangular pulses at two times threshold intensity). In the normoxic situation, the medium was equilibrated with 95% O\textsubscript{2}-5% CO\textsubscript{2} (P\textsubscript{O\textsubscript{2}} >500 mm Hg, pH 7.35) and with 95% N\textsubscript{2}-5% CO\textsubscript{2} (P\textsubscript{O\textsubscript{2}} <50 mm Hg, pH 7.35) during hypoxia. Transmembrane action potentials were recorded with standard microelectrodes filled with 3 M KCl (15–20 M\Omega). After dissection, the preparations were allowed to recover in the normoxic extracellular solution for at least 90 minutes.

**Perfusion Hearts**

**Perfusion protocol.** New Zealand White rabbits, guinea pigs of either sex, and male Wistar rats (average body weights of 2,500, 420, and 220 g, respectively) were anesthetized with 50–60 mg/kg sodium pentobarbital (intravenously in the rabbit and intraperitoneally in the rat and guinea pig), and heparin was administered intravenously. Hearts were rapidly removed and connected to a temperature-controlled (36–37°C), nonrecirculating perfusion system and retrogradely perfused at a perfusion pressure of 50 mm Hg (details are described elsewhere\textsuperscript{21,22}); composition of the perfusate was as follows (mM): NaCl 138, KCl 4.7, CaCl\textsubscript{2} 1.5, MgCl\textsubscript{2} 0.7, NaH\textsubscript{2}PO\textsubscript{4} 0.5, NaHCO\textsubscript{3} 28, and glucose 10, pH 7.4. Rat and guinea pig hearts were stimulated with a frequency of 4 Hz, and rabbit hearts were stimulated at 3.5 Hz throughout the experiment with a bipolar stimulus electrode attached to the right ventricle. Quiescence (0 Hz) of the hearts was obtained by crushing the atrioventricular node and by intraventricular application of phenol in the ventricles during a brief episode of global ischemia (10 seconds).\textsuperscript{21} This procedure caused destruction of a thin endocardial layer, including the conducting system, to a depth, in general, of 60 \mu m as verified by histological examination. K\textsuperscript{+}- and H\textsuperscript{+}-selective electrodes (see below) were inserted midurally in the left ventricular free wall.

All hearts were perfused with or without glibenclamide for a period of 30 minutes, during which in situ calibration of the ion-selective electrodes was performed. Subsequently, each heart was subjected to no more than one period of global ischemia, produced by complete interruption of aortic flow.

In the last minute before the onset of global ischemia, coronary flow was measured, and a sample of effluent was collected for determination of lactate by standard spectrophotometric analysis (kit No. 139084, Boehringer Mannheim B.V., Almere, The Netherlands). Lactate production is expressed as micromoles per minute per gram dry weight.

**Measurement of Extracellular Potassium and pH**

Details of the fabrication of K\textsuperscript{+}-selective electrodes (membranes containing valinomycin) and H\textsuperscript{+}-selective electrodes (membranes containing a proton cocktail) have been described previously.\textsuperscript{24,25} The changes in potential were referred to a silver/silver chloride reference electrode attached to the aortic root. In preliminary experiments in rat and rabbit hearts, no change in the direct current potential occurred during 30 minutes of global ischemia, indicating electrical homogeneity in the preparation. Therefore, local reference electrodes (the presence of which in regional ischemia is obligatory) were omitted. In each experiment two H\textsuperscript{+} and two K\textsuperscript{+} electrodes were used.

Electrodes were calibrated in vitro before and after each experiment and in situ by perfusing the heart for 2 minutes with oxygenated Tyrode’s solution containing an elevated concentration of K\textsuperscript{+} (11.5 mM) and a reduced concentration of HCO\textsubscript{3}\textsuperscript{−} (14 mM). Data from electrodes with an in vitro response of 55–61 mV for a 10-fold change in K\textsuperscript{+} and H\textsuperscript{+} concentration were accepted. A maximal difference of 2 mV between in situ and in vitro calibration was accepted. Data obtained by an electrode couple of one type were averaged, provided both electrodes met the acceptance criteria mentioned above.

**Drugs**

Glibenclamide, purchased from Sigma Chemical Co., St. Louis, was dissolved in NaOH. The pH
was readjusted after adding the sulfonylurea from the stock solution.

**Statistical Analysis**

When appropriate, differences between means were examined using either Student’s t test for unpaired observations (significance at a level of \(p<0.05\)) or nonoverlap of confidence intervals (±2 SEM was considered statistically significant).

**Results**

**Glibenclamide Blocks \(K^+_{\text{ATP}}\) Channels in Isolated Cardiac Myocytes**

In inside-out membrane patches, \(K^+_{\text{ATP}}\) channels were easily distinguished from inward rectifier \(K^+\) channels because of their larger conductance (70 pS versus 22 pS for the inward rectifier in symmetrical 140 mM \(K^+\)) and their high sensitivity to intracellular ATP. The open probability (\(P_{\text{open}}\)) of \(K^+_{\text{ATP}}\) channels was determined over recording periods of 30 seconds in the control ATP-free intracellular solution and in the presence of various concentrations of glibenclamide according to

\[
P_{\text{open}} = \frac{I(N-i)}{N}
\]

where \(I\) is the average channel current calculated as the integrated current divided by the total time of the sample, \(N\) is the total number of channels active in the patch, and \(i\) is the unit amplitude of the single-channel current. This quantitative analysis introduces an error due to the opening of inward rectifier \(K^+\) channels. However, since inward rectifier \(K^+\) channels were not affected by glibenclamide, the error can be considered as a constant during the recording periods analyzed. In 15 inside-out patches containing 4–12 single \(K^+_{\text{ATP}}\) channels, \(P_{\text{open}}\) measured in the absence of ATP immediately after patch excision (to minimize spontaneous rundown of the channel activity), was 0.91±0.05. It was decreased to 0.57±0.03 (\(n=3\)), 57±2 (\(n=3\)), and 16±3 (\(n=4\)) of its control value in the presence of 0.003, 0.03, and 0.3 \(\mu M\) glibenclamide, respectively. In five inside-out patches, channel openings invariably ceased with 3 \(\mu M\) glibenclamide. Figure 1 illustrates such an experiment in a membrane patch maintained at −50 mV, which contained at least four channels as indicated by occasional superposition of channel openings. Blockade of the channels with glibenclamide in inside-out patches was reversible upon washout of the drug. However, the complete recovery of the channel activity was slow and was usually impeded by concomitant spontaneous rundown; 5 minutes after washing out of 0.003, 0.03, 0.3, and 3 \(\mu M\) of the drug, \(P_{\text{open}}\) was only 92±6%, 70±6%, 50±5%, and 54±8%, respectively, of its control value determined before drug application. The action of glibenclamide was also explored in the whole-cell configuration. We have previously reported that glibenclamide lacks effects on both the instantaneous and the delayed rectifier \(K^+\) currents recorded in whole-cell voltage-clamped guinea pig ventricular myocytes. Since, in this configuration, \(K^+_{\text{ATP}}\) channels are normally blocked by the high intracellular ATP concentration, we first applied 2,4-dinitrophenol (DNP), a metabolic poison of the cell, to activate the channels. DNP reversibly produced a large increase in the outward current at voltages more positive than around −60 mV (Figure 2A). However, a marked variability in the effects of DNP was observed from cell to cell particularly concerning 1) the latency between DNP application and the increase in the outward current, 2) the amplitude of the outward current produced by DNP, and 3) the rate of rise of this outward current. Moreover, under our experimental conditions, we did not observe any plateauing of the DNP-induced current before the cell death occurred. Glibenclamide was thus applied before a steady state was reached. When applied soon (i.e., within 2 minutes) after the beginning of the increase in the outward current, 0.3 \(\mu M\) glibenclamide suppressed most of the effects of DNP. Under these experimental conditions, higher concentrations of glibenclamide (e.g., 1–3 \(\mu M\)) entirely reversed the effects of DNP on the outward current (\(n=9\); Figures 2B and 2C). As illustrated in Figure 2B, after glibenclamide was removed from the extracellular solution in the continuous presence of DNP, the outward current increased again, but this occurred after a delay of 3–5 minutes. When applied late (i.e., a few minutes), the increase in the outward current produced by DNP was irreversible, and glibenclamide did not exert any effect. From these data, it is concluded that externally applied glibenclamide (>1 \(\mu M\)) produces a complete blockade of \(K^+_{\text{ATP}}\) channels in guinea pig cardiac myocytes.

**Glibenclamide Prevents but Does Not Reverse the Action Potential Shortening in Hypoxic Papillary Muscles**

In a subsequent series of experiments, we investigated whether glibenclamide would prevent and/or reverse the shortening of the action potential duration produced by hypoxia in guinea pig papillary...
muscle. In six control experiments, 30- and 60-minute hypoxia produced, respectively, a 33.5±3.5% and 55.0±3.0% shortening of the action potential duration at 90% repolarization (APD$_{90}$) under our experimental conditions (Figure 3). Pretreatment of the preparation for 30 minutes with 30 μM glibenclamide did not significantly affect the action potential configuration under normoxic conditions (mean APD$_{90}$ 217.5±11.5 msec in control versus 222.0±9.5 msec with glibenclamide; n=5) but largely prevented the hypoxia-induced shortening (Figure 3B). In the five preparations pretreated with glibenclamide, the action potential duration was shortened by only 4.5±2.5% at 30 minutes and by 10.5±4.0% at 60 minutes of hypoxia (Figure 3C). By contrast, when glibenclamide was added after an initial 30-minute period of hypoxia, it was only found to produce slight effects on action potential duration (not illustrated). From these data obtained in papillary muscles, it is

**Figure 2.** Externally applied 1 μM glibenclamide (Glib) blockade of the outward current evoked by 50 μM of 2,4-dinitrophenol (DNP) in single guinea pig myocytes. Panel A: Current (I)-voltage (V) plot of the steady current recorded in the whole-cell configuration immediately before DNP (○), after 4 minutes with DNP (●), and after 6 minutes of washout (△). Depolarizing pulses, 1 second in duration, were applied every 3 seconds from −80 mV to the indicated voltage. DNP was directly microinjected onto the surface of the cell. Panel B: Amplitude of the outward current measured at the end of 1-second depolarizing pulses elicited from −80 to +40 mV plotted as a function of time. △, Control; * sorts of DNP; □, presence of DNP plus Glib. Panel C: Superimposed corresponding current tracings recorded in control (△), in the presence of DNP (*), and in the presence of DNP plus Glib (□). Tracing is from a cell not shown in panel A. The large increase in the outward current amplitude evoked by DNP was entirely reversed by 1 μM Glib.

**Figure 3.** Tracings and bar graph showing that glibenclamide (Glib) prevents the shortening of the action potential induced by hypoxia in guinea pig papillary muscles. Panel A: Effects of 30- and 60-minute hypoxia on the action potential configuration. Panel B: Effects of 30- and 60-minute hypoxia in another papillary muscle pretreated with Glib. Tracings recorded in the normoxic Tyrode’s solution before (control) and after 30-minute superfusion with 30 μM Glib were nearly identical. The superfusion medium was then switched to the hypoxic external solution for a 60-minute period of time. The continuous presence of Glib during the anoxic period largely improved the recovery of the preparation. Panel C: Action potential duration at 90% repolarization (APD90) measured before (control) and after 30- and 60-minute hypoxia. Values are mean±SEM obtained in six preparations superfused with standard Tyrode’s solution and in five preparations pretreated with 30 μM Glib. **p<0.01 and ***p<0.001 when compared with control values by paired t test.
concluded that glibenclamide largely prevents, but does not effectively reverse, the action potential shortening produced by hypoxia.

**Glibenclamide Partially Prevents Early Extracellular K⁺ Accumulation in Globally Ischemic Beating Hearts**

The left panels of Figure 4 show the extent to which [K⁺]₀ changes during a 30-minute period of global ischemia in the absence (open symbols) and presence (closed symbols) of 3 μM glibenclamide in rat, rabbit, and guinea pig hearts. In the absence of glibenclamide, the change in [K⁺]₀ is typically triphasic. In the presence of glibenclamide, K⁺ accumulation was less, especially during the first minutes of ischemia. Quantitatively this effect was least in the rat heart. The right panels of Figure 4, which show the rate of change of [K⁺]₀, more clearly demonstrate that the effect of glibenclamide on K⁺ accumulation is mainly confined to the first few minutes of ischemia. In this period there is no overlap between confidence intervals (±2 SEM), indicating statistical significance. Note that glibenclamide was capable of abolishing the increase of [K⁺]₀ only partially.

Ischemia-induced opening of K⁺_{ATP} channels per se can only contribute to (increased) K⁺ efflux and extracellular K⁺ accumulation when a driving force for K⁺ movement exists, that is, when the membrane potential deviates significantly from the equilibrium potential of K⁺ (see also Figure 2A). This can be tested by application of glibenclamide to quiescent ischemic cardiac tissue, in which membrane potential is close to the equilibrium potential for K⁺ and, consequently, the drug would have no effect on K⁺ accumulation. This is shown in Figure 5 for quiescent rat and rabbit hearts. In the first minutes, the extent and time course of change of [K⁺]₀ in quiescent hearts, which were respectively less and slower than in stimulated hearts, were completely insensitive to glibenclamide. These results support the view that opening of the K⁺_{ATP} channel is involved in early ischemic K⁺ loss.

Side effects of glibenclamide other than those mediated by blockade of the K⁺_{ATP} channel must be considered as a cause of the observed inhibition of increase of [K⁺]₀ in the intact heart. Results obtained in cardiac tissue with glibenclamide, reported so far, mainly deal with isolated cells and superfused tissue. In one report, tolbutamide, another sulfonylurea compound, was reported to stimulate glycolytic flux in the isolated perfused working rat heart. As shown in Table 1, we found that in all three species glibenclamide stimulated lactate production during normoxic perfusion. Moreover, we observed that the coronary flow rate was significantly decreased. To investigate whether these effects of glibenclamide could have also been responsible for the inhibition of increase of [K⁺]₀ during ischemia described above, we simultaneously measured the change of extracellular pH. We used this as an index of anaerobic glycolysis (i.e., anaerobic energy production). Figure 6 shows for the guinea pig heart that there is no difference in the decrease of pH between glibenclamide-treated and untreated hearts during
global ischemia. Similar results were obtained in rat and rabbit hearts. Therefore, an effect of glibenclamide on energy metabolism during ischemia is not likely. The preischemic reduction of coronary flow is discussed below.

**TABLE 1.** Effect of Glibenclamide (3 mM) on Coronary Flow and Lactate Production Before Ischemia

<table>
<thead>
<tr>
<th>Species</th>
<th>Flow (ml/min)</th>
<th>Lactate (μmol/g dry wt/min)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−Glib</td>
<td>4.8±0.43</td>
<td>1.7±0.15</td>
<td>6</td>
</tr>
<tr>
<td>+Glib</td>
<td>3.0±0.73</td>
<td>3.6±2.68</td>
<td>4</td>
</tr>
<tr>
<td>Rabbit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−Glib</td>
<td>28.7±5.77</td>
<td>1.1±0.42</td>
<td>4</td>
</tr>
<tr>
<td>+Glib</td>
<td>17.4±3.94</td>
<td>9.2±3.41</td>
<td>4</td>
</tr>
<tr>
<td>Guinea pig</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−Glib</td>
<td>17.1±3.42</td>
<td>0.8±0.67</td>
<td>4</td>
</tr>
<tr>
<td>+Glib</td>
<td>10.3±2.68</td>
<td>11.1±4.38</td>
<td>4</td>
</tr>
</tbody>
</table>

−Glib, absence of glibenclamide; +Glib, presence of glibenclamide.

**FIGURE 6.** Graph showing that glibenclamide does not influence the rate of extracellular acidification during ischemia. Change in pH (mean±2 SEM) during global ischemia in the absence (open symbols, n=4) and presence (closed symbols, n=4) of glibenclamide in the guinea pig heart (same hearts as described in Figure 4) is plotted.

**Discussion**

In a recent study, Weiss et al18 discussed the cause of K+ loss during inhibition of glycolysis, hypoxia, and ischemia and the role of lactate anion cotransport in maintenance of electroneutrality. They concluded that during ischemia a component of K+ loss is not related to anion efflux and suggested that an increase in membrane K+ conductance is the most likely cause of this "nonanion-linked K+ efflux." Increased sarcolemmal K+ conductance and consequent action potential shortening has indeed been found during exposure to hypoxia and/or metabolic inhibition in multicellular preparations5 and in single cardiac cells.6,17 In agreement with others,6,13,17 they indicated the possibility that activation of the K+ATP channel may underly the increased sarcolemmal K+ conductance and also the consequent action potential shortening and the related (or consequent?) increase in [K+]o. Evidence in support of this hypothesis is provided by the observation that specific blockade of this channel with sulfonylurea derivatives prevents shortening of the action potential induced by hypoxia12 (Figure 3) and by metabolic inhibition13 (Figures 2B and 2C).

Glibenclamide, a sulfonylurea derivative, is the most potent9 and specific10 blocker of the K+ATP channel known so far. We demonstrate that glibenclamide 1) reversibly prevented channel opening in inside-out membrane patches (Figure 1), 2) abolished the large increase in outward current evoked by DNP in whole-cell experiments (Figure 2), and 3) largely prevented hypoxia-induced shortening of the action potential (Figure 3). In addition, we demonstrate that the presence of glibenclamide influenced the time course of change and reduced the extent of extracellular K+ accumulation during ischemia in stimulated hearts (Figure 4). Therefore, we conclude that activation of the K+ATP channel is involved in the process of K+ loss during ischemia, provided nonspecific actions of glibenclamide could be disregarded (see below). However, we found that glibenclamide reduced K+ accumulation only in stimulated hearts (Figures 4 and 5), that only part of the
K⁺ accumulation could be abolished by the drug, and that this sensitivity to glibenclamide was restricted to the first few minutes of ischemia. Therefore, a quantitatively important component of K⁺ loss is not linked to opening of K⁺ₐ₅ₕ channels.

The net amount of K⁺ released into the extracellular space during ischemia (both in stimulated and in quiescent hearts) is so large that, for reasons of electroneutrality, an almost equal cotransport of anions or countertransport of cations is required (for discussion see References 14 and 18). Consequently, it should be realized that increased K⁺ conductance per se does not necessarily result in increased net K⁺ efflux and increased [K⁺]o. Although opened K⁺ₐ₅ₕ channels may provide the pathway for K⁺ to leave the cell, the associated co- and/or counter-ion movement is an essential part of the overall process of net K⁺ loss.

During ischemia, resting membrane potential in quiescent and stimulated hearts is not far from the potassium equilibrium potential. As a consequence, the electrochemical driving force on K⁺ is small, but the conductance is relatively high. Theoretically, blocking of open K⁺ₐ₅ₕ channels by glibenclamide would decrease membrane K⁺ permeability and thereby increase the difference between the equilibrium potential and membrane potential, and the net flux of K⁺ would be less than proportionate than the fall in membrane K⁺ permeability. Consequently, a role for K⁺ₐ₅ₕ channels in quiescent ischemic hearts cannot be excluded. However, we consider this unlikely because we observed (Figure 2A) that the cord conductance around the resting membrane potential is rather similar in the presence of metabolic inhibition (see also Reference 7) and insensitive to glibenclamide (not shown). The electrochemical driving force on the associated co- (or counter-) ions on the other side is relatively large. Therefore, it might be speculated that during diastole the driving force and permeability of the co- (or counter-) ions drive the process and determine the rate of net K⁺ loss.

In stimulated hearts, K⁺ loss associated with repolarization almost balances the Na⁺ gain associated with depolarization. A reasonable estimate of this quantity is 1.5 nmol/action potential/ml intracellular volume (calculated for an action potential amplitude of 100 mV, a membrane capacitance of 10⁻⁶ F/cm², and a surface/volume ratio of 1,500 cm²/ml). This quantity only depends on repolarization itself but not on action potential duration. If Na⁺/K⁺ pump activity were completely inhibited during ischemia (which in fact may not be so14), this amount would increase [K⁺], in a manner that depends on frequency and extracellular volume. In our “Tyrode”-perfused hearts, in which the intracellular/extracellular volume ratio is about 1 and in which heart rate is 4 Hz, the accumulated K⁺ loss during repolarization would then increase [K⁺], by about 0.6 mmol/l after 5 minutes of ischemia; allowing for sarcolemmal invaginations, this figure may be up to five times larger.

During the action potential plateau phase in normoxic hearts, the electrochemical driving force on K⁺ becomes relatively large, but K⁺ conductance considerably decreases. Activation of the K⁺ₐ₅ₕ channel by metabolic inhibition, hypoxia, or ischemia increases this K⁺ conductance,1 which, even during an action potential of reduced duration, allows a larger electroneutral K⁺ transport. The effects of glibenclamide on action potential duration (Figure 3) during hypoxia and on K⁺ accumulation during ischemia in stimulated hearts (Figure 4) support such an explanation. It may be speculated, however, that the glibenclamide-induced reduction in K⁺ accumulation underestimates the total K⁺ loss via the open K⁺ₐ₅ₕ channel. Because glibenclamide may prevent shortening of action potential duration in early ischemia (as in hypoxia, at relatively later intervals; Figure 3), the time-averaged driving force for K⁺ efflux via K⁺ₐ₅ₕ channels may be increased.

At this point the metabolic requirements for opening of the K⁺ₐ₅ₕ channel must be considered. In isolated patches of guinea pig1 and rat ventricular cells and in intact permeabilized rat ventricular cells,33 the K⁺ₐ₅ₕ channel only activates when the cellular ATP concentration falls below 0.5 mM. During the first 10 minutes of ischemia, however, the cytoplasmic concentration of ATP only slightly decreases (from about 10 to 8 mM). To account for this discrepancy, several explanations have been proposed (see also References 33 and 35). These include 1) possible compartmentation of ATP with local intracellular areas of low concentration, which might cause opening of the channel at an earlier time than that predicted from the overall cellular ATP content and 2) modification of the sensitivity of the channel for ATP by other factors pertinent to acute ischemia, among which the quite dramatic increase of free ADP concentration and the decrease in intracellular pH (as demonstrated in skeletal muscle in cardiac patches, unitary conductance of the channels was not modified by intracellular or extracellular protons (D. Thüringer, unpublished results). Free radical activity probably does not directly affect the ATP sensitivity of the channel in isolated cells.39 Finally, K⁺ₐ₅ₕ channels are prone to a voltage-dependence block by intracellular Mg²⁺ in such a way that the channel is largely inhibited by Mg²⁺ at positive potentials; at more negative potentials, the channel becomes less sensitive to the Mg²⁺ block.40 During ischemia, the importance of this phenomenon is difficult to assess: it may be exacerbated because the free intracellular Mg²⁺ concentration is expected to increase as a result of the release of Mg²⁺ bound to ATP, but on the other hand, it may be limited because the high [K⁺], may be sufficient to relieve the block produced by the intracellular Mg²⁺ concentration.40

Although glibenclamide is a specific blocker of the K⁺ₐ₅ₕ channel, the possible influence of reported side effects must be discussed. First, it has been...
found that sulfonylureas exhibit positive inotropic activity, both on hypoxic⁴¹ (possibly related to blockade of the K⁺<sub>ATP</sub> channel) and normoxic⁴² cardiac tissue. Interventions that are negatively inotropic and provide metabolic protection have been shown to decrease the rate of the [K⁺]<sub>o</sub> accumulation.⁴²,⁴³,⁴⁴ Therefore, it is more likely that the positive inotropic effect of the sulfonylureas would increase the rate of K⁺ release (which is opposite to our observations). Second, sulfonylureas have the property to stimulate glycolysis in normoxic conditions (Table 1). During ischemia this property seems of minor influence, because we measured that glibenclamide did not alter the rate of extracellular acidification (Figure 6), which can be considered an appropriate indicator of anaerobic lactate production. Third, we found, in agreement with others,³² that glibenclamide caused a reduction in coronary flow (Table 1). This could theoretically result in areas of local hypoxia or ischemia before the intervention. The existence of local hypoxia, however, is unlikely, because it can be calculated that, even when the coronary flow rate was reduced by 50%, O₂ supply would still be sufficient to adequately maintain oxidative metabolism.⁴⁶ Also, local preexisting ischemia is not very likely, because we never observed an increase of [K⁺]<sub>i</sub> during perfusion with glibenclamide. Moreover, it has been demonstrated that a period of reduced flow preceding total ischemia does not affect the rate of increase of [K⁺]<sub>i</sub>.²⁵

In regional ischemia, local inhomogeneity in [K⁺]<sub>i</sub>,²⁵ and refractoriness provide a substrate for reentrant arrhythmias.⁴⁷ Also, shortening of the action potential and the refractory period is a factor predisposing to reentry.⁴⁷ The results 1) demonstrate that glibenclamide prevented the hypoxia-induced shortening of the action potential and 2) suggest that glibenclamide might decrease severe inhomogeneity in [K⁺]<sub>i</sub> in the initial phase by reducing the rate of increase in [K⁺]<sub>i</sub>. Therefore, it may be speculated that glibenclamide potentially has antiarrhythmic properties (see References 32 and 48). We cannot exclusively decide on this because in the global ischemic heart, as studied at present, early ischemic arrhythmias (phase 1A arrhythmias) are only rarely observed.⁴⁹

We conclude that opening of K⁺<sub>ATP</sub> channels contributes to the increase of [K⁺]<sub>i</sub>, only during the first 5 minutes of global ischemia and only in hearts, in which during repetitive action potentials membrane potential substantially deviates from the equilibrium potential. The majority of K⁺ eventually released via K⁺<sub>ATP</sub> channels must be electrically compensated for by either anion cotransport or cation countertransport.

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References

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