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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 $2\times$ 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 ($18.5\pm1\%$ vs $43.7\pm3\%$ in control, $P<0.01$) as did Diaz ($22.2\pm4.7\%$, $P<0.01$). The protective actions of IP or Diaz were not abolished by Glim ($18.5\pm3\%$ in IP+Glim, $22.3\pm3\%$ 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.[1] 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 animal[4] and human[5] studies. For example, Geisen et al[4] 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.

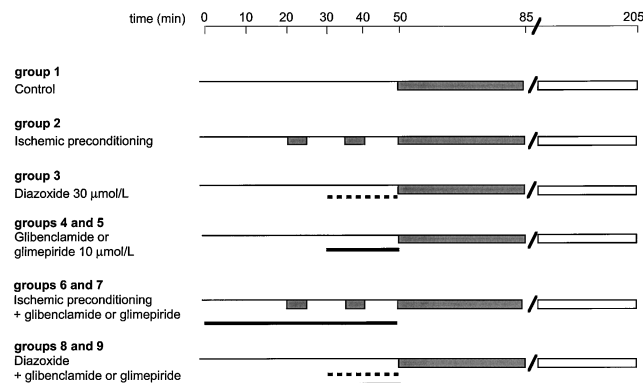*Circulation* **is available at http://www.circulationaha.org**

Figure 1. Experimental protocols for infarct studies.

Methods

Animals

Male Sprague-Dawley rats (350 ± 30 g body weight) were used. All animals were obtained from the same source (Charles River UK Limited, Margate, UK), fed a standard diet, housed under the same conditions, and received humane care in accordance with The Guidance on the Operation of the Animals (Scientific Procedures) Act 1986.

Isolated Heart Perfusion

Rats were anesthetized with sodium pentobarbital (55 mg/kg intraperitonally) and given heparin sodium (300 IU). Hearts were excised, arrested in ice-cold buffer, and mounted on a constant-pressure (80 mm Hg) Langendorff perfusion system. They were perfused retrogradely with a modified Krebs-Henseleit bicarbonate buffer containing the following chemicals (in mmol/L): NaCl 118.5, $NaHCO_3$ 25.0, KCl 4.8, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 1.7, and glucose 12.0. All solutions were filtered through a Whatman 2.0-μm microfilter and gassed with 95%O_2/5% CO_2 (pH 7.35 to 7.50 at 37°C). Temperature was continuously monitored by a thermoprobe inserted into the right ventricle. A latex, fluid-filled, isovolumic balloon was introduced into the left ventricle through the left atrial appendage and inflated to give a preload of 8 to 10 mm Hg. Left ventricular developed pressure, heart rate, and coronary flow were registered at regular intervals. A surgical needle was passed under the left main coronary artery, and the ends of the thread were passed through a small plastic tube to form a snare. Regional ischemia was induced by tightening the snare, and reperfusion was started by releasing the ends of the thread.

Treatment Protocols

The experimental protocols are presented in Figure 1. Glibenclamide, glimepiride, and diazoxide were dissolved in dimethyl sulfoxide and added to the Krebs-Henseleit buffer such that the final dimethyl sulfoxide concentration did not exceed 0.02%. The hearts were randomly assigned to 1 of 9 treatment groups: (1) Control hearts (n=9) were perfused with 0.02% dimethyl sulfoxide for 20 minutes during stabilization before 35 minutes of regional ischemia and 120 minutes of reperfusion. (2) IP hearts (n=7) were treated with 2 periods of 5 minutes each of global ischemia with a 10-minute intervening reperfusion before 35 minutes of regional ischemia and 120 of minutes reperfusion. (3) Hearts (n=9) were perfused with 30 μmol/L diazoxide for 20 minutes immediately before regional ischemia. (4) Hearts (n=6) were perfused with 10 μmol/L glimepiride for 20 minutes immediately before regional ischemia. (5) Hearts (n=6) were perfused with 10 μmol/L glibenclamide for 20 minutes immediately before regional ischemia. (6) Hearts (n=8) underwent the IP protocol in the presence of 10 μmol/L glimepiride. The drug was added to the perfusate 20 minutes before starting the preconditioning protocol and was present throughout this protocol. The buffer was switched to normal Krebs-Henseleit buffer after the

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.[8] 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_2PO_4 0.33, sodium pyruvate 5, and HEPES 10.0, 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.[9] 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-P-phenylene diamine. Aliquots of mitochondria were incubated with the mitochondrial membrane potential–sensitive dye tetramethylrhodamine methyl ester (TMRM, 200 nmol/L)[10] 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), glibencamide (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$\pm$SEM. Data were analyzed by 1-way ANOVA and Fisher's protected least significant difference
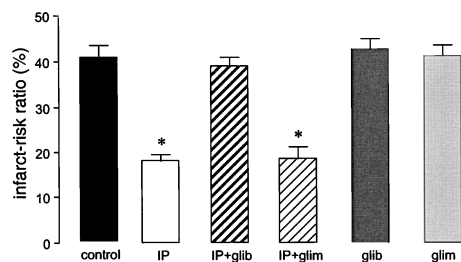
Figure 2. Infarction developed in risk zone in hearts preconditioned (IP) in presence of 10 μmol/L glibenclamide (glib) or glimepiride (glim). *$P < 0.001$.

test for multiple comparisons. Differences were considered significant for $P < 0.05$.

Results

Exclusions
We used a total of 76 rat hearts for the Langendorff perfusion study. Of these, 6 were excluded owing to poor function during stabilization.

Hemodynamic Data
Baseline data relating to cardiac function and coronary flow rates before regional ischemia where similar in all experimental groups. During regional ischemia, coronary flow and left ventricular developed pressure decreased to a similar extent in all groups. An increase in coronary flow during the first minutes of reperfusion was indicative of successful reflow, but coronary flow subsequently declined in all groups during the following 120-minute reperfusion period. During reperfusion, left ventricular developed pressure recovered gradually, though never reaching stabilization values.

Infarct Size Data
The risk zone volume was similar in all experimental groups, at ≈ 0.5 cm^3. Infarct size is represented as the percentage of tetrazolium-negative tissue in the ischemic risk zone. As expected, IP significantly reduced the amount of infarcted tissue in the risk zone compared with control hearts ($18.6 \pm 1.5\%$ vs $43.7 \pm 3.0\%$, $P < 0.01$; Figures 2 and 3). Glimepiride or glibenclamide alone did not influence infarct size (glibenclamide $44.7 \pm 5\%$, glimepiride $41.4 \pm 4.7\%$). However, when administered before and during the IP protocol, glibenclamide abolished the protective effect of preconditioning ($36.3 \pm 4\%$ in glibenclamide+IP vs $18.6 \pm 1.5\%$
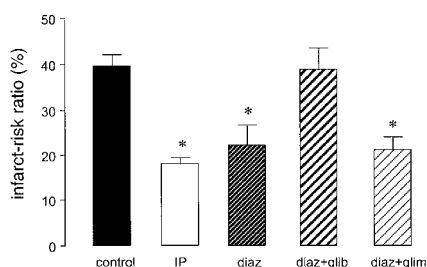

Figure 3. Infarction developed in risk zone in hearts pretreated with both 30 μmol/L diazoxide (diaz) and 10 μmol/L glibenclamide (glib) or glimepiride (glim) before regional ischemia. *$P < 0.001$.

in IP, $P < 0.05$), whereas glimepiride did not ($18.5 \pm 2.7\%$ in glimepiride+IP vs $18.6 \pm 1.5\%$ in IP; Figure 2).

With regard to potential effects on the mitochondria, we used the K_{ATP} channel opener diazoxide to investigate the differences between glibenclamide and glimepiride. Diazoxide alone given before ischemia also conferred protection against ischemia/reperfusion injury (infarct/risk zone, $22.2 \pm 4.7\%$; $P < 0.05$ vs control). This beneficial effect was lost in the presence of glibenclamide ($22.2 \pm 4.7\%$ in diazoxide vs $38.8 \pm 5\%$ in diazoxide+glibenclamide; $P < 0.05$) but not in the presence of glimepiride ($22.4 \pm 2.9\%$ in diazoxide+glimepiride vs $22.2 \pm 4.7\%$ 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 μmol/L glimepiride (Figure 4A) and 1 μmol/L glibenclamide (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 nmol/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 $14 \pm 0.9\%$ of the control value (Figure 5B). 5-Hydroxydecanoate, glimepiride, or glibenclamide alone had no effect on membrane potential (Figure 5B). Both glibencamide and 5-hydroxydecanoate prevented the changes in membrane potential induced by GTP (150 ± 4.7 and 150 ± 3.8
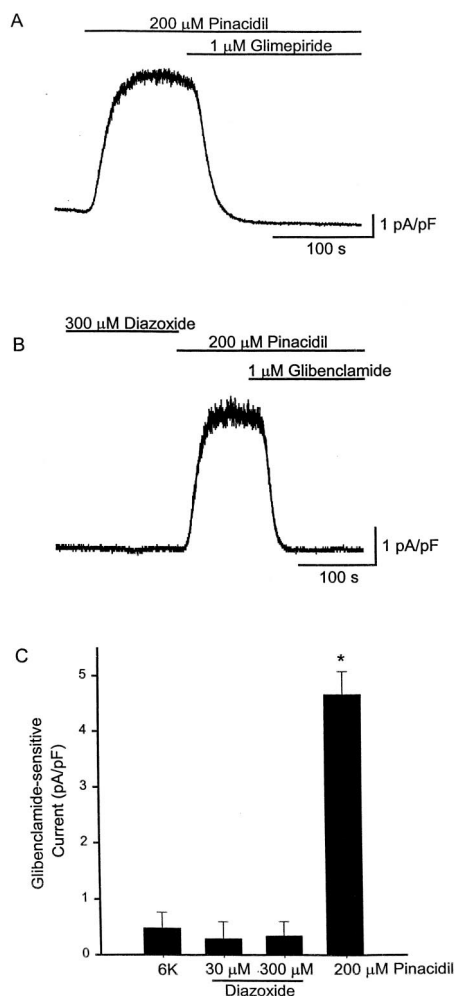
Figure 4. Effects of sulfonylureas and diazoxide on sarcolemmal K_{ATP} current. A, Membrane current recorded from isolated rat ventricular myocyte by using whole-cell patch-clamp technique. Cell was held at 0 mV throughout, and extracellular solution contained 6 mmol/L K^+. Application of K_{ATP} channel opener pinacidil activated outward K_{ATP} current, which was completely blocked by 1 μmol/L glimepiride. B, Recording from different cell under same conditions as in A. Diazoxide (300 μmol/L) did not activate K_{ATP} current, but subsequent application of pinacidil (200 μmol/L) activated substantial current that was inhibited by 1 μmol/L glibenclamide. C, Mean±SEM sarcolemmal K_{ATP} (glibenclamide-sensitive) current at 0 mV in absence of K_{ATP} channel openers (6K), in presence of diazoxide (30 and 300 μmol/L) or pinacidil (200 μmol/L). n=5 cells in each case. *$P<0.0001$ vs 6K, *t* test.

arbitrary units, respectively, compared with control 153±3.9), whereas glimepiride did not block these changes (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.[11] 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.[12] The most common treatment approach in type 2 diabetes is administration of oral sulfonylureas, such as glibenclamide, which block K_{ATP} channels, thereby stimulating insulin re-
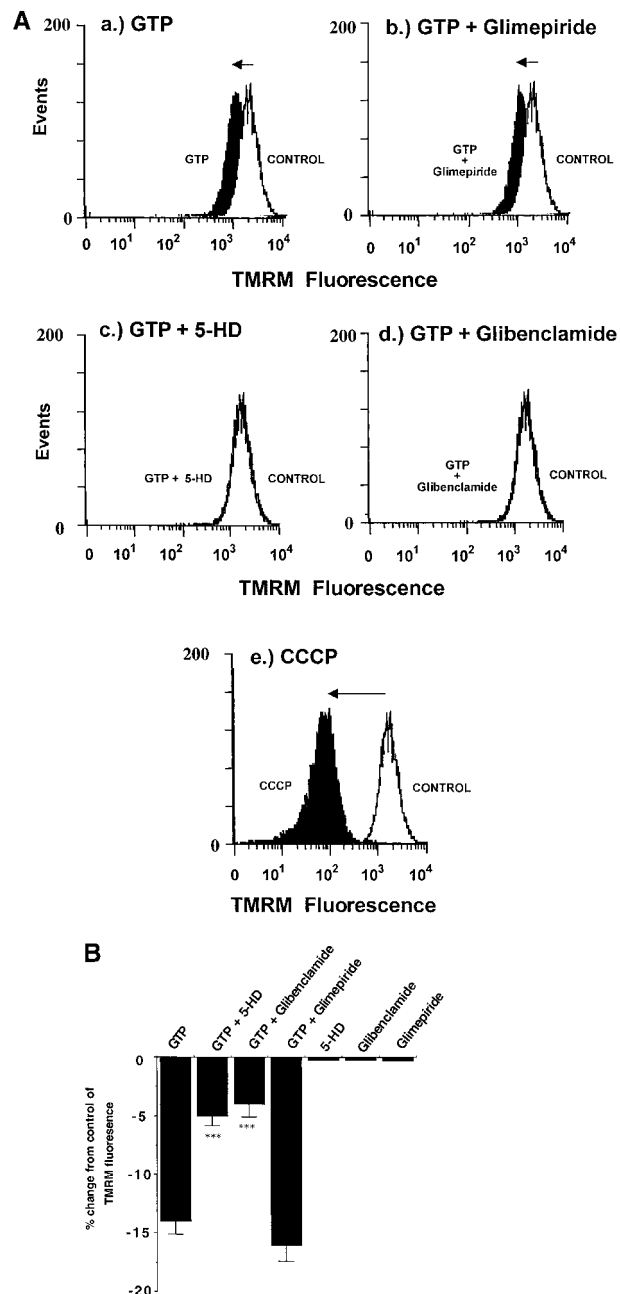

Figure 5. Effects of sulfonylureas and GTP on mitochondrial membrane potential. A, Representative flow cytometric profile of isolated cardiac mitochondria stained with TMRM showing mitochondrial membrane potential–associated fluorescence. Effect on control of (a) 50 μmol/L GTP, (b) GTP in presence of 10 μmol/L glimepiride, (c) GTP in presence of 100 μmol/L 5-hydroxydecanoate, (d) GTP in presence of 10 μmol/L glibenclamide, and (e) 1 μmol/L carbonyl cyanide *m*-chlorophenylhydrazone CCCP. B, Mean±SEM percent change from control of median TMRM fluorescence, n=6. ***$P<0.0001$.

lease by pancreatic β-cells. Unfortunately, K_{ATP} 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_{ATP} channels in a number of other cell types, including vascular smooth muscle cells, cardiac myocytes, and vascular endothelium, as well as the K_{ATP} channels situated on the inner membrane of mitochondria.[13]

There is also substantial evidence to suggest that in diabetic patients with acute myocardial infarction, these oral agents should be avoided.[14] 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.[15] 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,[16] 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.[17] This endogenous protective response has been demonstrated in all species, including humans,[18] 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_{ATP} channel opening as playing a central role in the acquisition of this protection.[3,19] Although it is not clearly established whether mitochondrial K_{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[18]). There are also data from human studies in which preconditioning has been examined with surrogate end points such as ST-segment deviation during repeated intracoronary balloon inflations that support the notion that glibenclamide blunts the preconditioning response.[20] 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.[1] Glimepiride, a second-generation sulfonylurea, has been shown to be more specific to the pancreas than to other tissues, especially the myocardium.[4] Furthermore, glimeperide 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.[13,21] 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.[22]

Our aim was to study the direct effect of these sulfonylurea drugs on the protection conferred by ischemic precondition-ing by using infarct size, which has been shown to be a valid end point in relation to experimental preconditioning studies.[23] 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_{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_{ATP} channels at specific doses.[7] It has been shown that diazoxide, when administered before ischemia, protects the infarcting myocardium;[19] this beneficial effect being lost in the presence of glibenclamide.[7] 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_{ATP} opener is given in the presence of this sulfonylurea. The most plausible explanation would be that glimepiride does not affect mitochondrial K_{ATP} opening, whereas glibenclamide blocks this channel. We do note, however, that 10 μmol/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.[3,24–26] Although diazoxide has been proposed to directly open mitochondrial K_{ATP} channels, it may in addition have a nonspecific effect on electron transport of the respiratory chain.[3,25,26] To concentrate on the mitochondrial K_{ATP} channel specifically, the physiological mitochondrial K_{ATP} channel opener GTP was therefore used to investigate the action of the two sulfonylureas.[27] 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_{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_{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.

Acknowledgments

References

1. Engler RL, Yellon DM. Sulfonylurea K_{ATP} blockade in type II diabetes and preconditioning in cardiovascular disease. *Circulation*. 1996;94: 2297–2301.

2. Cleveland JC, Meldrum DR, Cain BS, et al. Oral sulfonylurea hypoglycemic agents prevent ischemic preconditioning in human myocardium. *Circulation*. 1997;96:29–32.

3. Liu Y, Sato T, Seharaseyon J, et al. Mitochondrial $_{ATP}$- dependent potassium channels. Viable candidate effectors of ischemic preconditioning. *Ann NY Acad Sci*. 1999;874:27–37.

4. Geisen K, Vegh A, Krause E, Papp JG. Cardiovascular effects of conventional sulfonylureas and glimepiride. *Horm Metab Res*. 1996;28: 496–507.

5. Bijlstra PJ, Lutterman JA, Russel FGM, et al. Interaction of sulphonylurea derivatives with vascular $_{ATP}$-sensitive potassium channels in humans. *Diabetologia*. 1996;39:1083–1090.

6. Klepzig H, Kober G, Matter C, et al. Sulfonylurea and ischaemic preconditioning: a double-blind, placebo-controlled evaluation of glimepiride and glibenclamide. *Eur Heart J*. 1999;20:439–446.

7. Garlid KD, Paucek P, Yarov-Yarovoy V, et al. Cardioprotective effect of diazoxide and its interaction with mitochondrial $_{ATP}$-sensitive K^+ channels: possible mechanism of cardioprotection. *Circ Res*. 1997;81: 1072–1082.

8. Laurence CL, Rodrigo GC. A Na^+-activated K^+ current (Ik, Na) is present in guinea-pig but not rat ventricular myocytes. *Pflugers Arch*. 1999;437: 831–838.

9. Johnson N, Khan A, Virji S, et al. Import and processing of heart mitochondrial cyclophilin D. *Eur J Biochem*. 1999;263:353–359.

10. Scaduto RC, Grotyohann LW. Measurement of mitochondrial membrane potential using fluorescent rhodamine derivatives. *Biophys J*. 1999;76: 469–477.

11. Amos AF, McCarthy DJ, Simmet P. The raising global burden of diabetes and its complications: estimates and projections to the year 2010. *Diabet Med*. 1997;14:S1–S85.

12. Wikstrom G, Malmberg K, Ryden L. Improved knowledge of antidiabetic treatment: a necessity for the modern cardiologist. *Eur Heart J*. 1999;20: 403–405.

13. Geisen K. Special pharmacology of the new sulphonylurea glimepiride. *Arzneimittelforschung*. 1988;38:1120–1130.

14. Betteridge DJ, Close L. Diabetes, coronary heart disease and sulpnonylureas: not the final word. *Eur Heart J*. 2000;21:970–792.

15. University Group Diabetes Program. A study of the effects of hypoglycemic agents on vascular complications of patients with adult-onset diabetes. *Diabetes* 1976;25:1129–1153.

16. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes(UKPDS33). *Lancet*. 1998;352:837–853.

17. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation*. 1986; 74:1124–1136.

18. Yellon DM, Baxter GF, Garcia-Dorado D, et al. Ischaemic preconditioning: present position and future directions. *Cardiovasc Res*. 1998;37: 21–33.

19. Fryer RM, Eells JT, Hsu AK, et al. Ischemic preconditioning in rats: role of mitochondrial $K(_{ATP})$ channel in preservation of mitochondrial function. *Am J Physiol*. 2000;278:H305–H331.

20. Tomai F, Crea F, Gaspardone A, et al. Ischaemic preconditioning during coronary angioplasty is prevented by glibenclamide, a selective ATP-sensitive K^+ blocker. *Circulation*. 1994;90:700–705.

21. Raptis SA, Hatziagelaki E, Dimitriadis G, et al. Comparative effects of glimepiride and glibenclamide on blood glucose, C-peptide and insulin concentrations in the fasting and postprandial state in normal man. *Exp Clin Endocrinol Diabetes*. 1999;107:350–355.

22. Eckel J. Direct effects of glimepiride on protein expression of cardiac glucose transporters. *Horm Metab Res*. 1996;28:508–511.

23. Meeting report: Hatter Institute workshop on myocardial preconditioning. *Cardiovasc Drugs Ther*. 1998;12:529–531.

24. Grimmsmann T, Rustenbeck I. Direct effect of diazoxide on mitochondria in pancreatic B-cells and on isolated liver mitochondria. *Br J Pharm*. 1998;123:781–788.

25. Inoue I, Nagase H, Kishi K, et al. ATP-sensitive K^+ channel in the mitochondrial membrane. *Nature*. 1991;352:244–247.

26. Holmuhamedov EL, Jovanovic S, Dzeja PP, et al. Mitochondrial ATP-sensitive K^+ channels modulate cardiac mitochondrial function. *Am J Physiol*. 1998;275:H1567–H1576.

27. Jaburek M, Yarov-Yarovoy V, Paucek P, et al. State-dependent inhibition of the mitochondrial K_{ATP} channel by glyuride and 5-hydroxydecanoate. *J Biol Chem*. 1998;273:13578–13582.