ATP-Regulated K⁺ Channels Protect the Myocardium Against Ischemia/Reperfusion Damage

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The role of ATP-regulated K⁺ channels in protecting the myocardium against ischemia/reperfusion damage was explored using glibenclamide and pinacidil to block and activate the channels, respectively. Electrical and mechanical activity of arterially perfused guinea pig right ventricular walls was recorded simultaneously via an intracellular microelectrode and a force transducer. The preparations were subjected to either 1) 20 minutes of no-flow ischemia with or without glibenclamide (1 and 10 µM) followed by reperfusion, or 2) 30 minutes of no-flow ischemia with or without pinacidil (1 and 10 µM) followed by reperfusion. No-flow ischemia for 20 minutes produced changes in electrical and mechanical activity that were completely reversed on reperfusion; resting membrane potential declined by 13±1.2 mV, action potential duration at 90% repolarization (APD₉₀) decreased by 62%, and developed tension fell by >95%, but resting tension did not change significantly. Glibenclamide (10 µM) had no effect on activity during normal perfusion, but during ischemia, resting membrane potential fell slightly further (17±1.8 mV) and APD₉₀ declined by only 24%. Developed tension declined more slowly and to a lesser extent, but resting tension rose significantly between 10 and 20 minutes of ischemia. Reperfusion of glibenclamide-treated tissues elicited arrhythmias (extrasystoles and tachycardia), and the preparations failed to recover mechanical function. Glibenclamide at 1 µM produced qualitatively similar effects, albeit less severe. After 30 minutes of no-flow ischemia in untreated tissues, resting tension increased by ~130% during the no-flow period. Reperfusion caused arrhythmias (extrasystoles, tachyarrhythmias, and fibrillation) and failed to restore resting or developed tension to preischemic levels. Pinacidil at 1 µM did not affect electrical or contractile function, but at 10 µM it had a negative inotropic effect, decreasing APD₉₀ and developed tension by 5% and 18%, respectively. Both concentrations of the drug caused a faster and greater decline in APD₉₀ during the no-flow period. Resting tension did not change during 30 minutes of no-flow ischemia in the presence of pinacidil, and reperfusion led to 85% and complete recovery of electrical and mechanical activity at 1 and 10 µM, respectively. The data indicate that glibenclamide enhances whereas pinacidil reduces myocardial damage caused by ischemia/reperfusion. The results are consistent with the hypothesis that activation of ATP-regulated K⁺ channels during ischemia is an important adaptive mechanism for protecting the myocardium when blood flow to the tissue is compromised. (Circulation Research 1991;69:571–581)

It is well known that the plateau phase of the cardiac action potential (AP) shortens markedly and contractions decrease during metabolic depression elicited by ischemia, hypoxia, anoxia, or treatment with metabolic poisons. However, the mechanisms responsible for these functional alterations and their significance to myocardial survival during ischemia remain to be fully understood.

The more rapid repolarization of the AP could be due to 1) a decrease in inward current, 2) an increase in outward current, or 3) a combination of these changes. Voltage-clamp studies imply that the primary alteration in membrane currents during metabolic poisoning, anoxia, and hypoxia is the develop-

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ment of a very large time-independent outward $K^+$ conductance, resulting from the activation of ATP-regulated $K^+$ channels ($I_{\text{KATP}}$). $I_{\text{KATP}}$ was initially observed in excised membrane patches exposed to solutions lacking ATP. The channels are closed at physiological intracellular concentrations of ATP (5–10 mM), but when the concentration is decreased their open probability increases. Recent studies provide evidence that $I_{\text{KATP}}$ is also activated by so-called $K^+$ channel opening drugs such as nicorandil, cromakalim, and pinacidil. Moreover, inhibition of the channels is provided by the antidiabetic sulfonylurea drugs glibenclamide and tolbutamide. $K^+$ channel opening drugs shorten the cardiac AP markedly and cause a negative inotropism that can be reversed with the sulfonylureas.

Noma postulated that $I_{\text{KATP}}$ activation and a fall in AP duration might lead to a preservation of cellular ATP and prevention of irreversible ischemic injury. We reasoned that if $I_{\text{KATP}}$ were important for preservation, then differences in recovery should be evident with varied degrees of channel activation during ischemia. The role of $I_{\text{KATP}}$ in protecting the heart during ischemia was therefore explored in the present study by using glibenclamide to block and pinacidil to enhance channel activation. The effects of these agents on electrical and contractile activity during no-flow ischemia and reperfusion were assessed using arterially perfused right ventricular walls from guinea pigs and conventional microelectrode recording techniques.

**Materials and Methods**

**Preparation**

Guinea pigs (300–350 g) were anesthetized with CO$_2$ and killed by cervical dislocation. Their hearts were quickly removed and placed in a dish containing cold Krebs-Henseleit solution with the following composition (mM): NaCl 120, NaHCO$_3$ 25, KCl 4.8, NaH$_2$PO$_4$ 1.2, MgSO$_4$ 1.2, CaCl$_2$ 1.8, and glucose 11.0 with a pH of 7.4 when gassed with 95% O$_2$–5% CO$_2$. The right ventricular walls were prepared and maintained according to a procedure described by Pierce and coworkers. Briefly, the atria were removed, and the right ventricle was dissected from the heart by cutting along its border with the septum but leaving its attachment to the septum at its base intact. The stump of the aorta was opened by a single cut through its wall into the left ventricle. The aorta and right wall were then cut away from the left ventricle and septum. This dissection procedure leaves the coronary vasculature to the right ventricular wall intact. A fine cannula was placed in the aortic opening of the right coronary artery and sutured in place; the ventricular wall was perfused at a constant flow rate of 1.5 ml/min with 37°C Krebs-Henseleit solution. The dissection procedure causes minimal damage to the right wall, and all regions are well perfused by the coronary vasculature. For this reason, normal action potentials can be recorded even at the very periphery of the tissue, and arrhythmias (extrasystoles, tachycardia, or fibrillation) were never observed under normal control perfusion conditions after an equilibration period of 1–1.5 hours. The base of the wall was pinned to the bottom of a perspex bathing chamber (epicardial surface up), and its apex was attached to a force transducer (Gould Inc., Cleveland, Ohio) for recording mechanical activity. The muscles were stimulated by 2-msec square pulses delivered from a point source at a rate of 2 Hz. Resting tension was then adjusted to a level (generally 250–500 mg) that optimized developed tension.

Initial preliminary experiments demonstrated that 20 minutes of no-flow ischemia was the maximal time that consistently permitted fully reversible alterations in electrical and contractile activity, and a 30-minute no-flow period caused sustained postischemic dysfunction. Reversibility was judged as a complete recovery, perfusion of untreated tissues was stopped for 10, 15, 20, 25, or 30 minutes (two to four preparations at each time). Two series of experiments were conducted using the 20- and 30-minute no-flow periods. After control recordings were obtained, the muscles were either 1) exposed to 20 minutes of no-flow ischemia and 60 minutes of reperfusion or treated with glibenclamide (1–100 μM) (Sigma Chemical Co., St. Louis, Mo.) for 10–30 minutes before 20 minutes of no-flow ischemia and 60 minutes of reperfusion, or 2) exposed to 30 minutes of no-flow ischemia and 60 minutes of reperfusion or treated with pinacidil (1 and 10 μM) (a gift from Eli Lilly and Co., Indianapolis, Ind.) for 15 minutes before 30 minutes of ischemia and 60 minutes of reperfusion (without pinacidil). During no-flow ischemia the bathing chamber was gassed with 95% N$_2$–5% CO$_2$ to minimize O$_2$ reaching the muscle surface. Glibenclamide and pinacidil solutions were freshly prepared each day by dilution from 100 mM stock solutions in polyethylene glycol. This vehicle had no effect on electrical or mechanical activity at the concentrations used.

**Electrical Recording**

Transmembrane potentials were recorded by impaling myocytes with conventional intracellular glass microelectrodes inserted into the right ventricular walls from the epicardial surface. The micropipettes (20–30 MΩ) were pulled from filamented capillary tubes (1.2 mm o.d., World Precision Instruments, New Haven, Conn.) on a Sutter P-87 pipette puller (Sutter Instruments, Novato, Calif.), filled with 3 M KCl, and connected to a WPI Duo 773 electrometer. APs and resting and developed tension were recorded on VHS tape using a Vetter 420 analog recorder (A.R. Vetter Co., Rebersburg, Pa.). Elec-
trical and mechanical signals were digitized and stored on hard disk at a sampling frequency of 2 kHz using a TL-2-40 Labmaster A/D board (Axon Instruments, Foster City, Calif.). Axotape data acquisition software (Axon Instruments), and an IBM AT clone computer. The following parameters of the recorded APs and contractions were determined from the digitized records: RMP, AP amplitude, AP duration at 90% repolarization (APD$_{90}$), maximal rate of rise, resting tension, peak developed tension, and rates of tension development and relaxation. Maximal rates of change in voltage and tension were determined by converting the data from 12-bit digital to single precision floating representation before differentiation. Values for maximal rate of rise obtained using this technique were not different from those determined using a WPI differentiating circuit. Hard copies of the recordings were prepared using a LaserJet Series III printer (Hewlett-Packard Co., Palo Alto, Calif.).

**Results**

The characteristics of electrical activity recorded with intracellular microelectrodes inserted through the epicardial surface of perfused right ventricular walls under normal conditions were similar to those previously reported for other guinea pig cardiac muscle preparations.\textsuperscript{17-19} Control experiments demonstrated that the preparations maintained a consistent level of electrical and mechanical function without any evidence of arrhythmias (extrasystoles, tachycardia, or fibrillation) or contractile decline for more than 8 hours, so the preparations were stable over the course of the present experiments (~2.5–3.0 hours). In addition, because the arterial vasculature was not damaged during dissection, the entire ventricular walls were well perfused and there was no evidence of ischemic dysfunction even at the extreme edges of the preparations.

When perfusion of the ventricular walls was stopped in the absence of any treatment (i.e., control ischemia/reperfusion), several time-dependent changes in electrical and mechanical function were observed during the no-flow ischemic period. These changes were similar to those previously reported to occur in other models of ischemia using guinea pig myocardium.\textsuperscript{17,18} Initially, several experiments that would produce fully reversible effects on electrical and mechanical activity were conducted to determine the maximal no-flow period in this model. Complete recovery was consistently obtained until 20 minutes; thereafter, resting tension increased significantly during ischemia and severe arrhythmias and sustained postischemic contractile dysfunction were observed on reperfusion. A 20-minute no-flow period was used for comparison of control and glibenclamide-treated tissues, and a 30-minute period was used for control versus pinacidil-treated tissues.

Representative simultaneous recordings of electrical and mechanical activity at different times during a single control 20-minute no-flow ischemia/reperfusion experiment are shown in Figure 1. Mechanical movement of the preparations precluded continuous electrical recording from a single cell throughout the experiments. However, in some cases it was possible to maintain an impedance for some of the time points of interest during the experiments. The experiments presented in Figure 1 and subsequent figures below were chosen because the recordings at two or more of the time points were from the same cell (as indicated in the figure descriptions). The alterations in electrical and contractile parameters for several control 20-minute no-flow ischemia experiments are summarized in Figure 2. RMP fell by 7% within 2 minutes after flow to the preparations was stopped, reaching a stable value 10–12 mV less negative than under nonischemic conditions by 5–10 minutes, where it remained for the duration of the ischemic period (Figures 1 and 2). APD$_{90}$ decreased by 18% within the first 2 minutes and continued to decline throughout ischemia. By 20 minutes, the duration was ~35% of that under normal perfusion conditions (Figures 1 and 2). Similarly, the maximal rate of rise
and the amplitude of the APs were also reduced by ischemia (Figure 2); the maximal rate of rise and amplitude declined to $-55\%$ and $-75\%$, respectively, of their preischemic values by 10 minutes. Developed tension declined rapidly on initiation of ischemia, falling by 25\% during the first 2 minutes, and reached a level less than 5\% of nonischemic values by 20 minutes (Figures 1 and 2). Resting tension increased slightly but not significantly over the first 20 minutes of ischemia (Figures 1 and 2). Reperfusion after 20 minutes of control no-flow ischemia completely restored electrical and mechanical function (Figures 1 and 2). Resting tension increased slightly before return to pres ischemic level, and some extrasystoles (i.e., doublets in two of five preparations) occurred during the first 5–10 minutes of reflow, but tachyarrhythmias or fibrillation were not observed.

Glibenclamide had no effect on either electrical or mechanical function in normal, perfused preparations (Figures 2 and 3). In particular, in separate experiments it was demonstrated that at the concentrations used, the drug did not 1) increase AP duration, 2) cause depolarization, or 3) cause any alteration in mechanical function, either during 20 minutes of exposure or after more than 2 hours of washout. Similarly, preparations exposed to the drug at concentrations from 1 to 100 \( \mu \text{M} \) for more than 2 hours under normal perfusion conditions did not show any change in activity. The effects of no-flow ischemia on electrical and mechanical function in the presence of glibenclamide were different from those described above for control conditions. Moreover, the alterations in mechanical function produced by 20 minutes of ischemia in glibenclamide-treated tissues were not reversed on reflow. Figure 3 shows representative data from a single experiment using 10 \( \mu \text{M} \) glibenclamide, and results from several experiments are summarized in Figure 2. The most apparent difference between glibenclamide and control experiments was the failure of AP duration to decline to the same extent as in untreated preparations. Indeed, APD\(_{90}\) fell by only 24\% during the entire 20 minutes of ischemia compared with the 65\% decline.
noted in untreated preparations (Figure 2). A significant difference in AP duration was observed as early as 2 minutes after the start of ischemia (Figure 2). However, the decline in AP duration observed at later times during the no-flow period was not completely blocked by glibenclamide. APD₉₀ still declined by 20±3.8% (n=3) even when 100 μM of the drug was used (data not shown). This suggests that changes in membrane currents other than Iₖ(ATP) are also involved in the decline of AP duration in ischemia (e.g., Ca²⁺ current). However, because APD₉₀ was not significantly different from preischemic values until 5 minutes after the cessation of flow (similar to that noted at lower concentrations), it seems likely that alterations in other membrane currents occur after Iₖ(ATP) is already activated. RMP fell to -73 mV at 2 minutes of ischemia in tissues treated with 10 μM glibenclamide, a level that was not reached in untreated tissues until 10 minutes of ischemia (Figure 2). Moreover, the value reached at 20 minutes was -6 mV less negative than in untreated preparations, suggesting that Iₖ(ATP) may play a role in maintaining RMP during ischemia. The maximal rate of rise also declined to a greater extent, probably as a result of the greater depolarization. Resting tension rose significantly after 10–15 minutes of ischemia, reaching a value ~75% higher than the preischemic level (Figure 2). A similar rise in resting tension was consistently observed during ischemia in untreated preparations only after ~25 minutes of control ischemia (see below). Developed tension fell more slowly during ischemia compared with control experiments and was still greater than 10% of the level under nonischemic conditions (Figure 2). As noted above for AP duration and RMP, there was a significant difference between developed tension in untreated and glibenclamide-treated preparations after just 2 minutes of no-flow ischemia (Figure 2). Glibenclamide at 1 μM (n=4) produced qualitatively similar effects on electrical activity; APD₉₀ decreased by 28±3.5%, RMP fell to -70±1.9 mV, and resting tension rose by 21±4% by 20 minutes of ischemia.

Reperfusion of glibenclamide-treated tissues led to the development of arrhythmias in all preparations studied. These consisted of extra beats, usually doublets, as well as self-limiting episodes of triggered and sustained ventricular tachycardia. In some cases, the bouts of tachycardia lasted more than 8 minutes. Extra beats were observed in all 10 μM glibenclamide–treated preparations even after 30 minutes of reflow. Similar arrhythmias were observed in control experiments only if the ischemia period was continued between 25 and 45 minutes (see below). Tachyarrhythmia occurred in one preparation exposed to 1 μM glibenclamide but was less than 1 minute in duration, and extrasystoles were evident only during the first 30 minutes. The first 5–10 minutes of reflow was also associated with a decline in AP duration compared with that measured at 20 minutes of ischemia after treatment with glibenclamide, a feature not observed in untreated preparations (compare Figures 1 and 3). All electrical parameters had completely recovered by 60 minutes of reperfusion (Figure 2). There was only limited recovery of contractile function in tissues treated with glibenclamide; after 60 minutes of reperfusion, developed tension was only ~35% of that recorded before ischemia, and resting tension did not decline significantly from its ischemic level (Figure 3). At 1 μM glibenclamide (n=4), developed tension remained depressed, recovering only to 82.0±2.5% of its preischemic value, and resting tension remained elevated by 32.3±10.3%. Thus, although electrical activity in glibenclamide-treated tissues was restored, during the reflow period there was a sustained depression of mechanical function that was more severe at 10 μM.

In the second series of experiments, the no-flow period was prolonged to 30 minutes, a period that resulted in sustained postischemic dysfunction in untreated preparations. Representative recordings from a single experiment are shown in Figure 4, and results of several experiments are summarized in Figure 5. There were no differences in activity during the ischemic period beyond those described above for 20 minutes except that resting tension increased significantly between 20 and 30 minutes, reaching a value greater than twice that recorded before ischemia (Figure 5). Reperfusion led to severe arrhythmias in all preparations (tachyarrhythmias predominated) and elicited a further rise in resting tension beyond that observed at the end of the ischemic period (Figures 4 and 5). Self-limiting episodes of tachyarrhythmia were observed until 30–35 minutes of reflow, but extrasystoles were consistently present until 50–55 minutes. AP parameters (measured during nonarrhythmic periods) recovered to preischemic levels by 60 minutes of reflow, but extrasystoles were still observed in several preparations. Mechanical function did not recover during reperfusion; developed tension recovered only by ~40%, and resting tension remained elevated (Figure 5). In three preparations, reflow was continued for an additional 2 hours (total of 3 hours), but no further recovery occurred. Thus, untreated right ventricular walls of guinea pigs exposed to 30 minutes of no-flow ischemia followed by reperfusion showed a sustained postischemic depression of mechanical activity typical of myocardial “stunning.”

The effects of the Iₖ(ATP)-activating drug pinacidil on electrical and mechanical activity were then studied using the 30-minute no-flow ischemia/reperfusion protocol. Preliminary experiments demonstrated that 1) pinacidil above 5 μM consistently produced a fully reversible decrease in AP duration and had a negative inotropic effect in perfused tissues, similar to that described previously for cromakalim14,15; and 2) concentrations of the drug at or above 20 μM completely abolished electrical excitability during ischemia (i.e., RMP remained at ~75 mV and the cells could not be induced to fire APs by increasing stimulus strength). For this reason, concentrations of
1 and 10 \( \mu \text{M} \) pinacidil were chosen for these experiments. Representative recordings from single pinacidil experiments are shown in Figures 6 (1 \( \mu \text{M} \)) and 7 (10 \( \mu \text{M} \)), and data from several experiments are summarized in Figure 5. Pinacidil at 1 \( \mu \text{M} \) had no effect on electrical or contractile parameters (Figure 6), but 10 \( \mu \text{M} \) decreased APD\(_{90}\) by 5\( \pm \)3.4\% and developed tension by 18\( \pm \)4\% under normal perfusion conditions (Figures 5 and 7). There were several differences between the effects of no-flow ischemia/reperfusion in pinacidil compared with untreated preparations. 1) APD\(_{90}\) declined more rapidly during the first 5 minutes of ischemia and fell to a significantly lower value than that observed in untreated preparations (Figures 5–7). 2) Developed tension also declined faster, reaching a level within 2–3 minutes that was not observed in untreated preparations until after more than 10 minutes of ischemia (compare Figures 6 and 7). This enhanced rate of decline in developed tension was greater with 10 \( \mu \text{M} \) pinacidil. 3) There was no change in resting tension during the entire no-flow period compared with the significant increase in untreated tissues (Figures 5–7). However, RMP declined to similar levels during ischemia in pinacidil and untreated tissues (Figure 5).

After reperfusion, pinacidil-treated tissues exhibited enhanced recovery of electrical and mechanical function. Extrasystoles (predominantly doublets) were observed during the first 1–3 minutes of reflow in three of six preparations, but tachyarrhythmias and fibrillation were not observed. Pinacidil at 1 \( \mu \text{M} \) produced similar results except that two preparations had brief (<2 minutes) bouts of tachycardia. At 10 \( \mu \text{M} \) pinacidil there was no change in resting tension during the first 3–5 minutes of reperfusion and complete restoration of both electrical and mechanical function (Figures 5 and 7); at 1 \( \mu \text{M} \) pinacidil there was an average of 85.1\( \pm \)6.7\% recovery of

**Figure 4.** Electrical and mechanical activity of guinea pig right ventricular wall during 30 minutes of ischemia and reperfusion. Representative original recordings of transmembrane voltage and tension generation by ventricular wall in a single experiment; recordings were obtained before ischemia (control), at 2, 3, 5, 20, and 30 minutes of no-flow ischemia, and at 5 and 60 minutes after the start of reflow. The time at which each tracing was obtained is indicated above each panel. The recordings obtained at 2, 3, and 5 minutes of ischemia were from the same cell as the recordings at 20 and 30 minutes of ischemia. Note 1) the rise in resting tension between 20 and 30 minutes of ischemia, 2) the tachyarrhythmia and marked increase in resting tension at 5 minutes of reflow, and 3) the sustained postischemic mechanical dysfunction at 60 minutes of reflow (compare with Figures 6 and 7). The time base axis ticks indicate 50 msec; each panel is 350 msec in duration.

**Figure 5.** Alterations in electrical and mechanical activity in 30 minutes of ischemia and reperfusion with and without pinacidil (10 \( \mu \text{M} \)). Panels A–F: Mean\( \pm \)SEM changes in resting membrane potential (RMP), maximal rate of rise (\( V_{\text{max}} \)), action potential amplitude (AP\(_{\text{amp}}\)), action potential duration at 90\% repolarization (APD\(_{90}\)), resting tension (T\(_{\text{rest}}\)), and developed tension (T\(_{\text{devel}}\)), respectively, in untreated (○; \( n=8 \)) and 10 \( \mu \text{M} \) pinacidil–treated (●; \( n=6 \)) tissues in perfused conditions (C), after pinacidil (P), at 30 minutes of ischemia (I), and at 2, 5, 30, and 60 minutes after the start of reflow. *Significantly different (\( p<0.05 \)) from value measured before ischemia during normal perfusion. *Value for pinacidil-treated tissues was significantly different (\( p<0.05 \)) from that obtained for untreated preparations at the same time point.
developed tension. Thus, pretreatment with pinacidil and enhanced activation of $I_{\text{K}}(\text{ATP})$, during 30 minutes of no-flow ischemia led to a more rapid and greater decline in APD$_{90}$, a more rapid decline in developed tension, and protection of the myocardium against ischemia/reperfusion damage.

**Discussion**

In this study, arterially perfused preparations of guinea pig right ventricles were subjected to either 20 or 30 minutes of no-flow ischemia or in the presence of agents that influence $I_{\text{K}}(\text{ATP})$. The lengths of these no-flow periods were purposely chosen because post-ischemic dysfunction was not observed after 20 minutes but was pronounced after 30 minutes of ischemia in untreated preparations. In contrast, tissues subjected to 20 minutes of no-flow ischemia in the presence of glibenclamide, a potent blocker of $I_{\text{K}}(\text{ATP})$, did not recover function during reperfusion, and those subjected to 30 minutes of ischemia after
exposure to pinacidil, which activates the channels,12,13 recovered completely. Thus, glibenclamide provoked whereas pinacidil prevented ischemia/reperfusion damage. These data indicate that IK(ATP) may play a major role in myocardial recovery during ischemia.

The sulfonylurea drugs such as glibenclamide and tolbutamide apparently interact with a specific binding site or associated protein to block the channel.5 The limited change in AP duration during ischemia noted in glibenclamide-treated tissues in this study may have resulted from channel blockage arising from such a direct interaction. However, these drugs were also reported to affect cardiac metabolism. A stimulation of glycolytic ATP synthesis was reported for both sulfonylureas;20,21 for example, over a range of concentrations similar to those used in the present study, glibenclamide enhanced glycolytic metabolism ~50% in rat hearts but did not elicit a positive inotropic effect or change in oxygen consumption.21 Because IK(ATP) is known to be preferentially suppressed by ATP derived from glycolytic metabolism associated with the plasma membrane,22 inhibition of channel activity could have resulted from an indirect rather than a direct effect of the drug on channel activity. It is unlikely that the depressed postischemic recovery of contractile activity in glibenclamide-treated tissues was due to altered glycolytic metabolism for two reasons. First, enhanced generation of ATP would be expected to improve recovery by contributing to a maintenance of [ATP] during ischemia. Second, pinacidil had an opposite, positive influence on postischemic recovery, yet this drug has not been reported to influence glycolytic metabolism.

That activation of IK(ATP) may be involved in protecting the heart is supported by the present data with glibenclamide and pinacidil and by other studies that show that K+ channel openers reduce ischemia/reperfusion damage.23,24 Experiments using a variety of in vivo and in vitro models of ischemia indicate that there is a critical time window during which reperfusion of ischemic tissue will result in a complete recovery of mechanical function, the length of the window varying somewhat depending on the model of ischemia.25 Reperfusion after this time does not restore contractile activity and generally provokes further dysfunction. In the model of no-flow ischemia used in this study, reversible changes in activity were consistently obtained until 20 minutes, and after 30 minutes all preparations exhibited sustained postischemic dysfunction. Treatment with glibenclamide resulted in dysfunction after 20 minutes of ischemia, whereas exposure to pinacidil enhanced recovery after 30 minutes of ischemia. Thus, in the absence of IK(ATP) activation, the window for reversible ischemia was markedly shortened, and when activation of the channels was enhanced, the window was increased. Several lines of evidence imply that declining [ATP], elevated [Ca2+], and/or decline in pH may provoke contracture and injury during ischemia/reperfusion.25–28 The effects of glibenclamide and pinacidil on the length of the window for reversible ischemia suggest that IK(ATP)-mediated changes in electrical activity may influence the rate of change of cellular alterations important to this injury.

The changes in electrical activity noted after the cessation of flow to the myocardium include 1) a decline in RMP and AP amplitude, 2) a decrease in upstroke velocity, 3) a fall in AP duration, 4) an extracellular accumulation of K+ and positive shift in the K+ equilibrium potential (EK), 5) a greater adherence of RMP to EK, and 6) reduced propagation velocity.1,17 IK(ATP) contributes to some of these changes, with alterations in other membrane currents and/or cell-to-cell electrical coupling contributing to others. For example, this study provides evidence that activation of IK(ATP) contributes to a very large extent to the decline in AP duration during ischemia in guinea pig heart. Marked shortening of the AP has long been known to be a prominent characteristic of the changes in cellular electrical activity during metabolic suppression caused by a variety of causes. The mechanism for this decline has long been a matter of controversy, but it is now apparent that for hypoxic, anoxic, and metabolically poisoned cells, an increase in outward current caused by the activation of IK(ATP) plays a predominant role.3,6,7,29 Consistent with these observations, glibenclamide inhibited the fall in AP duration during ischemia, and pinacidil produced a more rapid and greater decline in APD90 when perfusion was stopped in the present study. It is unlikely that a decrease in L-type Ca2+ channel (ICa) activity accounts for the early changes in AP duration during ischemia, but an effect later during the no-flow period seems likely. Glibenclamide is not known to enhance ICa, so the absence of a change in APD90 in glibenclamide-treated tissues until ~5 minutes of ischemia most probably reflects blockage of IK(ATP) and not activation of an inward current. Moreover, there is only a slight depression of ICa associated with metabolic depression,7 or anoxia,6 and substantial depression of ICa apparently occurs after the activation of IK(ATP).7,29 It was apparent in this study, however, that glibenclamide could not prevent APD90 from declining at later times during ischemia (i.e., after 5 minutes). At the highest concentration used (100 μM) AP duration still declined by 20% at later times of ischemia. It is possible that a decrease in ICa accounts for this glibenclamide-insensitive decline.

Activation of IK(ATP) may be expected to contribute to extracellular K+ accumulation, a shift in EK, as well as maintenance and greater adherence of RMP to EK during ischemia. That IK(ATP) may be involved in maintaining RMP during ischemia is suggested by the greater depolarization observed in glibenclamide compared with untreated preparations. In the absence of activation of IK(ATP), resting K+ conductance through inward rectifier K+ channels (IK) may be insufficient to maintain a well-polarized RMP under no-flow conditions because this current is well known to be inhibited by a fall in pH and [ATP], or a rise in [Ca2+]i,3,30,31 such as those that occur in ischemia.
Activation of outward current through $I_{K_{\text{ATP}}}$ may be required to offset an ischemia-induced decline in $I_K$ and hold RMP close to $E_K$. It might be expected that $I_{K_{\text{ATP}}}$ would hyperpolarize the myocardium beyond the value of RMP observed under normal conditions. However, the accumulation of $K^+$ in the extracellular space under no-flow conditions and consequential positive shift in $E_K$ likely limits the maximal value to which $I_{K_{\text{ATP}}}$ can hyperpolarize the tissue, so depolarization is observed during ischemia despite activation of $I_{K_{\text{ATP}}}$.

The ability of $I_{K_{\text{ATP}}}$ to alter AP duration may be the most important mechanism by which these channels contribute to myocardial preservation during ischemia. The degree of ischemia/reperfusion damage was 1) greater in tissues that were treated with glibenclamide and failed to show marked decline in AP duration, and 2) less in pinacidil-treated tissues in which the AP declined very rapidly when perfusion was stopped. The role played by the $I_{K_{\text{ATP}}}$-induced shift in $E_K$ and maintenance of RMP during ischemia, as mentioned above, may be of lesser importance since pinacidil provided excellent protection against post-ischemic dysfunction, but the magnitude of depolarization during the no-flow period was the same in pinacidil-treated and untreated preparations.

The mechanism by which an $I_{K_{\text{ATP}}}$-induced decline in AP duration protects the myocardium during ischemia remains to be defined, but two possible mechanisms warrant consideration. It may 1) markedly reduce the time for $Ca^{2+}$ influx via voltage-sensitive $Ca^{2+}$ channels, and 2) increase the time during which the $Na^+-Ca^{2+}$ exchanger may operate in forward mode activity to extrude $Ca^{2+}$. Decreased $Ca^{2+}$ influx can be expected to directly improve the ability of cells to maintain appropriate [$Ca^{2+}$] levels by reducing the load on the $Ca^{2+}$ pump and the $Na^+-Ca^{2+}$ exchanger at a time when both of these $Ca^{2+}$ extrusion mechanisms are very likely depressed, the former because of reduced energy supply and the latter because of the marked depolarization that occurs during ischemia as a result of the shift in $E_K$.

The presence of a short AP also means that the cell will spend a greater period of time at membrane potentials negative to the reversal potential of the $Na^+-Ca^{2+}$ exchanger and favorable for $Ca^{2+}$ extrusion via forward mode activity.

A short AP and reduced $Ca^{2+}$ influx may also indirectly reduce the consumption of high energy phosphate stores. This energy would be used in contractile activity and, to a lesser extent, for the maintenance of [$Ca^{2+}$]. Noma$^8$ originally postulated that since contraction would be indirectly depressed by a decline in AP duration, $I_{K_{\text{ATP}}}$ activation would indirectly inhibit a major site of cellular energy consumption and reduce the rate of decline in [ATP] during the no-flow period. It is clear that contractile failure during anoxia$^8$ and metabolic poisoning$^7$ is chiefly the result of activation of $I_{K_{\text{ATP}}}$, $Ca^{2+}$ current, $Ca^{2+}$ release from the sarcoplasmic reticulum, and contractions were demonstrated to be little changed in cyanide- and deoxyglucose-poisoned cells treated with tolbutamide to block $I_{K_{\text{ATP}}}$.$^7$ The rate of decline of contractility during early ischemia was slowed with glibenclamide and enhanced with pinacidil in this study. Although suggestive of a role for $I_{K_{\text{ATP}}}$ in early contractile failure during ischemia, other changes such as a decline in pH, may be important.$^{32}$

According to Noma's$^8$ postulated role for $I_{K_{\text{ATP}}}$ in reducing the rate of decline in ATP$_a$, it would be expected that glibenclamide and $K^+$ channel opener treatment would result in significantly different rates of decline in high energy phosphate levels during ischemia. Evidence for this is conflicting at this time. Kantor et al$^{33}$ reported only a slight difference in ATP levels in rat hearts during ischemia in the presence and absence of glibenclamide (i.e., 1.6±0.3 versus 2.0±0.2 μmol/g tissue, respectively, compared with normoxia levels of 3.6±0.1). However, these measurements were performed after only 10 minutes of global low-flow ischemia. On the other hand, maintenance of ATP levels during ischemia was observed in studies using $K^+$ channel openers; both endocardial ATP and total adenine nucleotides were preserved in ischemic tissue after arterial ligation of dog hearts treated with nicorandil.$^{23}$

Any mechanism important for protecting the myocardium during ischemia would be expected to be activated very early after the cessation of flow to the heart. That an early activation of $I_{K_{\text{ATP}}}$ occurs in ischemia is supported by data obtained in this study. There was a significant difference in AP duration within the first 2 minutes of ischemia in glibenclamide- and pinacidil-treated tissues compared with untreated preparations. An early activation is also suggested by data showing that the enhanced efflux of $K^+$, which begins as early as 15–30 seconds after flow to the myocardium is stopped,$^{17,34}$ can be significantly inhibited by glibenclamide (i.e., 1 and 10 μM$^{33}$ or 30 μM$^{34}$). The factor(s) responsible for a rapid stimulation of $I_{K_{\text{ATP}}}$ activity during early ischemia is unknown. A decline in ATP, to less than 1 mM is generally regarded as required for substantial activation of $I_{K_{\text{ATP}}};$ however, it is extremely unlikely that ATP declined to this level within 2 minutes after the stoppage of flow.$^{25,26,35}$ Nichols and Lederer$^{36}$ argued that since $I_{K_{\text{ATP}}}$ is a relatively large conductance channel, only a very small fraction of the total number of channels need to be activated to account for the amount of outward current required for faster repolarization of the AP. Thus, it might be that only a very small change in ATP, is required to activate sufficient outward current to account for changes in AP duration. Alternatively, ATP might be compartmentalized within the myocyte, and activation of $I_{K_{\text{ATP}}}$ might be dependent on changes in glycolytic metabolism associated with the plasma membrane rather than bulk ATP levels.$^{22}$ It is also possible that the activation of $I_{K_{\text{ATP}}}$ is not solely dependent on [ATP]; there may be additional factors involved, such as ADP or the ATP/ADP ratio, adenosine, G proteins, intracellular cAMP, or catecholamines.$^{13,37,38}$
The sulfonylurea drugs, especially glibenclamide, were recently proposed to be useful drugs for the treatment of cardiac ischemia.5,39,40 Because glibenclamide can restore normal or near normal APs, it was reasoned that the drugs might counteract the arrhythmic activity resulting from the decline in AP duration and refractory period produced by activation of IKATP.39,40 Indeed, glibenclamide and tolbutamide reduced the incidence of ischemic arrhythmias, whereas the K+ channel opener cromakalim and pinacidil provoked arrhythmias in Langendorff-perfused rat heart.39 However, the present results provide a strong argument against the use of sulfonylureas or any other treatment that blocks activation of IKATP during ischemia. It is clear that the antiarrhythmic advantages of such treatments must be weighed against the possible deleterious effects of IKATP inhibition and a long-duration AP on postischemic recovery.

In conclusion, the present experiments demonstrate the effects of glibenclamide and pinacidil on electrical and contractile function of cardiac muscle during no-flow ischemia and reperfusion. The data provide evidence that activation of IKATP represents an important adaptive mechanism for protecting and preserving the myocardium during ischemia.

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