Sulfonylureas stimulate insulin secretion from pancreatic β-cells and are widely used to treat type 2 diabetes. Their principal target is the ATP-sensitive potassium (K<sub>ATP</sub>) channel, which plays a major role in controlling the β-cell membrane potential. Inhibition of K<sub>ATP</sub> channels by glucose or sulfonylureas causes depolarization of the β-cell membrane; in turn, this triggers the opening of voltage-gated Ca<sup>2+</sup> channels, eliciting Ca<sup>2+</sup> influx and a rise in intracellular Ca<sup>2+</sup> which stimulates the exocytosis of insulin-containing secretory granules (1). K<sub>ATP</sub> channels are also found at high density in a variety of other cell types, including cardiac, smooth, and skeletal muscle, and some brain neurons. In all these tissues, opening of K<sub>ATP</sub> channels in response to metabolic stress leads to inhibition of electrical activity. Thus they are involved in the response to both cardiac and cerebral ischemia (2). They are also important in neuronal regulation of glucose homeostasis (3), seizure protection (4), and the control of vascular smooth muscle tone (and, thereby, blood pressure) (5).

The K<sub>ATP</sub> channel is a hetero-octameric complex of two different types of protein subunits: an inwardly rectifying K<sup>+</sup> channel, Kir<sub>6.x</sub>, and a sulfonylurea receptor, SUR (6,7). Kir<sub>6.x</sub> belongs to the family of inwardly rectifying K<sup>+</sup> (Kir) channels and assembles as a tetramer to form the channel pore (Fig. 1A). Binding of ATP to the intracellular domains of this subunit produces channel inhibition (8). SUR is a member of the ABC transporter family, with 17 transmembrane helices (TMs), arranged as one group of 5 TMs, and two repeats each of 6 TMs followed by a large cytosolic loop containing consensus sequences for nucleotide binding and hydrolysis. Interaction of Mg nucleotides with the nucleotide-binding domains (NBDs) mediates activation of the K<sub>ATP</sub> channel (9–12). SUR also endows Kir<sub>6.2</sub> with sensitivity to certain drugs, such as the inhibitory sulfonylureas and the stimulatory K<sup>+</sup> channel openers (6,7).

More than one isoform exists for both Kir<sub>6.x</sub> (Kir<sub>6.1</sub>, Kir<sub>6.2</sub>) and SUR (SUR1, SUR2A, SUR2B). In most tissues, Kir<sub>6.2</sub> serves as the pore-forming subunit, but it associates with different SUR subunits; for example, it associates with SUR1 in the pancreas and brain; SUR2A in heart and skeletal muscle; and SUR2B in brain and smooth muscle (13–15). In vascular smooth muscle, the K<sub>ATP</sub> channel is composed of Kir<sub>6.1</sub> in association with SUR2B. Variation in the subunit composition of the K<sub>ATP</sub> channel accounts for the different metabolic and drug sensitivities of K<sub>ATP</sub> channels in different cells.

Inhibitors of K<sub>ATP</sub> channel activity fall into two groups: those that interact with Kir<sub>6.2</sub> and those that interact with SUR. Imidazolines (e.g., phenotolamine, cibenzoline) and antimalarials (e.g., quinine, mefloquine) block K<sub>ATP</sub> channels by binding to Kir<sub>6.2</sub> (16–18). In contrast, sulfonylureas (e.g., tolbutamide, gliclazide, glimepiride), and benzamido derivatives (e.g., meglinidine) close K<sub>ATP</sub> channels by binding with high affinity to SUR (19–21). Sulfonylureas also interact with Kir<sub>6.2</sub>, but with low affinity. All drugs that block K<sub>ATP</sub> channels stimulate insulin secretion, but only those that interact with the SUR subunit are used therapeutically to treat type 2 diabetes. Thus, in this review, we focus on how studies of recombinant K<sub>ATP</sub> channels have helped to elucidate the molecular mechanism of sulfonylurea inhibition.

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sites are occupied. Kir6.2 is expressed in the absence of SUR (20). The affinity site is independent of SUR, as a similar block is seen when described by a two-site model (Fig. 1 B). The low-affinity site is of no binding affinity, such action. An alternative possibility is that all channels bind drug with high affinity, but they are still able to open when drug is bound, albeit with lower probability. Single-channel currents recorded at sulfonylurea concentrations that saturate the high-affinity site (but do not interact with Kir6.2) can be used to distinguish between these possibilities. If some channels exist in a low-affinity state and others in a high-affinity state, and transitions between these states are relatively slow, there should be two kinetically distinct populations of channels—one with a reduced open probability (P_o) and another with kinetics similar to that observed in the absence of drug. On the other hand, if the reason for incomplete block by sulfonylureas is because the channel can still open when the drug is bound at the high-affinity site(s), then there will be only one type of single-channel kinetics. Under experimental conditions, only a single type of kinetic behavior has been observed in the presence of sulfonylureas (24; P.P., F.M.A., unpublished observations), supporting the idea that the channel can still open when drug is bound. Because it is thought that each SUR1 monomer binds drug independently, all four binding sites should be occupied at saturating drug concentrations of sulfonylureas, a property that can be used to distinguish between these possibilities.

SULFONYLUREAS BLOCK K\textsubscript{ATP} CHANNELS AT TWO SITES

When measured in excised patches in the absence of nucleotides, sulfonylurea block of K\textsubscript{ATP} channels is best described by a two-site model (Fig. 1B). The low-affinity site is independent of SUR, as a similar block is seen when Kir6.2 is expressed in the absence of SUR (20). The high-affinity site lies on SUR, as it is only present when SUR is coexpressed with Kir6.2. The presence of both low- and high-affinity sites for sulfonylureas on the K\textsubscript{ATP} channel introduces important considerations when interpreting experiments with these drugs. First, at high concentrations these drugs may mediate their effects via Kir6.2, and channel inhibition therefore cannot be taken to support the idea that they interact with the sulfonylurea receptor. Second, as discussed below, different SURs endow the K\textsubscript{ATP} channel with variable sensitivity to low concentrations of sulfonylureas, a property that can be used to distinguish the type of sulfonylurea receptor expressed in different tissues. Finally, to isolate effects mediated by the high-affinity site, it is best to use a drug that shows a wide separation between its low- and high-affinity actions, such as glipizide or glibenclamide. The low-affinity site is of no clinical relevance, because the concentrations required to inhibit the Kir6.2 subunit are much higher than those found in the plasma of patients treated with the drugs. Thus, in this review, we focus on the high-affinity drug-binding site.

STOICHIOMETRY

Because four SUR subunits contribute to the K\textsubscript{ATP} channel complex, the question arises as to how many subunits must bind drug to close the channel. This topic is currently controversial. Dörscher et al. (22) found that the affinity of sulfonylurea binding to Kir6.2/SUR1 or Kir6.2/SUR2B channels is between three- and sevenfold lower than their potency on channel inhibition (Table 1). Consequently, they concluded that occupation of a single binding site is sufficient to induce channel closure. In contrast, Russ et al. (23) found that the concentration-response relation for [\textsuperscript{3}H]glibenclamide binding to Kir6.1/SUR2B lies to the left of that for glibenclamide block of channel activity, so that the K\textsubscript{s} is sevenfold lower than the half-maximal inhibitory concentration on channel activity (IC\textsubscript{50}) (Table 1). They therefore suggested that glibenclamide can bind independently to four identical sites on the channel and that occupation of all four sites is required for channel closure (23). Interestingly, the different proposed stoichiometries arise from the different binding affinities measured by these groups (Table 1). It is not clear why the affinities were found to be so different, but possible confounding factors include the nature of the Kir6.x subunit (Kir6.2 or Kir6.1) and the nucleotide concentrations used. The conflicting results suggest that conclusions based on comparing binding affinities with potencies of channel inhibition may not be reliable.

SULFONYLUREA-BOUND CHANNELS CAN STILL OPEN

In the absence of added nucleotides, high-affinity inhibition of the K\textsubscript{ATP} current by sulfonylureas is not complete, but reaches a maximum of 60–80% (Fig. 1B). There are several possible explanations for this finding. One possibility is that some percentage of the channels are in a state in which they do not bind drug with high affinity. An alternative possibility is that all channels bind drug with high affinity, but they are still able to open when drug is bound, albeit with lower probability. Single-channel currents recorded at sulfonylurea concentrations that saturate the high-affinity site (but do not interact with Kir6.2) can be used to distinguish between these possibilities. If some channels exist in a low-affinity state and others in a high-affinity state, and transitions between these states are relatively slow, there should be two kinetically distinct populations of channels—one with a reduced open probability (P_o) and another with kinetics similar to that observed in the absence of drug. On the other hand, if the reason for incomplete block by sulfonylureas is because the channel can still open when the drug is bound at the high-affinity site(s), then there will be only one type of single-channel kinetics. Under experimental conditions, only a single type of kinetic behavior has been observed in the presence of sulfonylureas (24; P.P., F.M.A., unpublished observations), supporting the idea that the channel can still open when drug is bound. Because it is thought that each SUR1 monomer binds drug independently, all four binding sites should be occupied at saturating drug concentrations.
concentrations. Thus it appears that the $K_{ATP}$ channel can still open, even when each of its four SUR subunits has bound sulfonylurea.

The maximal extent of block at the high-affinity site can be modulated by mutations or truncations in either Kir6.2 or SUR (25–28), or by membrane phospholipids such as phosphatidylinositol bisphosphate (PIP$_2$) (29). All these modulators share the common property that they increase the channel $P_O$ (25–27,30,31). We therefore simulated the effect of P$_O$ on sulfonylurea block using the kinetic scheme given in Fig. 2B and rate constants calculated from single Kir6.2/SUR1 channel kinetics recorded in the absence and presence of gliclazide.

As Fig. 2A shows, $K_{ATP}$ channels exhibit bursts of openings separated by long closed periods. Gliclazide reduces the duration of the bursts of openings and increases the long closed intervals. The burst can be described by one open (O) and one short closed (C$_1$) state, and the long closings between bursts can be lumped together into a single long closed state (C$_2$). We assume that all these states can bind sulfonylurea, so that the channel can exist in both open and closed states when sulfonylurea is bound. We further assume that when drug is bound to SUR1, this either has no effect on channel activity (bound but untransduced states) or results in a conformational change that affects the channel kinetics (bound and transduced states). In the former case, the channel flickers between states O$_D$, C$_{1B}$, and C$_{2B}$ with the same rate constants as in the absence of drug. In the second case, the channel undergoes a conformational change that either results in channel closure (the $O_B$ to $C_T$ transition) or stabilization of the closed state (C$_{2B}$ to C$_{2T}$). We made the simplifying assumption that the major contribution to the channel $P_O$ in the presence of sulfonylurea is produced by states with only a single drug molecule bound (i.e., only one SUR in the tetramer is occupied).

This model explains the incomplete block by sulfonylureas, because, even when drug is bound, the channel does not inevitably enter the closed states C$_{2B}$ and C$_T$. Entry into these closed states is not, therefore, directly proportional to the drug concentration, and channel opening is still possible, even at very high concentrations of the drug.

The concentration-response curve for the high-affinity site is described by the following:

$$G/G_c = \frac{1 + L \times ([S]/IC_{50})}{1 + ([S]/IC_{50})^h}$$

(1)

where $G$ is the conductance in the presence of the drug, $G_c$ is the conductance in the absence of drug, $L$ is the fraction of current that remains unblocked in the presence of a saturating concentration of sulfonylurea, $h$ is the Hill coefficient, and IC$_{50}$ is the drug concentration ([S]) at which the fraction of the current inhibitable with high affinity is blocked by 50%. The kinetic scheme depicted in Fig. 2B allowed us to derive analytical equations that could be used to calculate the relation between the channel $P_O$ and $L$ (Fig. 2C), and between $P_O$ and IC$_{50}$ (Fig. 2D). It is clear that both $L$ and IC$_{50}$ increase with P$_O$. The value of $L$ reaches unity when the P$_O$ is maximal, which, in the case of the $K_{ATP}$ channel, is $\sim 0.85$, so that the model predicts that there will be no channel inhibition when P$_O$ is maximal. This has in fact been observed for channels containing the mutation C166S, which have a $P_O$ close to maximum (27). As predicted, reducing the P$_O$ of Kir6.2-C166S/SUR1 channels with ATP partially restored sulfonylurea sensitivity (25).

Deletion of the NH$_2$-terminus of Kir6.2 (Kir6.2AN) results in an increase in the channel P$_O$ and a concomitant

### Table 1

Comparison of the IC$_{50}$ for $K_{ATP}$ channel inhibition by various sulfonylureas with the $K_d$ for drug binding

<table>
<thead>
<tr>
<th></th>
<th>SUR1</th>
<th>SUR2A</th>
<th>SUR2B</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IC$_{50}$</strong></td>
<td><strong>$K_d$</strong></td>
<td><strong>IC$_{50}$</strong></td>
<td><strong>$K_d$</strong></td>
</tr>
<tr>
<td>(nmol/l)</td>
<td>(current)</td>
<td>(binding)</td>
<td>(current)</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>0.13 ±[22] $^\dagger$</td>
<td>$0.7^* ±[22] $^\dagger$</td>
<td>45 ±[22] $^\dagger$</td>
</tr>
<tr>
<td>Glipizide</td>
<td>4.2 ±[21] $^\dagger$</td>
<td>7.1 $^* ±[22] $^\dagger$</td>
<td>27 ±[21] $^\dagger$</td>
</tr>
<tr>
<td>Glimepiride</td>
<td>5.4 ±[21] $^\dagger$</td>
<td>$29^* ±[22] $^\dagger$</td>
<td>85 ±[22] $^\dagger$</td>
</tr>
<tr>
<td>Gliclazide</td>
<td>1.2 ±[22] $^\dagger$</td>
<td>$7^* ±[22] $^\dagger$</td>
<td>0.53 ±[21] $^\dagger$</td>
</tr>
<tr>
<td>Mitiglinide</td>
<td>3.8 ±[32] $^\dagger$</td>
<td>60 $^* ±[32]$</td>
<td>3200 $^* ±[33]$</td>
</tr>
<tr>
<td>Repaglinide</td>
<td>5.6 ±[53] $^\dagger$</td>
<td>1,600 $^* ±[32]$</td>
<td>2.2 ±[53] $^\dagger$</td>
</tr>
<tr>
<td>Nateglinide</td>
<td>0.8 ±[53] $^\dagger$</td>
<td>8 $^* ±[32]$</td>
<td>0.2 $^* ±[55]$</td>
</tr>
</tbody>
</table>

Only studies on recombinant SUR (indicated) are listed. * SURx expressed in the absence of Kir6.2; † SURx coexpressed with Kir6.1. The $K_d$ was measured by displacement of [3H] glibenclamide or, if no data were available, of [3H]P1075 (indicated). Numbers in square brackets indicate references.
reduction in tolbutamide block when Kir6.2ΔN is co-expressed with SUR1 (25). The reduction in block produced by deletion of up to nine residues can be accounted for simply by the increase in $P_O$. However, deletion of the first fourteen NH2-terminal residues produced a complete loss of tolbutamide inhibition, an observation that cannot be explained solely by an increase in $P_O$. In addition, decreasing $P_O$ with ATP was unable to restore the tolbutamide sensitivity of this deletion mutant (25). Thus it appears that NH2-terminal residues may be involved in transducing sulfonylurea binding to SUR1 into closure of the Kir6.2 pore.

**SPECIFICITY OF HIGH-AFFINITY SULFONYLUREA BLOCK**

Studies of cloned channels have revealed that certain $K_{ATP}$ channel inhibitors (tolbutamide, gliclazide, and mitoglinide) (19–21,32,33) block $K_{ATP}$ channels containing SUR1, but not SUR2A or SUR2B, subunits with high affinity. In contrast, meglitinide blocks all three types of cloned $K_{ATP}$ channel with similar affinities (21). This finding has been interpreted to indicate that sulfonylureas resembling tolbutamide in structure interact with a binding site that is specific to SUR1, whereas benzamido compounds and their derivatives (like meglitinide) interact with a site that is common to all SUR subtypes. Glibenclamide and glimepiride, which contain both sulfonylurea and nonsulfonylurea moieties and block both SUR1- and SUR2-containing channels, are postulated to interact with both sites on SUR1, but only a single (benzamido-derivative) site on SUR2 (21,34). The binding affinities and inhibitory potencies of these drugs for the high-affinity sites of different types of $K_{ATP}$ channels are illustrated in Table 1.

**LOCATION OF THE SULFONYLUREA BINDING SITE ON SUR**

A single residue within the COOH-terminal set of transmembrane domains accounts for the difference in tolbutamide sensitivity of SUR1- and SUR2-containing channels. Mutation of serine 1237 in the cytoplasmic loop between TMs 15 and 16 to tyrosine abolishes the high-affinity site for simply by the increase in $P_O$. However, deletion of the first fourteen NH2-terminal residues produced a complete loss of tolbutamide inhibition, an observation that cannot be explained solely by an increase in $P_O$. In addition, decreasing $P_O$ with ATP was unable to restore the tolbutamide sensitivity of this deletion mutant (25). Thus it appears that NH2-terminal residues may be involved in transducing sulfonylurea binding to SUR1 into closure of the Kir6.2 pore.

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β-cell K<sub>ATP</sub> channels, is not easily reversed in electrophysiological recordings, whereas glibenclamide block is readily reversed when SUR1 contains the S1237Y mutation. Kir6.2/SUR2 channels, which already possess a tyrosine at this position, also show reversible block by glibenclamide and glimepiride. In addition, glibenclamide binding to SUR1 is greatly decreased by the S1237Y mutation (35), whereas glibenclamide binding to SUR2B is enhanced by the reverse mutation (36,37). Thus S1237 must contribute to the binding site for sulfonylureas on SUR1. It is possible that the bulkier volume of the tyrosine side chain, as compared to serine, sterically hinders the binding of the sulfonylurea moiety, thereby accounting for its inability to interact with SUR2.

The fact that glibenclamide blocks Kir6.2/SUR1-S1237Y channels indicates that residues other than S1237 are critical for binding of this drug. Likewise, the block of Kir6.2/SUR1 by meglitinide and repaglinide, which do not possess a sulfonylurea moiety, is unaltered by the S1237Y mutation (35,53), suggesting that these drugs do not interact with this residue. Recent studies have shown that two nonadjacent cytosolic loops of SUR1, linking TMs 5 and 6 and TMs 15 and 16, are critical for [3H]glibenclamide binding (38). Because the TM 15–16 loop contains S1237, this raises the possibility that the TM 5–6 loop may interact with benzamido compounds and their derivatives. Interestingly, the drug-binding site of the related ABC transporter MRP1 also involves sites in both NH<sub>2</sub>- and COOH-terminal parts of the protein, and the TM 5–6 loop is essential for high-affinity drug binding (39).

These data do not imply that only residue S1237 in the TM 15–16 loop and unidentified residues in the TM 5–6 loop interact with sulfonylureas. However, it is clear that these residues are critical for high-affinity interactions. This suggests that despite their separate location in the primary structure of SUR, the cytosolic loops between TMs 5–6 and TMs 15–16 lie in close proximity in the three-dimensional structure of the channel. The maximal distance between them must be the length of the glibenclamide molecule (17 Å), but because the molecule is very flexible, they could be much closer. The location of the sulfonylurea binding site(s) within the cytosolic loops of SUR is consistent with earlier studies suggesting an intracellular site of action for sulfonylureas (40).

INTERACTIONS BETWEEN Kir6.2 AND SUR

It is well established that the sulfonylurea receptor can modulate the activity of Kir6.2, because binding of sulfonylureas or MgADP to SUR results in a decrease or increase of channel activity, respectively. There is growing evidence that Kir6.x can also affect the properties of SUR. Co-expression of Kir6.1 with SUR2A, or mutant SUR2B, has been shown to increase the affinity of [3H]glibenclamide binding by fivefold and fourfold, respectively (23,36). An even greater increase in affinity (eightfold) was found when Kir6.2 was co-expressed with mutant SUR2B (36). Coexpression of Kir6.2 with SUR1 has variously been reported to increase the affinity of [3H]glibenclamide binding (32) and to not affect it (22,41). Interestingly, Dörscner and colleagues (22) found no change in the [3H]glibenclamide binding affinity of SUR1 or SUR2 on co-expression with Kir6.x. These discrepancies may be a result of the different experimental conditions used by the groups or different fractions of uncoupled SUR subunits in the presence of Kir6.x. It is clear that additional experiments are needed to resolve this controversy.

MODULATION OF SULFONYLUREA BLOCK BY PIP<sub>2</sub>

The phospholipid PIP<sub>2</sub> reduces the maximal block produced by saturating concentrations of tolbutamide (28) and glibenclamide (29). It also increases the IC<sub>50</sub> of the drug-sensitive fraction of channels. The effects of PIP<sub>2</sub> are attenuated by the mutation R176A in Kir6.2 (29), which is believed to reduce PIP<sub>2</sub> binding to this subunit. Furthermore, negatively charged lipids reduce glibenclamide block with similar potency, whereas neutral or weakly charged lipids have no effect. As described above, the effects of PIP<sub>2</sub> on sulfonylurea sensitivity can be largely accounted for by the increase in channel P<sub>O</sub> produced by the phospholipid. However, it is also possible that PIP<sub>2</sub> (like the NH<sub>2</sub>-terminal deletions) has additional effects on sulfonylurea block that are masked by the effect on the channel P<sub>O</sub>. The amount of PIP<sub>2</sub> in the membrane can vary as a result of altered phospholipid metabolism. Thus PIP<sub>2</sub> is expected to fall in response to ischemia (because falling ATP levels reduce its generation from PIP by phosphorylation) and increase when phosphatidylinositol kinases are stimulated. This means that PIP<sub>2</sub> regulation of glibenclamide block will also be subject to regulation.

MODULATION OF SULFONYLUREA BLOCK BY Mg NUCLEOTIDES

As Fig. 1B shows, in the excised patch, the concentration-response curve for K<sub>ATP</sub> channel inhibition by sulfonylureas is best fit by assuming both high- and low-affinity sites, with a maximum of 60–70% block at the high-affinity site. This is not the case when sulfonylurea block is measured in whole cells. Remarkably, sulfonylureas produce complete (>90%) high-affinity inhibition of both the native β-cell K<sub>ATP</sub> channel (42,43) and its cloned counterpart, Kir6.2/SUR1 (44), when measured in the whole-cell configuration. This difference may arise because experiments on recombinant K<sub>ATP</sub> channels have largely been carried out in excised patches, whereas experiments on native K<sub>ATP</sub> channels have mainly been carried out in whole cells. We therefore compared the concentration-response curve for gliclazide block of Kir6.2/SUR1 currents when measured in the intact cell with that in the inside-out patch.

Figure 3A and B shows that there was no significant difference in the gliclazide concentration producing half-maximal block of Kir6.2/SUR1 channels or native β-cell K<sub>ATP</sub> channels when measured in the whole cell (108 ± 3 and 184 ± 30 nmol/l, respectively), and the maximum block was similar, being >90% in both cases. In contrast, there was a marked difference in the maximal extent of gliclazide block of Kir6.2/SUR1 currents when measured in the intact cell and in the excised patch, which amounted to 93 and 60%, respectively.

One explanation for the difference in the shape of the concentration-response curve in excised patches and whole cells is that it results from the presence of intracellular MgADP, which is always present in the intact cell, but not, unless deliberately added, in excised patches. Intrac-
Gliclazide block of Kir6.2/SUR1 currents in inside-out patches was also much greater in the presence of 100 μmol/l MgADP (Fig. 3C), and the concentration-inhibition relation was best fit with a single-site model with an IC_{50} of 204 ± 7 nmol/l (n = 5). This finding was consistent with the idea that the difference in the maximal block mediated via the high-affinity site in excised patch and whole-cell recordings results from the presence of MgADP in the intact cell.

MgADP has both stimulatory and inhibitory effects on the K_{ATP} channel, mediated by the SUR and Kir6.2 subunits, respectively (8). It has previously been hypothesized that the nucleotide produces an apparent enhancement of sulfonylurea block of Kir6.2/SUR1 currents, because the drug abolishes the stimulatory action of MgADP, while leaving the inhibitory effect untouched (20). Consequently, the combined inhibitory effects of sulfonylurea and nucleotide result in an enhanced inhibition. If this idea is correct, then gliclazide block of mutant K_{ATP} channels that show reduced nucleotide inhibition should be unaffected by MgADP.

Mutation of the arginine at position 50 of Kir6.2 to glycine (Kir6.2-R50G) strongly reduces nucleotide block at the inhibitory site on Kir6.2 (46). The extent of this reduction can be quantified by examining the effect of ADP in the absence of Mg^{2+}, because, in Mg-free solution, the stimulatory effect of ADP is abolished and the inhibitory effect of ADP is unmasked (20). As Fig. 4A shows, when tested in the absence of Mg^{2+}, ADP potently blocked Kir6.2/SUR1 currents, but blocked Kir6.2-R50G/SUR1 currents much less effectively (<15%). As a result of this reduction in nucleotide block, ADP produced an enhanced stimulation of Kir6.2-R50G/SUR1 currents (compared with control currents) when applied in the presence of Mg^{2+}.

In the absence of nucleotides, the extent of gliclazide block of Kir6.2-R50G/SUR1 was similar to that found for the wild-type channel, whether measured in the presence or absence of Mg^{2+} (Fig. 4B and C). Thus the R50G mutation did not interfere with the action of the drug. However, although MgADP enhanced gliclazide block of wild-type channels, this was not the case for Kir6.2-R50G/SUR1 channels; indeed, the currents were actually slightly larger in the presence of the drug (Fig. 4B). These data support the idea that gliclazide reduces the stimulatory action of MgADP on wild-type channels and unmasks the inhibitory effect of the nucleotide. The fact that Kir6.2-R50G/SUR1 currents are somewhat greater in the presence of MgADP than in the presence of Mg^{2+} suggests that MgADP reduces the stimulatory action of MgADP on wild-type channels and unmasks the inhibitory action of the nucleotide. An alternative explanation is that MgADP reduces the potency of gliclazide block, as 10 μmol/l gliclazide would not then saturate the high-affinity site.

If gliclazide prevents the stimulatory action of MgADP, and unmasks its inhibitory effect, then the combined block by gliclazide and ADP on wild-type channels should be similar in the absence and presence of Mg^{2+}. This was in fact observed (Fig. 4B and C). When Mg^{2+} was absent, Kir6.2-R50G/SUR1 currents were of similar amplitude in the presence of gliclazide or gliclazide plus ADP (Fig. 4C). This is presumably because ADP did not cause substantial block of the mutant channel. Because MgATP is the
predominant Mg nucleotide in vivo, we also examined the effect of gliclazide on MgATP activation. Kir6.2-R50G/SUR1 currents were activated by MgATP (Fig. 4A), because the inhibitory effect of the nucleotide was impaired by the R50G mutation (10). As with ADP, the stimulatory action of ATP in the presence of Mg$^{2+}$ was abolished by gliclazide (Fig. 4B).

The potency of gliclazide block was significantly different when measured in the absence and presence of 100 μmol/l MgADP in excised patches (IC$_{50}$ of 50 and 204 nmol/l, respectively). It is therefore possible that MgADP caused a small reduction in drug potency. This might also explain why gliclazide was somewhat less potent in intact oocytes (108 nmol/l). It also would be consistent with the fact that gliclazide block of Kir6.2-R50G/SUR1 currents is less in the presence of MgADP than in its absence. Although the effects of Mg nucleotides on gliclazide binding have not been investigated, it is known that $[^{3}H]$glibenclamide binding to native $\beta$-cell $K_{ATP}$ channels is decreased by MgADP (47). It is not clear whether gliclazide displaces MgADP from the nucleotide-binding domains of SUR1, or if it uncouples MgADP binding from channel activation. However, glibenclamide has been shown to cause unbinding of S-azido-$[^{32}P]$ATP to NBD1 of SUR1 in the presence of MgADP (48), lending some support to the former hypothesis.

**TRANSDUCTION**

Binding of sulfonylureas to the cytoplasmic domains of SUR1 ultimately results in closure of the pore formed by Kir6.2. Precisely how this transduction occurs is unknown. There must be a close physical association between the glibenclamide binding site on SUR and the Kir6.2 subunit, as both proteins can be photoaffinity labeled by $[^{32}P]$glibenclamide (7). Five residues within the first TM of Kir6.2 are essential for interaction with SUR (49), and recent studies have indicated that the first five TMs of SUR1 are also involved (50). However, although these regions are required for structural interactions, it is not clear whether they are also involved in functional coupling. Mutagenesis studies, however, have implicated the NH$_2$-terminus of Kir6.2 and the COOH-terminus of SUR1 in coupling sulfonylurea binding to channel inhibition (25,26).

**CONCLUSION AND PERSPECTIVES**

Sulfonylureas have been used for many years to treat type 2 diabetes, but it is only now that the details of their molecular mechanism of action are beginning to be unraveled. As first proposed by Henquin and colleagues (51), antidiabetogenic sulfonylureas, benzamido compounds, and their derivatives appear to interact with more than one part of the $K_{ATP}$ channel. This suggests that, like other ATP-binding cassette transporters, SUR possesses a large multifaceted drug-binding pocket that can accommodate several structurally distinct compounds. Individual compounds differ in the residues within this pocket with which they interact. Studies of recombinant $K_{ATP}$ channels suggest that the sulfonylurea moiety interacts with residues in the TM 15–16 linker of SUR1, whereas benzamido derivatives may interact with the TM 5–6 linker in the NH$_2$-terminal part of the protein. How these different parts of the sulfonylurea receptor contribute to drug binding, and how this is transduced into changes in channel activity, remains far from clear and awaits determination of the structure of SUR, and its relation to Kir6.2, at atomic resolution.

Currently, sulfonylureas are classified as first- and second-generation drugs, although there is no structural or functional basis for this classification. We now know that some sulfonylureas bind with high-affinity to SUR1, but not SUR2, whereas others interact with both types of SUR. We therefore propose that the classification of sulfonylureas, meglitinide derivatives, and structurally related compounds be changed to reflect the functional differences among these drugs, and that they be referred to instead as SUR1-specific and non-SUR1-specific.

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