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Glimepiride, a Novel Sulfonylurea, Does Not Abolish Myocardial Protection Afforded by Either Ischemic Preconditioning or Diazoxide

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Background—The sulfonylurea glibenclamide (Glib) abolishes the cardioprotective effect of ischemic preconditioning (IP), presumably by inhibiting mitochondrial K$_{ATP}$ channel opening in myocytes. Glimepiride (Glim) is a new sulfonylurea reported to affect nonpancreatic K$_{ATP}$ channels less than does Glib. We examined the effects of Glim on IP and on the protection afforded by diazoxide (Diaz), an opener of mitochondrial K$_{ATP}$ channels.

Methods and Results—Rat hearts were Langendorff-perfused, subjected to 35 minutes of regional ischemia and 120 minutes of reperfusion, and assigned to 1 of the following treatment groups: (1) control; (2) IP of 2× 5 minutes each of global ischemia before lethal ischemia; or pretreatment with (3) 30 μmol/L Diaz, (4) 10 μmol/L Glim, (5) 10 μmol/L Glib, (6) IP+Glim, (7) IP+Glib, (8) Diaz+Glim, or (9) Diaz+Glib. IP limited infarct size (18.5±1% vs 43.7±3% in control, P<0.01) as did Diaz (22.2±4.7%, P<0.01). The protective actions of IP or Diaz were not abolished by Glim (18.5±3% in IP+Glim, 22.3±3% in Diaz+Glim; P<0.01 vs control). However, Glib abolished the infarct-limiting effects of IP and Diaz. Patch-clamp studies in isolated rat ventricular myocytes confirmed that both Glim and Glib (each at 1 μmol/L) blocked sarcolemmal K$_{ATP}$ currents. However, in isolated cardiac mitochondria, Glim (10 μmol/L) failed to block the effects of K$_{ATP}$ opening by GTP, in contrast to the blockade caused by Glib.

Conclusions—Although it blocks sarcolemmal currents in rat cardiac myocytes, Glim does not block the beneficial effects of mitochondrial K$_{ATP}$ channel opening in the isolated rat heart. These data may have significant implications for the treatment of type 2 diabetes in patients with ongoing ischemic heart disease. (Circulation. 2001;103:3111-3116.)

Key Words: potassium • myocardial infarction • diabetes mellitus • ion channels

Sulfonylureas are drugs used in the treatment of type 2 diabetes. They inhibit ATP-sensitive potassium (K$_{ATP}$) channels, which induce membrane depolarization and an influx of calcium in pancreatic β-cells. Calcium acts as a second messenger, accounting for insulin release into the bloodstream. However, it has been suggested that classic sulfonylureas such as tolbutamide and glibenclamide (also known as glyburide) may have adverse effects on the cardiovascular system, mainly because they also close mitochondrial K$_{ATP}$ channels, thought to play a central role in ischemic preconditioning (IP) protection.1-3

Glimepiride is a newer sulfonylurea derivative demonstrated to have fewer cardiac actions than other sulfonylureas in both animal4 and human5 studies. For example, Geisen et al4 showed that glimepiride blocked K$_{ATP}$ currents in isolated rat cardiomyocytes at a concentration 5-fold higher than glibenclamide. There are also data providing indirect evidence that glibenclamide, but not glimepiride, prevents preconditioning in humans subjected to balloon angioplasty.6 If glimepiride has fewer cardiac actions than other sulfonylureas, then this would have important implications for its preferred use in the treatment of patients with type 2 diabetes with concurrent coronary artery disease.

The aim of this study was to compare the effect of glimepiride and the more conventionally used sulfonylurea glibenclamide on IP protection and on the protection afforded by one of the preconditioning mimetic agents, diazoxide. Diazoxide is a K$_{ATP}$ channel opener exhibiting selectivity for mitochondrial K$_{ATP}$ channels at concentrations up to 30 μmol/L.7 In this study, we used infarct size as the end point of injury, because this measure is a robust indicator of preconditioning-induced protection. We also assessed the effect of glimepiride and glibenclamide directly on sarcolemmal K$_{ATP}$ channel currents in isolated ventricular myocytes in addition to their effect on membrane potential in isolated cardiac mitochondria.

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onset of regional ischemia. (7) Hearts (n=7) underwent the IP protocol in the presence of 10 μmol/L glibenclamide as in group 6. (8) Hearts (n=9) were coperfused with 30 μmol/L diazoxide and 10 μmol/L glimepiride for 20 minutes immediately before regional ischemia. (9) Hearts (n=8) were coperfused with 30 μmol/L diazoxide and 10 μmol/L glibenclamide for 20 minutes immediately before regional ischemia.

### Infarct Size Measurement

At the end of the reperfusion period the snare was tightened to reocclude the coronary artery, and a saline solution of 0.12% Evans blue was infused slowly by way of the aorta. This procedure delineated the nonischemic zone of the myocardium as a dark blue area. After 1 to 4 hours at −20°C, hearts were sliced into 1-mm-thick transverse sections and incubated in triphenyltetrazolium chloride solution (1% in phosphate buffer, pH 7.4) at 37°C for 10 to 15 minutes. The tissue slices were then fixed in 10% formalin. At the end of this procedure, in the risk zone the viable tissue was stained red and the infarcted tissue appeared pale. The slices were drawn onto acetate sheets. With the use of a computerized planimetry package (Summa Sketch II, Summagraphics), the percentage of infarcted tissue within the volume of myocardium at risk was calculated.

### Patch-Clamp Studies

Ventricular myocytes were isolated from adult rat hearts by enzymatic dissociation as previously described. Cells were stored at 10°C to 12°C and bathed in a solution containing the following constituents (in mmol/L): NaCl 135.0, KCl 6.0, CaCl 2 2.0, MgCl 2 1.0, NaH 2 PO 4 0.33, sodium pyruvate 5, and HEPES 1.0, pH 7.4. The intracellular (pipette) solution contained the following constituents (in mmol/L): KCl 140.0, MgCl 2 1.0, EGTA 5.0, ATP 2.0, ADP 0.1, GTP 0.1, and HEPES 10.0, pH 7.2. Currents were recorded by using conventional patch-clamp techniques with an Axopatch 200B amplifier, analyzed with pCLAMP 8 software, and expressed relative to cell size as picoamps per picofarad. Experiments were performed at 30°C.

### Studies in Isolated Cardiac Mitochondria

Mitochondria were isolated from rat hearts by using a previously described technique. After extraction the mitochondria were kept on ice, and an aliquot was suspended in KCl buffer containing (in mmol/L) KCl 45.0, potassium acetate 25.4, TES 5.0, EGTA 0.1 (pH 7.4), MgCl 2 1.0, and 10 μmol/L cytochrome c. Substrates for respiration were 2.5 mmol/L ascorbate and 0.25 mmol/L N,N,N′,N′-tetramethyl-1-P-phenylene diamine. Aliquots of mitochondria were incubated with the mitochondrial membrane potential–sensitive dye tetramethylrhodamine methyl ester (TMRM, 200 nmol/L) at room temperature for 5 minutes before drug intervention. Where indicated, the physiological K ATP channel opener GTP (50 μmol/L) was added to the TMRM-stained mitochondria 2 minutes before measurements of fluorescence in the absence or presence of 5-hydroxydecanoate (a mitochondrial K ATP channel inhibitor, 100 μmol/L), glibenamide (10 μmol/L), and glimepiride (10 μmol/L). The mitochondrial uncoupler carbonyl cyanide m chlorophenylhydrazone (1 μmol/L) was used as a positive control. Cytofluorometric analysis was done on a Coulter Epics flow cytometer equipped with a 488-nm argon laser. The TMRM signal was analyzed in the FL2 channel equipped with a band-pass filter at 580±30 nm; the photomultiplier value of the detector was 631 V. Data were acquired on a logarithmic scale. Arithmetic mean values of the median fluorescence intensities were determined for each sample for graphic representation. Experiments were performed on mitochondria isolated from 6 individual rats, each experiment representing 15 000 mitochondria.

### Statistical Analysis

All values are expressed as mean±SEM. Data were analyzed by 1-way ANOVA and Fisher’s protected least significant difference test.
Glimepiride or glibenclamide alone did not influence infarct size in the ischemic risk zone. As expected, IP significantly reduced the amount of infarcted tissue in the risk zone compared with control hearts (18.6 ± 1.5% in IP vs 38.8 ± 5% in diazoxide + glibenclamide; P < 0.05) but not in the presence of glimepiride (22.4 ± 2.9% in diazoxide + glimepiride vs 22.2 ± 4.7% in diazoxide; P > 0.05; Figure 3).

Patch-Clamp Studies
To test whether glimepiride and diazoxide affect currents through sarcolemmal K$_{ATP}$ channels of rat ventricular myocytes, we used patch-clamp techniques to record whole-cell membrane currents at a holding potential of 0 mV in 6 mmol/L K$^+$ solution. Under these conditions, the K$_{ATP}$ channel opener pinacidil activated a substantial outward K$_{ATP}$ current, which was blocked by both 1 mmol/L glibenclamide (Figure 4A) and 1 mmol/L glimepiride (Figure 4B). The effectiveness of glimepiride in blocking sarcolemmal K$_{ATP}$ channels was confirmed in 16 additional cells. In experiments in which we tested different concentrations, half blockage occurred with ~10 mmol/L glimepiride. In similar experiments, we looked for current activation by diazoxide (at 30 and 300 μmol/L). Figure 4B shows that no activation of current was detectable in response to diazoxide at 300 μmol/L, but the subsequent application of pinacidil (200 μmol/L) to the same cell activated substantial K$_{ATP}$ current. The results from several cells (Figure 4C) show that diazoxide caused no activation of sarcolemmal K$_{ATP}$ current at either 30 or 300 μmol/L. These results suggest that glibenclamide and glimepiride are potent blockers of sarcolemmal K$_{ATP}$ channels in rat ventricular myocytes and that diazoxide does not activate these channels under our experimental conditions.

Mitochondrial Membrane Potential
Ascorbate was used in all experiments as a mitochondrial respiratory substrate. Application of ascorbate to the mitochondria caused an instantaneous increase in intensity of TMRM fluorescence, concomitant with mitochondrial membrane polarization. The mitochondrial uncoupler carbonyl cyanide m-chlorophenylhydrazone (1 μmol/L), used as a positive control to collapse membrane potential in the mitochondria, resulted in a large reduction in intensity of TMRM fluorescence (Figure 5A). Treatment of mitochondria with the physiological mitochondrial K$_{ATP}$ channel opener GTP (50 μmol/L) significantly (P < 0.0001) decreased the TMRM fluorescence from 153 ± 3.9 arbitrary fluorescence units in untreated mitochondria to 135 ± 2.9 (Figure 5A). GTP significantly decreased the mitochondrial membrane potential by 14 ± 0.9% of the control value (Figure 5B). 5-Hydroxydecanoate, glimepiride, or glibenclamide alone had no effect on membrane potential (Figure 5B). Both glibenclamide and 5-hydroxydecanoate prevented the changes in membrane potential induced by GTP (150 ± 4.7 and 150 ± 3.8 mV).
arbitrary units, respectively, compared with control (153 ± 6 versus 156 ± 3.9), whereas glimepiride did not block these changes (132 ± 6 versus 132 ± 2.9 arbitrary units; Figures 5A and 5B).

**Discussion**

Diabetes is a common and widespread disease. In the diabetic population, 90% of patients have type 2 diabetes. In these patients there is an increased risk of cardiovascular complications followed by higher morbidity and mortality than in a nondiabetic population with coronary artery disease. The most common treatment approach in type 2 diabetes is administration of oral sulfonylureas, such as glibenclamide, which block K<sub>ATP</sub> channels, thereby stimulating insulin release by pancreatic β-cells. Unfortunately, K<sub>ATP</sub> channel blockade is not specific to the pancreas and can affect other tissues as well. It is well established that glibenclamide can also block sarcolemmal K<sub>ATP</sub> channels in a number of other cell types, including vascular smooth muscle cells, cardiac myocytes, and vascular endothelium, as well as the K<sub>ATP</sub> channels situated on the inner membrane of mitochondria.
There is also substantial evidence to suggest that in diabetic patients with acute myocardial infarction, these oral agents should be avoided. Initial concern for issue this was raised in the early 1970s when the University Group Diabetes Program assessed the efficacy of oral hypoglycemic treatment compared with insulin and diet alone in the prevention of cardiovascular complications. They demonstrated a significantly higher cardiovascular mortality in patients on sulfonylureas compared with diet alone. Nonetheless, these agents have continued to be extensively used because, one suspects, of the lack of a plausible mechanism for the University Group Diabetes Program study results. The United Kingdom Prospective Diabetes Study, a large-scale clinical study of >5000 patients, attempted to answer the question of whether improved glycemic control reduced the risk of cardiovascular death in patients who were taking insulin and sulfonylureas. In that study no detrimental effect of sulfonylureas was noted, and the United Kingdom Prospective Diabetes Study is often cited as proof that sulfonylureas such as glibenclamide do not pose a risk to patients with type 2 diabetes. Unfortunately, what the study failed to ascertain was the effect that these agents had on these type 2 diabetic patients in the setting of acute coronary syndromes, ie, in patients directly at risk of myocardial infarction (presenting with chest pain or unstable angina).

In this context, one of the most potent mechanisms of protection against myocardial ischemia/reperfusion injury is ischemic preconditioning. This endogenous protective response has been demonstrated in all species, including humans, and has been described as the beneficial adaptive response of the myocardium to repeated episodes of sublethal ischemia. A substantial body of evidence implicates mitochondrial K\(_{\text{ATP}}\) channel opening as playing a central role in the acquisition of this protection. Although it is not clearly established whether mitochondrial K\(_{\text{ATP}}\) channel opening plays a trigger role (proximal event) or acts as a distal effector of protection, glibenclamide has been shown to attenuate this preconditioning response in animal studies (for a review, see Yellon et al). There are also data from human studies in which preconditioning has been examined with surrogate end points such as ST-segment deviation during repeated intra-coronary balloon inflations, which preconditioning has been examined with surrogate end points such as ST-segment deviation during repeated intra-coronary balloon inflations that support the notion that glibenclamide blunts the preconditioning response. Such observations have generated concern about the safety of sulfonylurea agents in diabetic patients with concurrent ischemic heart disease, because inhibition of the endogenous preconditioning mechanism by sulfonylureas might predispose to cell death. Glimepiride, a second-generation sulfonylurea, has been shown to be more specific to the pancreas than to other tissues, especially the myocardium. Furthermore, glimepiride was shown to have a more rapid as well as longer duration of action, and despite stimulating less insulin secretion in comparison with glibenclamide, it has been shown to have higher glucose-decreasing activity. This characteristic may be as a consequence of its having a direct effect on the expression of glucose transporters, such as Glut-1 and Glut-4.

Our aim was to study the direct effect of these sulfonylurea drugs on the protection conferred by ischemic preconditioning by using infarct size, which has been shown to be a valid end point in relation to experimental preconditioning studies. The results show that infarct size reduction due to ischemic preconditioning was not significantly changed when the preconditioning protocol took place in the presence of glimepiride. On the contrary, glibenclamide completely abolished this protection. A possible explanation would be that unlike glibenclamide, glimepiride does not block the mitochondrial K\(_{\text{ATP}}\) channels, known to play a crucial role in preconditioning protection. To examine this hypothesis, the second aim of our study was to ascertain whether glimepiride abolished the protective role of diazoxide, a known opener of mitochondrial K\(_{\text{ATP}}\) channels at specific doses. It has been shown that diazoxide, when administered before ischemia, protects the infarcting myocardium; this beneficial effect being lost in the presence of glibenclamide. Our results confirm these studies with respect to glibenclamide but also demonstrate that glimepiride does not appear to abolish this protective effect; ie, the protection conferred by diazoxide is not lost even when the mitochondrial K\(_{\text{ATP}}\) opener is given in the presence of this sulfonylurea. The most plausible explanation would be that glimepiride does not affect mitochondrial K\(_{\text{ATP}}\) opening, whereas glibenclamide blocks this channel. We do note, however, that 10 \(\mu\)mol/L glibenclamide may not be specific, and we cannot exclude the possibility that at this concentration, glibenclamide abolishes other mechanisms involved in preconditioning.

Diazoxide has been shown to cause a decrease in mitochondrial membrane potential, although the exact process by which it does so remains controversial. Although diazoxide has been proposed to directly open mitochondrial K\(_{\text{ATP}}\) channels, it may in addition have a nonspecific effect on electron transport of the respiratory chain. To concentrate on the mitochondrial K\(_{\text{ATP}}\) channel specifically, the physiological mitochondrial K\(_{\text{ATP}}\) channel opener GTP was therefore used to investigate the action of the two sulfonylureas. GTP produced a decrease in mitochondrial membrane potential, which was blocked by glibenclamide, as well as by a suitable agent known to block mitochondrial K\(_{\text{ATP}}\) channels, viz, 5-hydroxydecanoate. Under the same conditions, glimepiride failed to inhibit the effects of GTP on mitochondrial membrane potential. These data indicate that glimepiride has no effect on mitochondrial K\(_{\text{ATP}}\) channel opening by GTP.

We believe that more studies, basic as well as clinical, are needed to fully elucidate and characterize the role of this sulfonylurea. At present, we believe that our study undertaken in the isolated rat heart demonstrates that glimepiride appears to be significantly less harmful to the ischemic heart than is the more conventionally used sulfonylurea glibenclamide. Further work in other species and in vivo are warranted. However, the present data may have important implications for the treatment of type 2 diabetes patients at risk of myocardial infarction, and appropriate clinical studies would need to be designed to ascertain the true nature of the role and place of such sulfonylureas in ischemic heart disease patients.
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