The Impact of ATP-Sensitive \( K^+ \) Channel Subtype Selectivity of Insulin Secretagogues for the Coronary Vasculature and the Myocardium

Ulrich Quast, Damian Stephan, Susanne Bieger, and Ulrich Russ

Insulin secretagogues (sulfonylureas and glinides) increase insulin secretion by closing the ATP-sensitive \( K^+ \) channel (K\(_{ATP} \) channel) in the pancreatic \( \beta \)-cell membrane. K\(_{ATP} \) channels subserve important functions also in the heart. First, K\(_{ATP} \) channels in coronary myocytes contribute to the control of coronary blood flow at rest and in hypoxia. Second, K\(_{ATP} \) channels in the sarclemma of cardiomyocytes (sarcK\(_{ATP} \) channels) are required for adaptation of the heart to stress. In addition, the opening of sarcK\(_{ATP} \) channels and of K\(_{ATP} \) Channels in the inner membrane of mitochondria (mitoK\(_{ATP} \) channels) plays a central role in ischemic preconditioning. Opening of sarcK\(_{ATP} \) channels also underlies the ST-segment elevation of the electrocardiogram, the primary diagnostic tool for initiation of lysis therapy in acute myocardial infarction. Therefore, inhibition of cardiovascular K\(_{ATP} \) channels by insulin secretagogues is considered to increase cardiovascular risk. Electrophysiological experiments have shown that the secretagogues differ in their selectivity for the pancreatic over the cardiovascular K\(_{ATP} \) channels, being either highly selective (\( \sim 1,000 \times \) short sulfonylureas such as nateglinide and mitiglinide), moderately selective (10–20\( \times \) long sulfonylureas such as glibenclamide [glyburide]), or essentially nonselective (\( < 2 \times \) repaglinide). New binding studies presented here give broadly similar results. In clinical studies, these differences are not yet taken into account. The hypothesis that the in vitro selectivity of the insulin secretagogues is of importance for the cardiovascular outcome of diabetic patients with coronary artery disease needs to be tested. Diabetics 53 (Suppl. 3):S156–S164, 2004

Insulin secretagogues are widely prescribed in the treatment of type 2 diabetes. They close the ATP-sensitive \( K^+ \) channel (K\(_{ATP} \) channel) in the membrane of the pancreatic \( \beta \)-cell, thereby depolarizing the cell and triggering insulin secretion. K\(_{ATP} \) channels are gated by intracellular nucleotides with ATP inducing channel closure and MgADP channel opening. The \( \beta \)-cell is special in that physiological changes in plasma glucose change the intracellular ATP and ADP concentrations such that the channel opens and closes; hence, the channel functions as the glucose sensor in this cell (1–3). K\(_{ATP} \) channel subtypes are found in many cell types. The generation of mice in which the genes for the K\(_{ATP} \) channel subunits were deleted have shed new light on the diverse functions of the K\(_{ATP} \) channels in various tissues in physiological and pathophysiological conditions (1). In brain, K\(_{ATP} \) channels are involved in actions as diverse as the control of glucose homeostasis and the regulation of neuronal excitability in hypoxia (1); however, the insulin secretagogues do not cross the blood-brain barrier easily enough to affect these channels at therapeutic plasma levels (4). In several vascular beds, the K\(_{ATP} \) channel in the vascular myocytes is involved in the regulation of vessel tone; opening is triggered in particular by stimuli increasing cAMP (5). In general, the channel opens in ischemia and hypoxia when the ADP-to-ATP ratio increases; this clamps the cell at the K\(^+ \) equilibrium potential and brings the cell to rest.

K\(_{ATP} \) channels are composed of two types of subunits, inwardly rectifying K\(^+ \) channels (Kir6.x) and sulfonylurea receptors (SURx), arranged as tetradimeric complexes, (Kir6.x/SURx)\(_4 \) (Fig. 1) (rev. in 1–3,6). The Kir6.x subunits form the pore of the channel. SURs function as regulatory subunits. They are members of the ATP-binding cassette protein superfamily, carry binding sites for nucleotides, and exhibit ATPase activity. In addition, SUR is endowed with the binding sites for the sulfonylureas and the K\(_{ATP} \) channel openers (Fig. 1). Both Kir6.x and SURx are encoded by two genes; this, together with alternative splicing of SUR, gives rise to various combinations of Kir6.x/SURx, which account for the differences in the biophysical and pharmacological properties of the K\(_{ATP} \) channels in the various tissues. Of particular importance for the following discussion are the K\(_{ATP} \) channels in pancreatic \( \beta \)-cells (Kir6.2/SUR1), in cardiomyocytes

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K\(_{ATP} \) channel, ATP-sensitive \( K^+ \) channel; Kir channel, inwardly rectifying \( K^+ \) channel; mitoK\(_{ATP} \) channel, K\(_{ATP} \) channel in the inner membrane of mitochondria; sarcK\(_{ATP} \) channel, K\(_{ATP} \) Channel in the sarcolemma of cardiomyocytes; SUR, sulfonylurea receptor.

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The Kir6.x subtypes differ mainly in their single-channel conductance (30 vs. 80 pS in high symmetrical K⁺ solution for Kir6.1 and Kir6.2, respectively) and in their sensitivity to block by ATP. Kir6.1 is only weakly sensitive ($K_i \sim 1$ mmol/l), whereas for Kir6.2, the $K_i$ is $\sim 100$ μmol/l (1). The sensitivity of Kir6.2 to inhibition by ATP is increased by coexpression with SUR. In the Kir6.2-containing channels, inhibition by ATP is mediated by ATP binding to Kir6.2, whereas channel activation by MgADP is conferred by SUR (1–3,6). The SUR subtypes, SUR1 and SUR2, give rise to several isoforms due to alternative splicing; of major importance are SUR2A and SUR2B, which differ in the last exon (i.e., by 44 amino acids) (6). Generally, SUR1 has a higher affinity for sulfonylureas and glinides than the SUR2 isoforms, whereas SUR2 has a higher affinity for the K⁺ATP channel openers (1,6). There are notable exceptions to both rules. The selectivity ranking of the sulfonylureas and glinides for the pancreatic over the cardiac K⁺ATP channels is detailed in Table 1; in addition, a sulfonylthiourea, HMR 1883 (HMR 1098), that is highly selective for SUR2 has been synthesized (7). Regarding the openers, diazoxide has been found to affect the K⁺ATP channels in the β-cell and in the vascular myocyte at similar concentrations (6), and, recently, openers with excellent selectivity for SUR1 over SUR2 have been synthesized (8).

In addition to these K⁺ATP channels in the cell membrane, there is evidence for a K⁺ATP channel in the inner membrane of mitochondria (the mitoK⁺ATP channel). This channel was discovered in 1991 in electrophysiological experiments; however, despite intensive effort, its molecular identity remains unknown (9,10). This channel has been proposed to play a central role in ischemic preconditioning (i.e., the mechanism by which brief ischemic episodes “condition” the heart to better survive subsequent longer periods of ischemic insult) (9,10).

In the following sections, we will first consider the selectivity of representative insulin secretagogues for the K⁺ATP channel in the β-cell over that in the cardiomyocyte and present new binding studies dealing with this question. Then the role of the K⁺ATP channels in the (human) coronary vascular bed and in cardiac myocytes is discussed. In the last section, we examine the available clinical evidence that therapy with insulin secretagogues

![Diagram of K⁺ATP channels and its subunits](image)

**FIG. 1. Structure of K⁺ATP channels and its subunits. The binding regions of SUR for the insulinotropes and K⁺ATP channel openers are indicated (see text for details). CL, cytosolic loop; NBF, nucleotide binding fold.**

### Table 1
Inhibition of [³H]glibenclamide binding to recombinant K⁺ATP channels subtypes by sulfonylureas and glinides

<table>
<thead>
<tr>
<th>Secretagogue</th>
<th>SUR subsite</th>
<th>$K_i$ (nmol/l)</th>
<th>Selectivity pancreas/heart*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Kir6.2/SUR1</td>
<td>Kir6.2/SUR2A</td>
</tr>
<tr>
<td>Glibornuride</td>
<td>A</td>
<td>360 (330–400)</td>
<td>6,800 (6,200–7,400)</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>A+B</td>
<td>0.45 (0.31–0.65)</td>
<td>6.2 (5.6–6.8)†</td>
</tr>
<tr>
<td>Glimepiride</td>
<td>A+B</td>
<td>0.58 (0.53–0.60)</td>
<td>11 (10–12)</td>
</tr>
<tr>
<td>Meglitinide</td>
<td>B</td>
<td>3,200 (2,800–3,600)</td>
<td>830 (660–1,000)</td>
</tr>
<tr>
<td>(-)AZ-DF 265</td>
<td>B</td>
<td>3.7 (2.9–4.9)‡</td>
<td>25 (23–28)</td>
</tr>
<tr>
<td>Repaglinide</td>
<td>B</td>
<td>0.72 (0.60–0.87)</td>
<td>1.5 (1.4–1.7)</td>
</tr>
<tr>
<td>Nateglinide</td>
<td>A</td>
<td>350 (330–360)</td>
<td>10,000 (8,500–12,000)</td>
</tr>
</tbody>
</table>

Data are $K_i$ values (95% CIs). Experiments were performed as described in Fig. 3. Hill coefficients were $\sim 1$. $K_i$ values are followed by the 95% CI in parentheses. †Calculated as $10^{\Delta pK}$ with $\Delta pK = pK_i$(Kir6.2/SUR1) – $pK_i$(Kir6.2/SUR2A), taking propagation of errors into account. ‡For the recombinant vascular K⁺ATP channel, a value of 5.8 (5.2–6.2) nmol/l was obtained (20). $K_i$ value of high-affinity component, which comprised 87% of specific binding; the low-affinity component (13%) gave $K_i = 180$ nmol/l.

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may affect the cardiovascular outcome of type 2 diabetic patients.

THE SUR BINDING SITE(S) FOR SULFONYLUREAS AND GLINIDES

In the sulfonylureas (for selected structures, see Fig. 2), the nitrogen next to the sulfoxide is acidic with pKₐ values ranging from 5.3 (glibenclamide and tolbutamide) to 6.3 (glimepiride). Sulfonylureas act from the inside of the cell; they cross the cell membrane in the undissociated form, but it is the negatively charged form that binds to SUR (11). The first-generation sulfonylureas were the short-chain sulfonylureas such as tolbutamide, glibornuride, and gliclazide. The second generation comprises the long-chain sulfonylureas such as glibenclamide and glimepiride, with a new aromatic moiety attached to the short chain via a carboxamido group (–CONH–) (Fig. 2). These compounds bound to SUR1 with a higher affinity than the short-chain sulfonylureas, and it was hypothesized that the binding pocket of glibenclamide comprises two parts (or subsites): site A, which accommodates the old (short-chain sulfonylurea) part of the compound, and site B for the new carboxamido part (12). This “two-sites hypothesis” of glibenclamide binding was supported by the finding that meglitinide, the carboxamido-part of glibenclamide with the negative charge provided by benzoic acid (Fig. 2), also blocked the channel, albeit with lower potency. New glinides in clinical use or development are repaglinide (a benzoic acid derivative), nateglinide (a D-phenylalanine derivative), and mitiglinide (a 3-phenylpropionic acid derivative) (Fig. 2). The sulfonylureas and glinides have in common the negative charge and the central phenyl ring, suggesting that the binding sites for a type A ligand such as tolbutamide and for a type B ligand such as meglitinide overlap to accommodate these parts (12).

The two-sites hypothesis of glibenclamide binding to SUR1 is now supported by structure-function analysis of cloned SURs. Exploiting the potency difference between tolbutamide blocking the Kir6.2/SUR1 and Kir6.2/SUR2A channels, Ashfield et al. (13) showed that transmembrane segments 14–16 were important for high-affinity block (Fig. 1). In particular, exchange of one amino acid, Ser 1237, in the cytoplasmic loop linking transmembrane segments 15 and 16, by Tyr (which is the corresponding amino acid in SUR2) abolished the block by tolbutamide, rendered the block by glibenclamide readily reversible, and abolished high-affinity [3H]glibenclamide binding to SUR1(S1237Y). However, the block by the benzoic acid derivative meglitinide was unaltered (13). This led to the hypothesis that the short-chain sulfonylureas bound to the region surrounding S1237 of SUR1 (i.e., site A) and that the bulkier Tyr residue in SUR2 resulted in steric hindrance of binding to this site; the binding site of meglitinide was assumed to be in another region of SUR (13). In the photoaffinity labeling studies leading to the cloning of SUR1, it was found that [125I]glibenclamide labeled the NH₂-terminal portion of SUR (rev. in 6), and Mikhailow et al. (14) showed that in SUR1, the third cytosolic loop (CL3), which links the transmembrane domains 0 and 1 (Fig. 1), was essential for [3H]glibenclamide binding. This suggested that CL3 was (an essential part of) the binding region for the carboxamido part of glibenclamide (i.e., site B) (2,3). The region accommodating the negative charge remains unknown.

Comparing the block of wild-type (Kir6.2/SUR1) and mutant (Kir6.2/SUR1(S1237Y)) channels by sulfonylureas and glinides has led to a classification of the secretagogues as interacting with sites A, A+B, or B ligands. Sulfonylureas act from the inside of the cell; they cross the cell membrane in the undissociated form, but it is the negatively charged form that binds to SUR (11). The first-generation sulfonylureas were the short-chain sulfonylureas such as tolbutamide, glibornuride, and gliclazide. The second generation comprises the long-chain sulfonylureas such as glibenclamide and glimepiride, with a new aromatic moiety attached to the short chain via a carboxamido group (–CONH–) (Fig. 2). These compounds bound to SUR1 with a 1,000× higher affinity than the short-chain sulfonylureas. The “two-sites hypothesis” of glibenclamide binding was supported by the finding that meglitinide, the carboxamido-part of glibenclamide with the negative charge provided by benzoic acid (Fig. 2), also blocked the channel, albeit with lower potency. New glinides in clinical use or development are repaglinide (a benzoic acid derivative), nateglinide (a D-phenylalanine derivative), and mitiglinide (a 3-phenylpropionic acid derivative) (Fig. 2). The sulfonylureas and glinides have in common the negative charge and the central phenyl ring, suggesting that the binding sites for a type A ligand such as tolbutamide and for a type B ligand such as meglitinide overlap to accommodate these parts (12).
(or Kir6.2) not reached by glibenclamide or the other compounds.

**K\textsubscript{ATP} CHANNEL SUBTYPE SELECTIVITY**

The \textit{K\textsubscript{ATP}} channel subtype selectivity of the insulin secretagogues has been extensively studied in electrophysiological experiments mostly using recombinant channels expressed in \textit{Xenopus} oocytes (rev. in 2); some data from isolated organs are also available (19). Among the glinides, nateglinide (17) and mitiglinide (18) were shown to be \textit{selective} for the Kir6.2/SUR1 over the Kir6.2/SUR2 channels. This exquisite selectivity is in line with the classification of these secretagogues as type A ligands and is in sharp contrast to the benzoic acid derivatives, meglitinide and repaglinide, which are type B ligands and do not discriminate much between the channel subtypes (2,16). For repaglinide, half-maximal inhibitory concentration (IC\textsubscript{50}) values of 7.4, 8.7, and 10 nmol/l were reported for inhibition of the channels formed by Kir6.2 with SUR1, SUR2A, and SUR2B, respectively, in azide-treated intact \textit{Xenopus} oocytes; in inside-out patches in the absence of nucleotides, IC\textsubscript{50} values of 5.6, 2.2, and 2.0 nmol/l were obtained for inhibition of the respective channels (16). An intermediate selectivity was found for the long-chain sulfonylureas as type A ligands and is in sharp contrast to the benzoic acid derivatives, meglitinide and repaglinide, which are type B ligands and do not discriminate much between the channel subtypes (2,16). For repaglinide, half-maximal inhibitory concentration (IC\textsubscript{50}) values of 7.4, 8.7, and 10 nmol/l were reported for inhibition of the channels formed by Kir6.2 with SUR1, SUR2A, and SUR2B, respectively, in azide-treated intact \textit{Xenopus} oocytes; in inside-out patches in the absence of nucleotides, IC\textsubscript{50} values of 5.6, 2.2, and 2.0 nmol/l were obtained for inhibition of the respective channels (16). An intermediate selectivity was found for the long-chain sulfonylureas as type A + B ligands (rev. in 2). For glibenclamide, IC\textsubscript{50} values of 0.13, 45, and 42 nmol/l were determined in isolated patches from COS cells transfected with Kir6.2 + SUR1, 2A, and 2B, respectively; in macropatches from \textit{Xenopus} oocytes. IC\textsubscript{50} values were 4 and 27 nmol/l for the Kir6.2/SUR1 and/SUR2A channels, respectively (2). The whole-cell current passing through the recombinant vascular channel Kir6.1/SUR2B expressed in HEK cells was inhibited by glibenclamide, with an IC\textsubscript{50} value of 43 nmol/l (value at 37°C) (20).

Regarding the binding step, data for the interaction of secretagogues with Kir6.2/SUR1 are available (2); however, little is known about the binding to the cardiovascular channels. Due to the lower affinity of these channels for glibenclamide, binding studies were generally performed using the opener [\textsuperscript{3}H]P1075 as the radioligand. However, [\textsuperscript{3}H]P1075 senses the binding of glibenclamide to SUR2 by negative allosteric interactions and, in such experiments, the true affinity of SUR2 for glibenclamide (and probably for the other secretagogues, too) is underestimated (20,21). For these reasons we have studied here the binding of selected sulfonylureas and glinides to the Kir6.2/SUR1 and Kir6.2/SUR2A channels, using [\textsuperscript{3}H]glibenclamide as the radioligand. Experiments were performed at 37°C in intact HEK cells transiently expressing the respective channels as described earlier (20,21).
the relatively low ratios for the pure type A ligands, glibornuride and nateglinide. For glibornuride, the binding study gave a selectivity ratio of 14, which is in agreement with that obtained for the comparison with the recombinant vascular KATP channel, Kir6.1/SUR2B ($K_\text{i} = 5.8 \text{nmol/l}$) (20). For repaglinide, the lack of selectivity found in the electrophysiological studies (16) is confirmed (Table 1, Fig. 4). Regarding the three type B ligands [repaglinide, meglitinide, and (−)AZ-DF 265], Fig. 4 shows that they lie near, above, or below the line of identity. This indicates that ligands for the B site can exhibit some selectivity for either the cardiac or the pancreatic channel with selectivity ratios varying from 0.26 for meglitinide (i.e., $4 \times$ cardioselective) to 6.8 for (−)AZ-DF 265. The relative cardioselectivity of meglitinide makes a modest contribution to the exquisite selectivity (>10,000) of HMR 1883 for the cardiovascular over the pancreatic channel (7).

**FUNCTION OF K$_{\text{ATP}}$ CHANNELS IN THE CORONARY VASCULAR BED IN NORMOXIA AND ISCHEMIA**

The functionally important K$_{\text{ATP}}$ channel in vascular myocytes is Kir6.1/SUR2B. In several vascular beds, it is opened by local or circulating vasodilator transmitters (5), and its opening leads to vasorelaxation by a variety of pathways (22). In addition, there is a K$_{\text{ATP}}$ channel in endothelial cells consisting of SUR2B + Kir6.1 and/or Kir6.2 (23). Opening of this channel hyperpolarizes the endothelial cell and increases Ca$^{2+}$ entry (24), which in turn promotes the synthesis of nitric oxide (NO) and other vasorelaxant factors and increases the permeability of the wall (23, 24). That pinacidil (a K$_{\text{ATP}}$ channel opener) did not decrease blood pressure in the Kir6.1 knockout mouse (25) argues that the endothelial K$_{\text{ATP}}$ channel has the same composition as that in vascular myocytes (i.e., Kir6.1/SUR2B).

Under resting conditions, the coronary vasculature of several species responds to glibenclamide with vasoconstriction and a reduction of coronary blood flow (5). The importance of K$_{\text{ATP}}$ channels for the regulation of coronary tone is increased in various pathological states (e.g., in hyperlipidemic pigs [26] and in dogs with alloxan-induced diabetes [27], left ventricular hypertrophy [28], or pacing-induced heart failure [29]). To knock out the vascular channel, both Kir6.1$^{-/-}$ (25) and SUR2B$^{-/-}$ mice (30) were generated. Both phenotypes showed spontaneous ischemic ST elevations in the electrocardiogram, arteriovenous (AV) blocks of various degrees, and, finally, asystole (i.e., sudden cardiac death) (25, 30) due to transient coronary vasospasms (30). This phenotype resembles Prinzmetal (vasospastic) angina in humans (25, 30), and it suggests that, in humans, loss-of-function mutations in the Kir6.1 or SUR2B genes may genetically predispose for Prinzmetal angina. In patients with coronary artery disease, infusion of glibenclamide (40 μg/min) in a coronary conduit artery decreased vessel diameter by 7.2%, increased coronary resistance by 28%, and reduced flow by 18%; higher doses were not given in order to avoid confounding effects on insulin levels and blood glucose (31). However, another study showed that at therapeutic doses of glibenclamide (50 μg/kg i.v.), insulin levels were increased but coronary blood flow and vasodilator response to adenosine and papaverine were unchanged in poststenotic and angiographically normal coronary arteries of patients with coronary artery disease (32).

During exercise, coronary blood flow increases. In exercising swine and dog, adenosine, NO, and glibenclamide-sensitive K$_{\text{ATP}}$ channels are major players in recruiting coronary reserve. In various species (5), including the human (33), the reactive hyperemia following transient cardiac ischemia and hypoxia is strongly glibenclamide sensitive. In pressurized human coronary arterioles dissected from right atrial appendages, hypoxia hyperpolarized the vascular smooth muscle cells and induced vasodilation in an endothelium-independent manner; glibenclamide (1 μmol/l) completely reversed the hyperpolarization and reduced the vasodilator response by 65% (33). In coronary arterioles from patients with type 1 and type 2 diabetes, the responses to hypoxia and to the K$_{\text{ATP}}$ channel opener aprikalim were greatly attenuated (33). Under the proviso that these observations in atrial arterioles are representative for the coronary resistance vessels in general, these results indicate that the vascular K$_{\text{ATP}}$ channel plays a major role in the autoregulation of coronary blood flow in the human heart in hypoxia and that the function of this channel is impaired in patients with type 1 or type 2 diabetes (33).

**K$_{\text{ATP}}$ CHANNELS IN CARDIAC MYOCYTES: FUNCTION IN THE ADAPTIVE RESPONSE TO STRESS AND ROLES IN ISCHEMIA**

Experiments with the Kir6.2$^{-/-}$ mouse have shown that functional sarcK$_{\text{ATP}}$ channels (Kir6.2/SUR2A) are required for adaptation of the heart to stress (34). In severe ischemia and hypoxia, the decrease in the ratio of ATP to ADP (rev. in 35) and the increase in oleoyl-coenzyme A...
ester levels (36) lead to a massive opening of sarcK\textsubscript{ATP} channels. Simultaneously, a variety of tissue hormones (among them adenosine) are released, which triggers the process of ischemic preconditioning. This mechanism was first linked to the opening of the sarcK\textsubscript{ATP} channel, later to the opening of the mitoK\textsubscript{ATP} channel, and then to the opening of both channels (9,10). In the following, the physiological consequences of the opening of these channels are discussed with emphasis on the human heart.

sarcK\textsubscript{ATP} CHANNELS AND ADAPTATION OF THE HEART TO STRESS

Until recently, it was thought that under physiological conditions, sarcK\textsubscript{ATP} channels had no function since the high ATP concentrations in the healthy cardiomyocyte kept them locked in the closed state. This notion changed dramatically when it was found that Kir6.2\textsuperscript{−/−} mice were much less tolerant to physical and sympathetic stress than wild-type mice (34). The sarcK\textsubscript{ATP} channel-deficient mice performed poorly in the treadmill exercise test. Under vigorous β-adrenergic stimulation, these animals died from arrhythmia; cardiomyocytes showed a diminished reduction in action potential duration, Ca\textsuperscript{2+} overload, and myocardial contraction bands (34). Such phenomena may not be restricted to the mouse with its high heart rate (>600 bpm); in humans, defective regulation of channel activity due to mutations in SUR2A has been linked to the occurrence of dilated cardiomyopathy (37).

OPENING OF sarcK\textsubscript{ATP} CHANNELS IN CARDIAC HYPOXIA AND ISCHEMIA: PATHOPHYSIOLOGICAL AND CLINICAL IMPLICATIONS

The opening of sarcK\textsubscript{ATP} channels in cardiac hypoxia and ischemia reduces action potential duration and clamps the cardiomyocyte at the potassium equilibrium potential, rendering the cell unexcitable. Whereas this may salvage ATP and preserve the structural integrity of the cell, it also increases the electrical heterogeneity of the heart and promotes reentry arrhythmias (35). In addition, a prolonged opening of sarcK\textsubscript{ATP} channels leads to the accumulation of extracellular K\textsuperscript{+} in the ischemic zone, depolarizes the cell, and induces cytotoxic Ca\textsuperscript{2+} entry (35). Along these lines, prevention of the opening of these channels in ischemia was shown to protect against ischemia-induced ventricular fibrillation (35). It may be considered a proof of concept that the selective sarcK\textsubscript{ATP} channel blocker, HMR 1883, afforded effective protection against sudden cardiac death in rats, pigs, and dogs subjected to cardiac ischemia after coronary artery ligation (7).

Using the selective block of sarcK\textsubscript{ATP} channels as an antiarrhythmic strategy in cardiac ischemia, however, raises two points of concern. First, the opening of sarcK\textsubscript{ATP} channels in cardiac ischemia leads to heterogeneity of the plateau phase of myocardial action potentials and induces the deviation (elevation or depression) of the ST-segment of the electrocardiogram observed in cardiac ischemia (38). Accordingly, in animals with experimental cardiac ischemia, pretreatment with sulfonylureas or HMR 1883 (7,35) blunted the deviation. In a recent retrospective study, the electrocardiogram (ECG) charts from 88 patients with type 2 diabetes and myocardial infarction were analyzed (39). In patients treated with “sulfonylureas of all kinds,” the ischemia-induced ST elevation was reduced and these patients were significantly less likely to meet the standard ECG criteria for admission to thrombolytic therapy (39). The small number of patients did not allow the authors to take into account the differing selectivity ratios of the insulin secretagogues for the K\textsubscript{ATP} channel subtypes (nor the different plasma half-lives of the drugs). In an accompanying commentary, Brady and Jovanovic (40) pointed out several limitations of the study. In the light of this new evidence, however, they recommended to discontinue sulfonylurea treatment in type 2 diabetic patients with suspected acute coronary syndrome and to infuse insulin instead, if necessary, until myocardial ischemia was ruled out (40); this opinion is expressed frequently (see CLINICAL PERSPECTIVES).

The second point of concern is that functional sarcK\textsubscript{ATP} channels are required for the adaptation of the heart to stress. In addition, recent experiments in mice have clearly shown that opening of the sarcK\textsubscript{ATP} channel plays a central role in ischemic preconditioning, at least in this species (1,10).

ISCHEMIC PRECONDITIONING: sarcK\textsubscript{ATP} AND mitoK\textsubscript{ATP} CHANNELS

Prolonged cardiac ischemia leads to the death of cardiomyocytes by necrosis and apoptosis. Ischemic preconditioning (i.e., the protection that a short ischemic event, or stimulus, affords against a prolonged ischemic insult and subsequent reperfusion) occurs in two temporal windows. The early phase lasts 1–3 h after the stimulus (classic preconditioning); the delayed phase (second window) of protection appears ~18 h later and lasts for up to 3 days (9,10). The triggers for the two phases of ischemic preconditioning are essentially the same: release of adenosine, bradykinin, norepinephrine, and opioids. NO, however, is more effective in triggering the delayed response. Great progress has been made in delineating the complex signaling chains that mediate the two windows of preconditioning (9,10). Work in Kir6.2\textsuperscript{−/−} mice demonstrated that opening of the sarcK\textsubscript{ATP} channel (perhaps via protein kinases C [PKC] and an adenosine pathway [10]) is a necessary step in the first phase of preconditioning in this species (1); in wild-type mice, pharmacological analysis showed that the delayed phase of ischemic preconditioning is also mediated via sarcK\textsubscript{ATP} channel opening (10).

There is, however, overwhelming pharmacological evidence also for a mitochondrial pathway of ischemic preconditioning (9,10,41). This is mainly based on the differential effects of the “mitoK\textsubscript{ATP} channel–selective” blocker 5-hydroxydecanoate versus the sarcK\textsubscript{ATP} channel–selective blocker HMR 1883 or on the effects of the “mitoK\textsubscript{ATP} channel–selective” opener diazoxide (9,41). The selectivity of 5-hydroxydecanoate and diazoxide for the mitoK\textsubscript{ATP} channel is, however, not established beyond doubt (41). At reasonable concentrations, both substances target also proteins other than K\textsubscript{ATP} channels, and the very existence of the mitoK\textsubscript{ATP} channel has been called into question (42). On the other hand, an opener with improved selectivity for the mitoK\textsubscript{ATP} channel has been characterized recently (43). Whatever the pathway, mitochondria play an essential part in ischemic preconditioning.
Unfortunately, little is known about the ability of the clinically used insulin secretagogues to interfere with ischemic preconditioning with the exception of glibenclamide and glimepiride. In the Langendorff-perfused rat heart, glibenclamide (10 μmol/l) abolished ischemic preconditioning completely, whereas glimepiride (10 μmol/l) was ineffective (44).

Ischemic preconditioning also occurs in the human heart. The most direct evidence stems from patients undergoing coronary artery bypass grafting. If prolonged global ischemia (by aortic clamping) or cardioplegic arrest were preceded by a preconditioning stimulus and reperfusion, the ischemic damage as judged by troponin C release or decrease in ATP content in cardiac muscle was alleviated (9). Experiments in human ventricular myocytes and atrial trabeculae have provided evidence for both the early and delayed phases of ischemic preconditioning in the human heart and for the involvement of the mitochondrial pathway in these phenomena (9). As an example, recovery of contractile force of atrial trabeculae obtained from nondiabetic patients or from diabetic patients taking insulin was augmented by ischemic preconditioning; however, ischemic preconditioning was ineffective when applied to trabeculae from type 2 diabetic patients taking glibenclamide (2×5 mg/day; n = 6) or glipizide (10 mg/day; n = 1). This suggests that chronic treatment with these sulfonylureas abolishes ischemic preconditioning (45). Ample but indirect evidence for ischemic preconditioning in patients with coronary artery disease is provided by phenomena such as the “warm-up” or “first effort” angina (i.e., the conditioning of the heart by a first exercise-induced angina attack for subsequent exercise) or by the fact that a first angina attack limits the consequences of a following infarction if the time window between the two events is 24–72 h (9). A large body of literature treats the effects of repeated balloon inflation in patients undergoing coronary angioplasty. If the duration of the conditioning inflation exceeded 60–90 s, indicators of myocardial ischemia such as chest pain, ST-segment elevation, or lactate production were attenuated during subsequent inflations (9). Pretreatment with glibenclamide was repeatedly shown to abolish or reduce this effect, whereas glimepiride at equivalent hypoglycemic doses did not (46–48). These studies must, however, be interpreted with caution since ST-segment elevation was used as the only (46,47) or a major (48) indicator of the ischemic burden. As mentioned above, pretreatment with sulfonylureas may blunt the ST-segment deviation in humans (39), and, at least in rabbits, ST-segment elevation is a parameter dissociated from ischemic preconditioning (49). These controversies show that ischemic preconditioning is difficult to quantify in the clinical setting; in addition, aging reduces the effectiveness of preconditioning in animal models and, possibly, in patients (9). The effect of most clinically used insulin secretagogues on ischemic preconditioning in the human heart remains unknown.

**CLINICAL PERSPECTIVES**

Type 2 diabetic patients have an increased risk of cardiovascular complications (50). They are generally treated with insulin secretagogues, and, as outlined above, these drugs differ greatly in their selectivity for the pancreatic over the cardiovascular K_\text{ATP} channels. Hence, some of these drugs may inadvertently reduce coronary blood flow at rest (31) and in hypoxia (33). In addition, they may interfere with the ability of the heart to adapt to stress (34), with ischemic preconditioning (44–48), and with the diagnostically important electrocardiographic ST elevation in acute myocardial infarction (39).

Two large-scale prospective and randomized clinical studies have addressed the question of whether chronic treatment of type 2 diabetic patients with sulfonylureas affects cardiovascular outcome. The University Group Diabetes Program (UGDP) study in one arm tested tolbutamide and provided evidence for an increased risk of cardiovascular mortality of ~1% per year as compared with the other arms (51). This result is difficult to reconcile with the high selectivity of tolbutamide for the pancreatic K_\text{ATP} channel, and arguments were raised that the study may be flawed (52). However, the effect of tolbutamide on the mitoK_\text{ATP} channel is unknown, and an interference with ischemic preconditioning could explain the increase in cardiovascular mortality. The U.K. Prospective Diabetes Study (UKPDS) has shown that there was no difference in cardiovascular outcome between the groups assigned to intensive treatment with glibenclamide, chlorpropamide, or insulin (53). Hence, chlorpropamide (a short-chain sulfonylurea) and glibenclamide seem to be acquitted. However, concern about glibenclamide and sulfonylureas in general continues to be raised. As an example, one small-scale study with type 2 diabetic patients suggested that sulfonylureas increase the risk of in-hospital mortality after coronary angioplasty for acute myocardial infarction (54). Another study suggested that patients taking sulfonylureas have an increased incidence of coronary events after a prior myocardial infarction compared with patients treated with insulin, metformin, or diet alone (55). Based on studies of this kind, it is often recommended to discontinue sulfonylurea treatment in clinical situations such as acute coronary syndromes, coronary angioplasty, and coronary artery bypass grafting (29). However, concern about glibenclamide and sulfonylureas in general continues to be raised. As an example, one small-scale study with type 2 diabetic patients suggested that sulfonylureas increase the risk of in-hospital mortality after coronary angioplasty (54). Another study suggested that patients taking sulfonylureas have an increased incidence of coronary events after a prior myocardial infarction compared with patients treated with insulin, metformin, or diet alone (55). Based on studies of this kind, it is often recommended to discontinue sulfonylurea treatment in clinical situations such as acute coronary syndromes, coronary angioplasty, and coronary artery bypass grafting (29). In contrast to these reports, a recent analysis of the UKPDS showed that patients taking a sulfonylurea at the time of a myocardial infarction had the same case fatality (51%) as those not taking a sulfonylurea (53%) (56). Hence, sulfonylureas do definitely not increase case fatality in this group of patients at high cardiovascular risk.

From a survey of the clinical literature, three points emerge: First, in therapy with insulin secretagogues, much emphasis is placed on a potential interference with ischemic preconditioning; however, the effect of most clinically used insulin secretagogues on this phenomenon is unknown. Second, the interference of insulin secretagogues with coronary blood flow is considered more rarely (notable exceptions are refs. 31–33,50,55). The dramatic phenotype of the mice lacking the vascular K_\text{ATP} channel (25,30) may stimulate more work on the channel in the human coronary circulation. Third, in many small-scale studies, all insulin secretagogues were put in the same basket, regardless of their K_\text{ATP} channel subtype selectivity; often the names of the prescribed drugs could not be retrieved. It has been hypothesized that subtype selectivity is of importance for the cardiovascular outcome of diabetic patients (50); however, randomized prospective stud-
ies addressing this point are lacking. At this point, it remains unknown whether the selectivity ratios of the insulin secretagogues determined for the recombinant K\textsubscript{ATP} channels reflect the selectivity for the native human channels in physiological and pathophysiological conditions. In the cardiac myocyte, the sarcK\textsubscript{ATP} channel is physically associated with adenylate and creatine kinase, thereby facilitating delivery of metabolic signals from the mitochondria to the plasma membrane.}

In the diabetic state, vascular K\textsubscript{ATP} channel function is depressed, and the sympathetic surge that patients generally experience at the time of a myocardial infarction may dramatically change the state of the cardiovascular K\textsubscript{ATP} channels (e.g., by phosphorylation). Clearly, these points are not reflected in the in vitro experiments using recombinant channels. Hence, in view of the widely differing in vitro selectivity ratios of repaglinide and nateglinide, a clinical study comparing these two glinides in type 2 diabetic patients with coronary artery disease would be of great interest for both therapeutic and merely scientific reasons.

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