Molecular Mechanism of Sulphonylurea Block of \( K_{ATP} \) Channels Carrying Mutations That Impair ATP Inhibition and Cause Neonatal Diabetes

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Intracellular adenine nucleotides influence \( K_{ATP} \) channel activity in multiple ways. Binding of ATP (or ADP) to Kir6.2 results in channel closure (9). Interaction of MgATP or MgADP with the two nucleotide-binding sites (NBS1, NBS2) of SUR1 stimulates channel activity by increasing the channel open probability (\( P_o \)), thereby masking the inhibitory effect of ATP and indirectly reducing ATP block (10–13). It is believed that this interaction is mediated by MgADP occupancy of NBS2 and that MgATP is first hydrolyzed to MgADP (14). MgATP interaction with SUR1 also reduces ATP inhibition at Kir6.2 by most likely allosterically reducing ATP binding (15,16).

In the absence of nucleotides, sulphonylureas bind to SUR1 act as partial agonists and produce a maximum block of \( \approx 50\% \)–80\% (Fig. 1B) (17,18). Like ATP, they reduce the mean open time and burst duration of the channel and increase the frequency and duration of the interburst closures (19,20). Sulphonylureas also decrease Mg-nucleotide binding to SUR1 and, consequently, abolish channel stimulation (Fig. 1A) (17). This unmasks the inhibitory effect of ATP at Kir6.2, which adds to the sulphonylurea block and produces an apparent increase in the inhibitory effect of the drug (Fig. 1B).

Sulphonylurea block, therefore, is predicted to depend on both cell metabolism and the ATP sensitivity of the \( K_{ATP} \) channel. By impairing the latter, ND mutations are expected to reduce the efficacy of sulphonylurea block (Fig. 1A). We explored the effect of a range of ND mutations on sulphonylurea block of the \( K_{ATP} \) channel. These results explain why patients with some mutations respond to sulphonylurea therapy and why those with other mutations do not.

RESEARCH DESIGN AND METHODS

Oocyte studies. For heterologous expression, we used human Kir6.2 (Genbank NM000525 with E23 and I377) and rat SUR1 (Genbank L40624). Site-directed mutagenesis, preparation of mRNA, and isolation and injection of *Xenopus laevis* oocytes with mRNA have been described previously (21). Macroscopic currents were recorded from giant inside-out patches at 20 kHz and digitized at 20 kHz. The pipette solution contained (mmol/L) 140 KCl, 2.0 MgCl\(_2\), 2.6 CaCl\(_2\), and 10 HEPES (pH 7.4 with KOH). The standard internal solution obtained by averaging the current before and after application, 

\[
\frac{I_X}{I_C} = a + \frac{1 - a}{1 + \left(\frac{|X|}{R_{50}}\right)^n}
\]  

(Eq. 1)

where \( I_X \) is the steady-state \( K_{ATP} \) current in the presence of the test nucleotide or drug concentration [\( X \)], \( I_C \) is the current in nucleotide-free (or drug-free) solution obtained by averaging the current before and after application, \( R_{50} \) is the nucleotide (or drug) concentration at which the inhibition is half maximal,
$h$ is the Hill coefficient, and $a$ is the fraction of $K_{ATP}$ current remaining at glilizide concentrations that saturate the high-affinity binding site on SUR1 ($a = 0$ for ATP concentration-inhibition relations).

For noise analysis, the macroscopic mean current ($I$) and variance ($s^2$) were determined from 1 s data segments. Control data were recorded immediately before glilizide application, and test data were recorded once a steady-state condition was reached. $P_0$ and $N$ (number of active channels in the patch) were calculated as described previously (16).

**β-Cell studies.** Mice expressing the Kir6.2-V59M mutation specifically in their pancreatic β-cells (β-V50M) were mated by crossing mice carrying a floxed Kir6.2-V50M gene (8) with RIP-Cre-ER mice (22). Mice were maintained as described previously (8). Tamoxifen (0.4 mL of 20 mg/mL in corn oil) was injected at 12 weeks of age to induce Kir6.2-V50M gene expression. Three days later when blood glucose concentration was >20 mmol/L, islets were isolated and dissociated into single β-cells, and cells were cultured for 1–3 days as previously described (8). Control mice were injected with corn oil alone. All experiments were conducted in accordance with the U.K. Animals (Scientific Procedures) Act 1986 and University of Oxford ethical guidelines.

Whole-cell $K_{ATP}$ currents were recorded from isolated β-cells using the standard whole-cell configuration. β-Cells were distinguished from α-cells by
their size and (in the case of mutant β-cells) their larger steady-state whole-cell $K_{ATP}$ currents. The pipette solution contained (mmol/L) 107 KCl, 1 CaCl₂, 1 MgCl₂, 10 EGTA, 10 HEPES, and 0.3 ATP (pH 7.2 with KOH). The extracellular solution contained (mmol/L) 138 NaCl, 5.6 KCl, 1.2 MgCl₂, 2.6 CaCl₂, and 10 HEPES (pH 7.4 with NaOH). A 100 mmol/L stock solution of glibenclamide was made in DMSO and diluted in extracellular solution as required. Concentration-response curves in Fig. 1B (open circles) and Fig. 7D were fit with

$$I_x = \frac{a}{1 + \frac{1}{IC_{50(1)}^x}} = \frac{1}{1 + \left(\frac{1}{IC_{50(2)}^x}\right)^{h_x}} \quad \text{(Eq. 2)}$$

where $I_x$ and $I_C$ are the current amplitudes in the presence and absence of the drug, respectively; $IC_{50(1)}$ and $IC_{50(2)}$ are the concentrations ($[x]$) at which inhibition is half maximal for the high-affinity and low-affinity sites, respectively; $h_1$ and $h_2$ are slope factors; and $a$ is the fraction of unblocked current at the high-affinity site at saturating drug concentration. For fitting, $h_2$ was fixed at 1.0.

**Statistical analysis.** All values are presented as mean ± SEM. Statistical significance was determined by Student t-test.

**RESULTS**

To explore the effects of sulphonylureas on $K_{ATP}$ channels containing ND mutations, we used the sulphonylurea gliclazide. Gliclazide is technically advantageous because its effects are readily reversible, and when measured in inside-out patches, its concentration-inhibition relationship exhibits a clearly defined separation between block at the high-affinity ($IC_{50} \sim 5$ mmol/L) site on SUR1 and block at the low-affinity ($IC_{50} \sim 3$ mmol/L) site on Kir6.2 (Fig. 1B) (23). We confined our studies to high-affinity block at gliclazide concentrations ≤100 μmol/L because only this is of clinical importance (Fig. 1B) (24).

We also restricted our analysis to mutations in Kir6.2 because these are associated with the most severe reductions in ATP inhibition (7.25–30), and some are known to confer a significantly reduced block by sulphonylureas (28,30,31). We selected four Kir6.2 ND mutations, all of which reduce the ability of ATP to block the channel. Two (R201C and G334D) are located in the putative ATP-binding site (25,26), and two (V59M and E296L) reduce ATP inhibition indirectly by impairing channel gating (20,27). We refer to channels containing these mutations by the name of the mutation (e.g., Kir6.2-R201C/SUR1 is R201C).

**Effects of gliclazide on ATP block of mutant channels.** We first examined the effect of gliclazide on ATP block of R201C, V59M, and E296L channels heterologously expressed in *Xenopus* oocytes. We could not study G334D channels because they are not inhibited by ATP (25,28).

Figure 2 shows $K_{ATP}$ currents and ATP concentration-inhibition relationships for wild-type and R201C channels measured in inside-out patches in the presence and absence of Mg$^{2+}$ or gliclazide. The mutation increased the $IC_{50}$ of ATP from 7 to 98 μmol/L in the absence of Mg$^{2+}$ and from 16 μmol/L to 2 mmol/L in the presence of Mg$^{2+}$ (Table 1). These values are similar to those reported previously for R201C channels expressed in *Xenopus* oocytes (21,26).

In the absence of Mg$^{2+}$, gliclazide caused a small, but significant increase in ATP block of wild-type channels (Fig. 2A and E and Table 1). One possible explanation for this finding is that ATP binding to NBS1 of SUR1 (in an Mg-independent manner) reduces MgATP inhibition and that this effect is reversed by gliclazide. Gliclazide did not alter the $IC_{50}$ for ATP inhibition of R201C channels in the absence of Mg$^{2+}$ (Fig. 2F and Table 1), suggesting that the mutation might impair the ability of ATP binding to SUR1-NBS1 to cause channel closure (and because this is absent, gliclazide has no effect). Why gliclazide reduces the slope of the ATP concentration-inhibition curve is unclear.

Addition of Mg$^{2+}$ reduced ATP block of both wild-type and R201C channels, but the effect was much greater for the mutant channels (Fig. 2E and F), as shown previously (21). In the presence of gliclazide, the MgATP and ATP concentration-inhibition relationships of R201C channels were identical (Fig. 2F and Table 1), suggesting that gliclazide abolishes channel activation by MgATP at SUR1 as it does for wild-type channels.

We next examined the effect of gliclazide on V59M and E296L channels. In the absence of Mg$^{2+}$, gliclazide enhanced ATP block of V59M channels (Fig. 3A and Table 1), suggesting that, as proposed for wild-type channels, ATP binding to SUR1-NBS1 might decrease block at Kir6.2-V59M and that gliclazide abolishes this effect. The ATP sensitivity of V59M and E296L channels were markedly reduced by Mg$^{2+}$, which is expected if MgATP interaction with SUR1 increases $P_0$ and reduces ATP block at Kir6.2. The latter probably makes the greatest contribution because the intrinsic $P_0$ (i.e., in the absence of nucleotide binding) of these channels is extremely high (>0.8) and cannot be increased much more. In the presence of gliclazide, MgATP inhibition of both channels was similar to that in the absence of both drug and Mg$^{2+}$, which is consistent with gliclazide preventing MgATP activation.

**Modification of high-affinity gliclazide block by MgATP.** We next compared the effect of MgATP on gliclazide block of wild-type and mutant $K_{ATP}$ channels. To enable a quantitative comparison, we used an ATP concentration that produces an approximately half-maximal block at Kir6.2. This was obtained from the ATP concentration-inhibition relationship in the absence of Mg$^{2+}$ (Table 1). We used 15 μmol/L for wild-type, 100 μmol/L for R201C and V59M, and 3 mmol/L for E296L channels. For G334D channels, we used 1 mmol/L MgATP because this concentration produced near-maximal activation (16), and in the present study, higher concentrations of ATP (without Mg$^{2+}$) sometimes caused a slight block.

In the absence of MgATP, wild-type, R201C, and G334D channels exhibited similar gliclazide concentration-inhibition curves. The $IC_{50}$ lay between 50 and 70 mmol/L, and inhibition reached a plateau at 50–60% of maximum (Fig. 4), indicating that there is significant channel activity (i.e., $P_0$ is not 0) even when drug-binding sites are fully occupied. These results are similar to previous findings for sulphonylurea block of wild-type (23) and G334D (28) channels in the absence of intracellular nucleotides and indicate that neither the R201C nor the G334D mutation alters the direct block of the channel by gliclazide.

In contrast, gliclazide inhibited V59M channels very poorly and was almost without effect on E296L channels (Fig. 5A and B). These mutations impaired $K_{ATP}$ channel gating, increasing the intrinsic $P_0$ from 0.36 ± 0.06 (n = 6) for wild-type channels to 0.77 ± 0.02 (n = 6) for V59M and 0.85 ± 0.01 (n = 6) for E296L channels. Previous reports indicated that an increased $P_0$ is correlated with reduced sulphonylurea sensitivity (30,32,33). We simulated the effect of $P_0$ on gliclazide block with a Monod-Wyman-Changeux model (see Fig. 6C and D legend for details).

This model predicts that as $P_0$ is increased, the extent of block is reduced, and the $IC_{50}$ increases. For a $P_0$ of 0.77, the maximal predicted block was 22%, which agrees well with that measured for V59M channels (20%). A similar correlation between measured and predicted values was found for I296L channels. Thus, in the absence of
nucleotides, the $P_o$ largely determines the maximal extent of sulphonylurea block.

In the presence of MgATP, the maximal extent of high-affinity gliclazide block of all channels was enhanced (Figs. 4 and 5). The current reached a plateau at a level that was ~50% of that in the absence of MgATP. This finding is expected because we used an ATP concentration close to the IC$_{50}$. Of note, however, is that the ATP concentration required to produce this additional block was substantially greater for mutant channels (e.g., 3 mmol/L MgATP for I296L compared with 15 mmol/L MgATP for wild-type channels).

It has been previously proposed that sulphonylureas abolish the stimulatory effects of Mg-nucleotides on wild-type channels and thus unmask ATP inhibition at Kir6.2, leading to an apparent enhancement of sulphonylurea block (Fig. 1A) (17,33). The present results show the same is true for V59M, I296L, and R201C channels. However, because these channels show less inhibition at Kir6.2, the ability of nucleotides to enhance sulphonylurea block is correspondingly reduced, explaining why much more ATP is required to produce the same maximal block.

Figure 6 plots the current in the presence of gliclazide as a fraction of that in the absence of both drug and nucleotide, showing that with the exception of G334D channels (which are activated) and R201C channels (which are unaltered), MgATP inhibits both wild-type and ND channels. These differences are dictated by the extent to which

![FIG. 2. Effect of gliclazide on ATP block of wild-type and R201C K$_{ATP}$ channels. A-D: Representative Kir6.2/SUR1 (A and C) and Kir6.2-R201C/SUR1 (B and D) currents recorded at −60 mV in the absence (A and B) and presence (C and D) of Mg$^{2+}$. Gliclazide and ATP were added as indicated by the bars. The dotted line indicates the zero current level. E and F: ATP concentration-inhibition curves for Kir6.2/SUR1 (E) and Kir6.2-R201C/SUR1 (F) channels measured in the absence of both gliclazide (SU) and Mg$^{2+}$ (□) and in the absence of gliclazide but in the presence of 2 mmol/L Mg$^{2+}$ (■), and 30 μmol/L gliclazide plus 2 mmol/L Mg$^{2+}$ (●). The lines are the best fit of Eq. 1 to the mean data with the following parameters: Kir6.2/SUR1 (E) IC$_{50}$ = 6.8 μmol/L, $h$ = 1.0 (□); IC$_{50}$ = 4 μmol/L, $h$ = 1.0 (●); IC$_{50}$ = 16 μmol/L, $h$ = 1.0 (▲); and IC$_{50}$ = 4 μmol/L, $h$ = 0.97 (■) and Kir6.2-R201C/SUR1 (F): IC$_{50}$ = 98 μmol/L, $h$ = 1.4 (□); IC$_{50}$ = 2.0 mmol/L, $h$ = 1.1 (○); IC$_{50}$ = 97 μmol/L, $h$ = 0.87 (■); and IC$_{50}$ = 107 μmol/L, $h$ = 0.90 (●). The dotted line is the concentration-inhibition curve for Kir6.2/SUR1 channels in the absence of Mg$^{2+}$ and gliclazide (Fig. 2E) (□). SU, sulfonylurea; WT, wild-type.]

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**FIG. 2. Effect of gliclazide on ATP block of wild-type and R201C K$_{ATP}$ channels.** A–D: Representative Kir6.2/SUR1 (A and C) and Kir6.2-R201C/SUR1 (B and D) currents recorded at −60 mV in the absence (A and B) and presence (C and D) of Mg$^{2+}$. Gliclazide and ATP were added as indicated by the bars. The dotted line indicates the zero current level. E and F: ATP concentration-inhibition curves for Kir6.2/SUR1 (E) and Kir6.2-R201C/SUR1 (F) channels measured in the absence of both gliclazide (SU) and Mg$^{2+}$ (□) and in the absence of gliclazide but in the presence of 2 mmol/L Mg$^{2+}$ (■), and 30 μmol/L gliclazide plus 2 mmol/L Mg$^{2+}$ (●). The lines are the best fit of Eq. 1 to the mean data with the following parameters: Kir6.2/SUR1 (E) IC$_{50}$ = 6.8 μmol/L, $h$ = 1.0 (□); IC$_{50}$ = 4 μmol/L, $h$ = 1.0 (●); IC$_{50}$ = 16 μmol/L, $h$ = 1.0 (▲); and IC$_{50}$ = 4 μmol/L, $h$ = 0.97 (■) and Kir6.2-R201C/SUR1 (F): IC$_{50}$ = 98 μmol/L, $h$ = 1.4 (□); IC$_{50}$ = 2.0 mmol/L, $h$ = 1.1 (○); IC$_{50}$ = 97 μmol/L, $h$ = 0.87 (■); and IC$_{50}$ = 107 μmol/L, $h$ = 0.90 (●). The dotted line is the concentration-inhibition curve for Kir6.2/SUR1 channels in the absence of Mg$^{2+}$ and gliclazide (Fig. 2E) (□). SU, sulfonylurea; WT, wild-type.
MgATP inhibits channel activity at Kir6.2. Wild-type channels, which are the most ATP sensitive, show the greatest block. In contrast, G334D channels, which show no block at Kir6.2 (25), are activated by MgATP (16).

The addition of gliclazide results in further inhibition, the extent of which varies with the ATP sensitivity of the channel. Independent of the molecular mechanism of action of the mutation, channels that are inhibited most strongly by MgATP are also most strongly blocked by gliclazide in the presence of the nucleotide (Fig. 6F and G). For example, R201C and V59M channels are blocked by approximately the same amount (~70%) because the magnitude of ATP inhibition is similar, even though the mechanism by which the mutation affects ATP inhibition differs.

In the case of G334D channels, which are activated by MgATP but not blocked by ATP, gliclazide removes channel activation by 1 mmol/L MgATP but has no further effect. Because the channel is not blocked by ATP, the maximal block is the same whether or not MgATP is present (Fig. 6C). It is also substantially smaller than that found for wild-type channels in 1 mmol/L MgATP but no gliclazide (Fig. 6G). This finding explains why patients with this mutation fail to respond to sulphonylurea therapy (28).

Mg-nucleotides influence gliclazide binding. The IC_{50} for gliclazide block of G334D and R201C channels increased threefold when MgATP was added (Fig. 4B and C). The most likely explanation for this finding is that MgATP binding to the NBSs of SUR1 causes partial displacement of gliclazide from its binding site. Indeed, nucleotide-induced displacement of glibenclamide binding to SUR1 has been reported (34,35). An alternative idea is that MgATP leads to the generation of phosphatidylinositol 4,5-bisphosphate (PIP_{2}), which either impairs gliclazide binding directly or by increasing P_{O} (36).

To distinguish between these possibilities, we measured the effect of various agents on the magnitude of G334D currents in the presence of 100 nmol/L gliclazide (Fig. 7A). At this concentration, the difference in block in the presence (22%) and absence (37%) of 1 mmol/L MgATP is evident (Fig. 4B). Wortmannin (10 μmol/L), which prevents PI_{2} production through inhibition of phosphatidylinositol kinases (37), did not affect block by 1 mmol/L ATP (Fig. 7A), suggesting that PI_{2} is not involved. The addition of 1 mmol/L MgADP, which should not stimulate PI_{2} production, produced an even greater suppression of block than MgATP. Taken together, the data favor the idea that the reduced IC_{50} is attributable to nucleotide binding to the NBSs of SUR1, which produces a conformational change that displaces gliclazide binding. This effect requires Mg^{2+} because it is abolished in Mg-free solution (Fig. 7A). Because MgADP is more effective that MgATP, we postulate that MgADP binding to SUR1 displaces gliclazide binding and that MgATP must be converted to MgADP to be effective.

If the reduction in IC_{50} arises because MgADP interacts with SUR1 to decrease gliclazide binding, it should be abolished by mutations that impair MgADP binding at SUR1. Mutation of the Walker A lysine in either NBD1 or NBD2 of SUR1 markedly reduces the ability of MgADP (or MgATP) to stimulate wild-type channels (10) and impairs MgADP binding (38). We therefore simultaneously mutated the Walker A lysine in both NBD1 and NBD2 of SUR1 to alanine (SUR1-KAKA) and coexpressed SUR1-KAKA with SUR1 to decrease gliclazide binding.
with Kir6.2-G334D. As Fig. 7A shows, the KAKA mutation prevented MgADP from reducing gliclazide block.

In the absence of nucleotide (but presence of Mg²⁺), the gliclazide concentration-inhibition curve for Kir6.2-G334D/SUR1-KAKA channels was similar to that of wild-type channels (Fig. 7B), indicating that the KAKA mutations do not affect drug binding. There was no effect of 1 mmol/L MgADP on either the IC₅₀ or the maximal extent of gliclazide block, which is expected if MgADP binding is abolished by the KAKA mutation. The data are consistent, therefore, with the idea that MgADP can displace gliclazide binding (and vice versa).

Surprisingly, in the presence of 1 mmol/L MgATP, the IC₅₀ was reduced from 63 to 15 nmol/L. Because the KA mutations impair MgATP hydrolysis but not binding (39), this finding suggests that MgATP binding to SUR1-KAKA increases gliclazide binding, which is in contrast to Kir6.2-G334D/SUR1 channels where MgATP binding decreases gliclazide binding to SUR1 (Fig. 4B).

Figure 7C shows the relationship between gliclazide concentration and Pₒ for Kir6.2-G334D/SUR1 and Kir6.2-G334D/SUR1-KAKA channels in cell-attached patches, where channel activity is determined by the balance between the stimulatory and inhibitory effects of intracellular ligands. In the absence of gliclazide, Pₒ was 0.76 ± 0.03 (n = 6) for Kir6.2-G334D/SUR1 and 0.59 ± 0.02 (n = 6) for Kir6.2-G334D/SUR1-KAKA channels. We assume that the latter represents the intrinsic Pₒ because this channel is largely insensitive to either ATP block or MgATP activation. The greater Pₒ of Kir6.2-G334D/SUR1 channels must, therefore, be produced by MgATP hydrolysis at NBS2. Of note, the increase in Pₒ is quite small: only ~25% of total. The maximal block of Kir6.2-G334D/SUR1 and Kir6.2-G334D/SUR1-KAKA channels was only 50 and 30%, respectively, because they are insensitive to ATP inhibition, and thus, only the direct block by gliclazide was observed following abolition of channel activation by the drug.

The IC₅₀ for gliclazide block of Kir6.2-G334D/SUR1 channels in cell-attached patches was 440 nmol/L (Fig. 7C). This is approximately sixfold greater than that measured in the presence of 1 mmol/L MgATP (in excised patches) and can be attributed to the higher Pₒ (0.76 in the cell-attached patch vs. <0.4 in the inside-out patch). The IC₅₀ for gliclazide block of Kir6.2-G334D/SUR1-KAKA channels was substantially smaller, consistent with what was observed in excised patches (Fig. 7B).

High-affinity block of V59M channels in pancreatic β-cells is not complete. Patients with severe ND mutations are treated with much higher doses (~10-fold) of sulphonylureas than those with type 2 diabetes, yet they rarely experience hypoglycemia (2,40–42). A possible explanation is that therapeutic concentrations of sulphonylureas do not completely block β-cell KᵦTMP channels so that excessive insulin secretion is prevented. To explore this hypothesis, we tested the effect of glibenclamide, which is commonly used to treat ND (2,40), on whole-cell channels in the absence (○) and presence (●) of MgATP. Currents are expressed relative to those in the absence of gliclazide. The MgATP concentration was 15 μmol/L for Kir6.2/SUR1, 1 mmol/L for Kir6.2-G334D/SUR1, and 100 μmol/L for Kir6.2-R201C/SUR1, respectively. The IC₅₀ and IC₅₀ are given for each channel type with the following parameters: Kir6.2/SUR1 (A): IC₅₀ = 67 nmol/L, h = 1.3, α = 0.45 (○) and IC₅₀ = 71 nmol/L, h = 1.0, α = 0.20 (●); Kir6.2-G334D/SUR1 (B): IC₅₀ = 67 nmol/L, h = 1.1, α = 0.39 (○) and IC₅₀ = 213 nmol/L, h = 1.0, α = 0.21 (●); and Kir6.2-R201C/SUR1 (C): IC₅₀ = 49 nmol/L, h = 1.2, α = 0.48 (○) and IC₅₀ = 190 nmol/L, h = 1.2, α = 0.27 (●). n = 6 in all experiments.
K\textsubscript{ATP} currents in β-cells isolated from mice expressing a β-cell–specific Kir6.2-V59M mutation (ib-V59M mice) (8).

Figure 7D shows the relationship between glibenclamide concentration and whole-cell K\textsubscript{ATP} current in control and ib-V59M β-cells. Both datasets were best fit by the sum of a high-affinity and a low-affinity inhibitory site. Wild-type currents were almost fully blocked by 100 nmol/L glibenclamide, with an IC\textsubscript{50} for high-affinity block of 2.1 ± 0.2 nmol/L (n = 6 patches, three mice). Occupation of the high-affinity site produced 97 ± 1% inhibition. In contrast, the IC\textsubscript{50} for high-affinity block of ib-V59M β-cells was 7.5 ± 3.5 nmol/L (n = 6 patches, three mice), and 10 ± 1% of current remained at drug concentrations that saturated the high-affinity site. Thus, there was a significant increase in the IC\textsubscript{50} for channel inhibition by glibenclamide, as was observed for gliclazide block of V59M channels in Xenopus oocytes (Fig. 5A). Furthermore, in agreement with our hypothesis, a clear pedestal of current was observed for mutant channels, and there was only a small reduction in block between glibenclamide concentrations of 100 nmol/L and 10 μmol/L.

**DISCUSSION**

These data demonstrate that MgATP increases high-affinity block of ND channels by sulphonylurea, as it does for wild-type channels (17,33). However, because mutant channels are less sensitive to ATP inhibition, much higher...
FIG. 6. ATP modulation of gliclazide block of wild-type and mutant K\textsubscript{ATP} channels. A–E: Gliclazide concentration-inhibition relations for wild-type and mutant channels in the absence (○) and presence (●) of MgATP. Currents are expressed relative to those in the absence of both MgATP and gliclazide. MgATP concentrations were 15 μmol/L (A, Kir6.2/SUR1), 100 μmol/L (B, Kir6.2-R201C/SUR1), 1 mmol/L (C, Kir6.2-G334D/SUR1), 100 μmol/L (D, Kir6.2-V59M/SUR1), and 3 mmol/L (E, Kir6.2-I296L/SUR1). The lines are the best fit of Eq. 1 to the mean data with the following parameters: Kir6.2/SUR1 (A): $IC_{50} = 67$ nmol/L, $h = 1.3$, $a = 0.45$ (○) and $IC_{50} = 71$ nmol/L, $h = 1.0$, $a = 0.10$ (●); Kir6.2-R201C/SUR1 (B): $IC_{50} = 67$ nmol/L, $h = 1.1$, $a = 0.39$ (○) and $IC_{50} = 190$ nmol/L, $h = 1.2$, $a = 0.25$ (●); Kir6.2-G334D/SUR1 (C): $IC_{50} = 140$ nmol/L, $h = 0.88$, $a = 0.31$ (○) and $IC_{50} = 213$ nmol/L, $h = 1.0$, $a = 0.39$ (●); Kir6.2-V59M/SUR1 (D): $IC_{50} = 200$ nmol/L, $h = 0.92$, $a = 0.79$ (○) and $IC_{50} = 49$ nmol/L, $h = 1.2$, $a = 0.48$ (●); and Kir6.2-I296L/SUR1 (E): $IC_{50} = 930$ nmol/L, $h = 1.5$, $a = 0.95$ (○) and $IC_{50} = 1,200$ nmol/L, $h = 0.98$, $a = 0.38$ (●). $n = 6$ in all experiments. Note that in the absence of gliclazide Kir6.2-G334D/SUR1 currents are greater in the presence of ATP than in the absence of ATP because the G334D mutation abolishes the inhibitory effect of ATP at Kir6.2, leaving only the stimulatory effect at SUR1 (C). In contrast,
ATP concentrations are needed to produce the same degree of enhancement. As a consequence, some ND channels are not fully blocked by the drug, even at physiological ATP concentrations, which explains why diabetes cannot be managed by sulphonylureas in patients with certain ND mutations and why hypoglycemia is less common in patients with severe ND mutations than in patients with type 2 diabetes.

**Effects of Mg-nucleotides on gliclazide inhibition of mutant K$_{ATP}$ channels.** The present results are consistent with a mutual antagonism between binding of gliclazide and Mg-nucleotides to SUR1. At low sulphonylurea concentrations, suppression of gliclazide binding by physiological (mmol/L) MgATP concentrations increases the IC$_{50}$ for high-affinity gliclazide block of K$_{ATP}$ currents. Conversely, at high drug concentrations, gliclazide displaces Mg nucleotide binding to NBS2 of SUR1, abolishing the ability of the nucleotide to stimulate channel activity (10–13) and reduce ATP inhibition at Kir6.2 (11,15), which results in an apparent enhancement of the maximal sulphonylurea block at the high-affinity site on SUR1. The magnitude of this increased block is determined by the extent of ATP

currents are smaller in the presence of MgATP for all other channels because both inhibition and activation are present. F and G: Current remaining in the presence of 100 mmol/L MgATP or 1 mmol/L MgATP in the absence (open bars) and presence (solid bars) of 30 μmol/L gliclazide for wild-type channels and K$_{ATP}$ channels carrying ND mutations. The current is expressed as a fraction of that in drug- and nucleotide-free solution. $n = 6$ in all experiments. WT, wild-type.
inhibition at Kir6.2 (as measured in Mg-free solution). This phenomenon predicts that the magnitude of the enhanced block depends on the metabolic state of the cell being smaller when metabolism and ATP levels are low (although whether ATP levels fall sufficiently under physiological conditions for this to occur is unclear). This phenomenon also accounts for the reduced gliclazide block of ND channels, which have impaired ATP sensitivity.

As the present data show, ND channels that have an intermediate reduction in ATP sensitivity (IC$_{50}$ ~ 100 μmol/L in Mg-free solutions), such as R201C and V59M channels, are almost fully blocked by sulphonylureas at physiological ATP concentrations. Those that are poorly blocked (IC$_{50}$ > 1 mmol/L in Mg-free solutions), such as E296L, or not inhibited at all, such as G334D, show little or no enhancement of sulphonylurea block, and sulphonylureas can never effectively inhibit these channels. Although most of our experiments were performed with homeric K$_{ATP}$ channels heterologously expressed in Xenopus oocytes, a similar reduction in sulphonylurea block was observed in β-cells of mice hemizygously expressing V59M channels.

Clinical relevance. These results resolve the conundrum of why G334D channels show normal block by sulphonylureas in excised patches in the absence of nucleotides but patients are unresponsive to drug therapy (28). Under physiological conditions (i.e., in the presence of intracellular adenine nucleotides), G334D channels are poorly blocked by sulphonylureas. The IC$_{50}$ is increased, and the maximal block is less. The present data demonstrate that any mutation that dramatically reduces the sensitivity of the channel to ATP might also impair sulphonylurea block sufficiently to prevent sulphonylurea control of glucose homeostasis in patients with these mutations; this might also account for why patients with the I296L mutation cannot transfer to drug therapy (2).

In the presence of physiological levels of MgATP, ND mutations that decrease ATP binding produce an increase in the IC$_{50}$ for both gliclazide and glibenclamide block (Figs. 4 and 7) (28) and might explain why higher doses of drug are required to treat ND than to treat type 2 diabetes. In addition, the fact that K$_{ATP}$ currents are predicted to be larger in mutant β-cells might be a factor.

K$_{ATP}$ channels are largely blocked at resting blood glucose concentrations (5–7 mmol/L) (43–46), so β-cells sit poised on the cusp of secretion, and only a small reduction in P$_{O}$ is needed to trigger electrical activity and insulin secretion (45). We found that 2 μmol/L glibenclamide blocked K$_{ATP}$ currents in ib-V59M β-cells to the same extent as ~7 mmol/L glucose in wild-type mice (44–46). Thus, it appears that the drug restores P$_{O}$ to the level found for wild-type channels at resting blood glucose levels. Consequently, metabolic amplifying pathways, which couple glucose metabolism to insulin secretion downstream of K$_{ATP}$ channel closure-mediated Ca$^{2+}$ entry into the β-cell, are enabled (47), explaining why insulin secretion is meal dependent in ND patients (2) as it is in nondiabetic individuals.

For channels containing severe ND mutations, maximal inhibition at the high-affinity site is incomplete even at physiological ATP levels, which might explain why human patients with these mutations rarely experience hypoglycemia (2,40–42): Because channel activity is never completely abolished, insulin secretion is limited. The present data show that increasing glibenclamide has little effect on the extent of block over a wide range of concentrations; however, this will only apply to those Kir6.2 mutations that show pronounced ATP insensitivity. Kir6.2 mutations that reduce ATP sensitivity only slightly will be completely blocked by glibenclamide at physiological ATP levels, and patients with these mutations will be more susceptible to hypoglycemia.

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P.P. performed the data analysis and modeling. P.P. and H.d.W. performed the experiments. P.P., H.d.W., and F.M.A. wrote and reviewed the manuscript. P.P. and F.M.A. designed the experiments. P.P. and F.M.A. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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