ATP and Sulfonylurea Sensitivity of Mutant ATP-Sensitive K⁺ Channels in Neonatal Diabetes
Implications for Pharmacogenomic Therapy

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The prediction that overactivity of the pancreatic ATP-sensitive K⁺ channel (KᵦTᵦP channel) underlies reduced insulin secretion and causes a diabetic phenotype in humans has recently been borne out by genetic studies implicating “activating” mutations in the Kir6.2 subunit of KᵦTᵦP as causal in both permanent and transient neonatal diabetes. Here we characterize the channel properties of Kir6.2 mutations that underlie transient neonatal diabetes (I182V) or more severe forms of permanent neonatal diabetes (V59M, Q52R, and I296L). In all cases, the mutations result in a significant decrease in sensitivity to inhibitory ATP, which correlates with channel “overactivity” in intact cells. Mutations can be separated into those that directly affect ATP affinity (I182V) and those that stabilize the open conformation of the channel and indirectly reduce ATP sensitivity (V59M, Q52R, and I296L). With respect to the latter group, alterations in channel gating are also reflected in a functional “uncoupling” of sulfonylurea (SU) block: SU sensitivity of I182V is similar to that of wild-type mutants, but the SU sensitivity of all gating mutants is reduced, with the I296L mutant being resistant to block by tolbutamide (≤10 mmol/l). These results have important implications for the use of insulinotropic SU drugs as an alternative therapy to insulin injections.

In the pancreatic β-cell, the ATP-sensitive K⁺ channel (KᵦTᵦP channel) couples membrane excitability to insulin secretion (1). Increased glucose metabolism leads to elevated cytosolic ATP/ADP, closure of KᵦTᵦP channels at the plasma membrane, and membrane depolarization. The resulting activation of voltage-sensitive Ca²⁺ channels causes a rise in [Ca²⁺], which serves as the stimulus for insulin vesicle exocytosis. Sulfonylureas (SUs), which are major hypoglycemic agents used in treating type 2 diabetes, promote insulin secretion by specifically binding the regulatory SUR1 subunit of KᵦTᵦP and inhibiting KᵦTᵦP current (2), thus underscoring the central role of KᵦTᵦP in the regulation of insulin secretion.

Implicit in the paradigm of excitation-secretion coupling is that alterations in KᵦTᵦP currents should disrupt electrical signaling in the β-cell and, thereby, alter insulin release. Specifically, decreased metabolic flux, decreased sensitivity of KᵦTᵦP to inhibitory ATP, or increased density of KᵦTᵦP channels should all lead to abnormally high KᵦTᵦP activity and relative hypoinsulinism. In such scenarios, a diabetic phenotype is predicted; indeed, a severe neonatal diabetic phenotype is observed in mice lacking the β-cell glucokinase gene (3,4) and transgenic mice expressing β-cell KᵦTᵦP channels with reduced sensitivity to inhibitory ATP (i.e., “overactive” KᵦTᵦP) (5). A series of genetic studies have recently demonstrated that heterozygous, missense mutations in the pore-forming Kir6.2 subunit of the KᵦTᵦP channel underlie neonatal diabetes in humans, accounting for both permanent and transient forms of the disease (6–11). Rare in its occurrence (1:400,000 births), neonatal diabetes is usually diagnosed within the first 3 months of life and requires insulin administration to treat the hyperglycemia (12). In the milder, transient neonatal diabetes (TND), hyperglycemia usually resolves within 18 months after the diagnosis, whereas the equally common permanent neonatal diabetes (PND) requires insulin treatment for life. In both PND and TND, β-cell dysfunction is likely the primary lesion. However, in TND, it is proposed that expansion of β-cell mass compensates for the β-cell defect and underlies the remission phase (13). In one important finding, the administration of SUs to several neonatal diabetic patients carrying Kir6.2 mutations was sufficient to treat the hyperglycemia, thereby implicating KᵦTᵦP “overactivity” as causal in the suppressed insulin release (6,14).

All neonatal diabetes—associated Kir6.2 mutations examined to date have been shown to decrease ATP sensitivity of reconstituted KᵦTᵦP channels (7,10,15). Some of the mutations are in putative ATP-binding residues (e.g., R50, I182, R201), but others lie well away from any predicted binding regions. These latter mutations are likely to reduce ATP sensitivity by stabilizing the open state relative to the closed state (16); in fact, this has been demonstrated...
for some PND mutations (15). Little attention has been paid to the sensitivity of different mutations to insulinotropic SU drugs. This is an important issue given that other mutations that alter open-state stability have been shown to dramatically affect SU sensitivity (17,18) and that SU therapy holds the promise of an alternative to insulin treatment for neonatal diabetes patients (6,14).

In the present study, we characterized Kir6.2 mutations that arguably underlie the mildest form of neonatal diabetes, TND (1182V), as well as the more severe, permanent, forms of the disease (V59M, Q52R, and I296L). In all cases, mutations led to decreased ATP sensitivity. The mutations can be mechanistically classified as those that appear to directly alter ATP binding (1182V) and those that indirectly alter ATP sensitivity through changes in gating behavior (V59M, Q52R, and I296L). With respect to the latter group, a concomitant decrease in the sensitivity of reconstituted KATP channels to two clinically prescribed SUs (tolbutamide and glibenclamide) was observed in intact cells and excised patches. These findings have potentially important implications for the treatment of diabetic patients carrying Kir6.2 mutations and suggest that SU dosing may need to be considered on a case-by-case basis.

RESEARCH DESIGN AND METHODS

Molecular biology. Kir6.2 was cloned into the EcoR/U/Clu 1 site of pCMV6b, and the parental plasmid DNA was used to generate Kir6.2 mutations using the QuickChange Site Directed Mutagenesis kit (Stratagene, La Jolla, CA). SUR1 was cloned into the pECE expression vector. The nucleotide sequences of the mutant Kir6.2 constructs were verified by fluorescence-based cycle sequencing using AmpliTaq DNA polymerase FS (PerkinElmer, Foster City, CA) and an ABI Prism DNA sequencer (PerkinElmer).

Expression of KATP channels in COS76 cells. COS76 cells were plated at a density of ~2.5 × 10^5 cells/well (30-mm, six-well dishes) and cultured in Dulbecco’s modified Eagle’s medium with 10 mmol/l glucose supplemented with FCS (10%). The following day, a transfection cocktail was prepared by adding 6 µl of PuGENE 6 Transfection Reagent (Roche, Indianapolis, IN), 600 ng each of pCMV6b-Kir6.2 and pECE–green fluorescent protein plasmid, and 1 µg pECE/SUR1 (per well). After a 45-min incubation at room temperature, the cocktail was added directly to the growth medium on the plated cells. Cells were assayed for KATP currents by patch-clamp measurements 2–4 days after transfection.

Rubidium flux experiments. 2.5 mCi/ml of 86Rb was added in fresh growth medium 48 h after transfection, and cells were incubated for an additional 24 h before Rb+ flux was assayed. For efflux measurements, cells were preincubated for 2 min at 25°C in Krebs-Ringer solution with or without metabolic inhibitors (2.5 µg/ml oligomycin plus 1 mmol/l 2-deoxyglucose (Sigma, St. Louis, MO)). At selected time points, the solution was aspirated from the wells and replaced with fresh solution. The 2.5 µCi/ml in the aspirated solution was counted in a scintillation solution. Rubidium efflux curves were fit by assuming that a single non-KATP conductance was active in untransfected cells, determined as

\[ \text{Rb efflux} = \exp(-k_r \cdot t) \]  (1)

where \( k_r \) is the rate constant for efflux through the endogenous, non-KATP pathway.

In transfected cells, efflux curves were fit by assuming that both a KATP and a non-KATP conductance pathway were active in parallel and determined as

\[ \text{Rb efflux} = \exp\{-k_r \cdot t\} + \exp\{-k_{\text{KATP}} \cdot t\} \]  (2)

where \( k_r \) is the rate constant for efflux through the endogenous, non-KATP pathway (obtained from untransfected cells by Eq 1) and \( k_{\text{KATP}} \) is the rate constant for efflux through the KATP pathway. \( k_r \) is then directly proportional to the KATP conductance.

Patch-clamp measurements. Patch-clamp studies were performed at room temperature in an oil-gate chamber that allowed the solution bathing the exposed surface of the isolated patch to be changed rapidly. The standard bath (intracellular) and pipette (extracellular) solution (K-INT) used in these experiments was comprised of 140 mmol/l KCl, 10 mmol/l K-HEPES, and 1 mmol/l K-EGTA (pH 7.3). Microelectrode resistance was typically 0.5–1 MΩ when filled with K-INT solution. Membrane patches were voltage clamped with an Axopatch 1B patch-clamp amplifier (Axon, Foster City, CA). Phosphatidylinositol 4,5-bisphosphate (PIP2; 5 µg/ml in K-INT) was bath sonicated in ice for 30 min before being used. Glibenclamide was dissolved as a 1 mmol/l stock solution in DMSO and diluted to <1% DMSO in K-INT. Tolbutamide was prepared as a 100 mmol/l stock solution in 150 mmol/l KOH and diluted to the appropriate working concentration in K-INT (pH 7.3). PIP2 was obtained from Boehringer Mannheim. Tolbutamide, glibenclamide, and ATP were purchased from Sigma. All currents were measured at a membrane potential of ~50 mV (pipette voltage = +50 mV). Data were normally filtered at 0.5–3 kHz, and signals were digitized at 22 KHz (Neurocorder; Neurodata, New York, NY).

Experiments were digitized into a microcomputer using Clampex 8.2 software (Axon), and off-line analysis was performed using Clampfit 8.2 (Axon) and Microsoft Excel programs. Wherever possible, data are presented as means ± SE. Microsoft Solver was used to fit data by a least-squares algorithm.

Estimation of \( P_{\text{a,zero}} \). Two approaches, one indirect and one direct, were used to calculate the open probability in zero ATP (\( P_{\text{a,zero}} \)) after isolated membrane patches containing multiple channels were excised (19). \( P_{\text{a,zero}} \) method (indirect). \( P_{\text{a,zero}} \) was added to the patch until the current reached a saturating level (\( I_{\text{max}} \)). This was assumed to represent a maximum \( P_{\text{a,zero}} \) of ~0.9 (20). The fractional change in current was calculated as

\[ \frac{\text{Fractional change}}{P_{\text{a,zero}}/P_{\text{P2}}} = \frac{I_{\text{max}}}{I_{\text{max}}/P_{\text{PT2}}} \]  (3)

where \( I_{\text{initial}} \) is the initial current. \( P_{\text{a,zero}} \) was then estimated as

\[ P_{\text{a,zero}} = 0.9 \times \text{fractional change} \]  (4)

Noise analysis method (direct). In addition, the mean \( P_{\text{a,zero}} \) was estimated from stationary fluctuation analysis of macroscopic currents (21,22) on short (<1 s) recordings of currents in zero or 5 mmol/l ATP or 10 mmol/l spermine (for estimation of ATP-independent noise). Currents (corresponding to ~1–1000 channels) were filtered at 1 kHz and digitized at 3 kHz with 12-bit resolution. The mean patch current (1) and variance (2) in the absence of ATP were obtained by subtracting the mean current and variance in 5 mmol/l ATP or 10 mmol/l spermine (i.e., assuming all channels were fully closed or blocked). The single channel current (\( I \)) was assumed to be ~3.75 pA at ~50 mV, corresponding to a wild-type single channel conductance of 75 pS (23). \( P_{\text{a,zero}} \) was then estimated as

\[ P_{\text{a,zero}} = 1 - \frac{\sigma^2}{\bar{I}^2} \]  (5)

Three-state model for KATP channel activity. Detailed kinetic analysis of KATP channel activity predicts complex models, but a simplified three-state model (19,20) can account qualitatively and quantitatively for the essential features of macroscopic KATP channel activity. Accordingly, we examined whether this simple model (Fig. 1), in accounting for the effects of mutations on gating behavior and nucleotide sensitivity, could be used predictively to account for observed changes in drug sensitivity.

The ATP binding affinity (\( K_a \)), and open-close state equilibrium (\( K_{\text{CO}} \)) for wild-type channels were assumed to be ~3 mmol/l and 0.60, respectively. To obtain predictions of behavior for mutant channels (as in Fig. 8), only one or the other parameter was changed. For 1182V, it was necessary to change \( K_a \) (increased by sixfold) without changing \( K_{\text{CO}} \). For Q52R, V59M, and I296L mutations, it was necessary to change \( K_{\text{CO}} \) (increased by 40, 60, and 800-fold, respectively) without changing \( K_a \).

Dose-response curves for SU (glibenclamide and tolbutamide) inhibition were described by the sum of two Hill equations (reflecting two components of inhibition):}

\[ G_{\text{rel}} = f(1 + [SU/K_a]) + (1 - f)[1 + (SU/K_b)], \]  (6)

where \( G_{\text{rel}} \) is the relative conductance, \( f \) is the fraction of high-affinity block, and \( K_a \) and \( K_b \) are the half-maximal concentration of high- and low-affinity block, respectively.

It is clear that the relative magnitude of the two components was changed by the mutations as was the apparent affinities. We postulated, based on previous findings (17,18), that these changes resulted directly from changes in open-state stability. As shown in Fig. 8, to model the effects of different mutations on SU sensitivity, it was necessary to assume only that the fraction

\[ K_A \quad K_CO \]

ATP

\[ C \quad C \quad O \]
inhibition reduced the Rb$^+$ efflux rate in untransfected cells; Rb$^+$ efflux time courses in basal and metabolically inhibited conditions for untransfected cells were thus fit by Eq. 1, which assumes a single non-K$_{ATP}$ conductance is active in each condition. The rate constants thus obtained were used to estimate rate constants for K$_{ATP}$ conductance under basal conditions and metabolic inhibition in transfected cells using Eq. 2. These rate constants were then assumed to be directly proportional to K$_{ATP}$ conductance. Figure 4A shows the estimated K$_{ATP}$ conductance under basal and metabolically inhibited conditions for each transfected construct. Maximal activated conductance was similar for all constructs except I296L. This mutation gave consistently lower fluxes, a point considered below (see DISCUSSION), but the basal flux was almost maximal.

We next examined K$_{ATP}$ channel activity directly in voltage-clamped membrane patches from transfected cells. For each patch, we estimated the current in basal physiological conditions (on-cell current) and the measured maximal K$_{ATP}$ current after patch excision (data not shown). In Fig. 4B, relative K$_{ATP}$ current activation on-cell from patch-clamp analysis is plotted against the relative K$_{ATP}$ activation in intact cells estimated by Rb$^+$ efflux for each mutation. Both measures give correlated activities, suggesting that each method reflects the degree of overactivity in intact cells. To examine the correlation between increased K$_{ATP}$ activity in intact cells and ATP sensitivity, the latter was measured directly in excised membrane patches (Fig. 5). All mutant K$_{ATP}$ channels exhibited a significant decrease in ATP inhibition, ranging from twofold (V59M) to >100-fold (I296L) (Table 1). Channel overactivity in intact cells correlated reasonably well with reduction in ATP sensitivity, but a direct correlation between ATP insensitivity and severity of the neonatal diabetes was not absolute, as the TND-causing I182V mutation was approximately twofold less sensitive than V59M, a mutation that is associated with severe, syndromic PND (see DISCUSSION). It is conceivable that channel trafficking was altered by neonatal diabetes mutations so that a higher current may partly reflect higher expression levels. As shown in Table 1, patch current density and calculated channel density in this recombinant system were not significantly different between wild-type and mutant channels.

**Mutations may affect ATP sensitivity by reduced ATP binding or indirectly by changes in the relative stability of the open state.** Mutations could mechanistically reduce ATP sensitivity directly by reducing ATP affinity or indirectly by affecting the intrinsic opening ability. Previous studies have characterized mutations throughout Kir6.2 that stabilize the open state of the channel relative to the closed state, even in the absence of ATP, and thereby decrease the apparent ATP sensitivity (19,20,24, 25). To elucidate potential mechanisms in neonatal diabetes mutations, we measured the open probability in the absence of ATP ($P_{o,zero}$), using two independent methods: PiP$_2$ activation (19) and nonstationary noise analysis (22) (see RESEARCH DESIGN AND METHODS). Each method gave very similar results (see Fig. 8A). $P_{o,zero}$ of wild-type channels was ~0.4 and was not altered by the TND-associated I1182V mutation (which reduced ATP sensitivity approximately
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As shown in Fig. 6, glibenclamide almost completely inhibited Rb⁺ efflux through wild-type channels at clinically effective doses (i.e., in the nanomolar range). It has previously been demonstrated that SU inhibition in excised membrane patches is biphasic, with high- and low-affinity components (17,26). Although a biphasic sensitivity has not been previously demonstrated in intact tissue, it is evident in the SU sensitivity of mutant K<sub>ATP</sub> channels in intact COS cells (Fig. 6), where Rb⁺ efflux is inhibited to a plateau level by micromolar glibenclamide. For mutants that exhibit increased open-state stability, the high-affinity component decreases (Q52R, V59M, I296L) and the affinity itself decreases (Q52R, I296L). The highest open-state stability mutant (I296L) is almost completely insensitive to glibenclamide in the nanomolar range, but a reduced high-affinity component and lower affinity is also quite clear for the Q52R mutant.

Glibenclamide is a hydrophobic drug (27), and inhibition of K<sub>ATP</sub> channels in excised patches was largely irreversible over the experimental period. However, tolbutamide, a first generation SU, is more hydrophilic, can be rapidly reversed, and has been a useful model SU in excised membrane patch experiments. As shown in Fig. 7, wild-type channels showed typical biphasic block by tolbutamide in inside-out membrane patches (17,26), and sensitivity was unaltered in the I182V mutation (Fig. 7B). This finding is again consistent with the interpretation that the I182V substitution directly alters ATP binding/trans-
production but does not affect gating (28), and thus the mutant channels remain functionally "coupled" to SUR1.

In sharp contrast, functional uncoupling from the SUR1 subunit is again reflected by a decrease in high-affinity fractional SU block in each of the mutations that increase the open-state stability (Q52R, V59M, I296L) (Table I). It is interesting that I296L channels, which show the highest open-state stability, are completely insensitive to inhibition by tolbutamide at concentrations as high as 10 mmol/l. However, when wild-type Kir6.2 and I296L subunits were coexpressed to generate heterozygous channels, the SU sensitivity was partially restored, but with a significant decrease in the high-affinity component of block (17% high-affinity tolbutamide block for heterozygous I296L:wild-type channels vs. 55% block for homozygous wild-type channels) (Fig. 7B).

**DISCUSSION**

**Reduced ATP sensitivity underlies both TND and PND.** It is now clear that mutations in the Kir6.2 subunit of $K_{ATP}$ represent a common cause of neonatal diabetes in humans (6–11). To better understand the mechanism(s) by which these mutations alter excitation-secretion coupling and their effect on SU sensitivity, we chose to characterize Kir6.2 mutations implicated in the mildest, transient form of the disease (I182V) (10) and those associated with more severe PND (Q52R, V59M, I296L) (6–9). In all cases, the sensitivity of homomeric mutant $K_{ATP}$ to inhibitory ATP was reduced relative to wild-type $K_{ATP}$, with the following order of sensitivity: WT > V59M > I182V > Q52R > I296L. The syndromic PND-linked V59M mutation exhibited comparable if not greater sensitivity to ATP than did the TND-associated I182V ($K_{1/2ATP} = 15$ [V59M] and 31 [I182V] μmol/l), reflected in a similar degree of "overactivity" in intact cells (Fig. 4B). Assuming the behavior of reconstituted channels reflects $K_{ATP}$ activity in the β-cell, these data do not demonstrate an absolute correlation between the severity of the diabetes (TND, PND, and syndromic PND) and the degree of ATP sensitivity. Such a positive correlation has been previously suggested based on a comparison of the less severe functional defects of a PND-associated mutation (R201C) with syndromic PND-associated mutations (Q52R, V59G) (15). Taken together, these data suggest a complex phenotype in which the primary defect is at the level of the β-cell and altered insulin secretion, but they also indicate that additional genetic and/or environmental factors may modulate the severity of the disease (TND, PND, and syndromic PND). It is interesting that $K_{ATP}$ in skeletal muscle (Kir6.2 + SUR2A) is implicated in peripheral insulin sensitivity based on the observation that both Kir6.2- and SUR2A-null mice exhibit increased glucose uptake in skeletal muscle and adipose tissue (29,30). The mouse models, therefore, predict that "overactive" $K_{ATP}$, as observed in neonatal diabetes, may suppress glucose uptake and contribute to the hyperglycemia. Whether neonatal diabetes patients, in addition to having suppressed insulin release, also exhibit insulin insensitivity remains to be determined.

An important distinction between the present study and that of Proks et al. (15) is that the order of sensitivity in Proks et al.'s report was based on the ATP sensitivity of heteromeric channels (generated by the expression of channels from equal amounts of wild-type Kir6.2 and mutant Kir6.2 cRNA), whereas our analysis is of homomeric channels. We have found that in similar experiments with mixed expression of wild-type and mutant Kir6.2 subunits, the rank order of mutant ATP sensitivity is unaltered (data not shown). However, a quantitative analysis of mixed expression is very complicated, given the fact that six distinct combinations of wild-type and mutant subunits will generate six different channel types, each with its own ATP sensitivity. Whether mutant alleles of Kir6.2 will be expressed equally in vivo and in different tissues is also not known. Given these complexities, the analysis of homomeric channels may be the most straightforward approach to understanding mutational effects on channel properties.

Finally, the I296L mutant channel consistently exhibited a lower activity in Rb⁺ efflux experiments, despite a channel density that was similar to that of wild-type channels in excised patches. This finding may reflect a lower transfection efficiency. However, because the I296L mutation resulted in the highest on-cell activity of all the neonatal diabetes mutants, it is also possible that this
elevated K\textsuperscript+ conductance was toxic to the cells, so that we were experimentally selecting for a subpopulation of cells that are resistant to elevated K\textsuperscript+ conductance in excised patch experiments.

**Differential SU sensitivity of neonatal diabetes mutations.** SUs block pancreatic K\textsubscript{ATP} currents and stimulate insulin release. The high-affinity component of block by glibenclamide (IC\textsubscript{50} \sim 1 \text{mmol/l}) or tolbutamide (IC\textsubscript{50} \sim 1 \text{\textmu mol/l}) results from binding to the SUR1 subunit (26), whereas the Kir6.2 subunit underlies the low-affinity component (IC\textsubscript{50} \sim 1,000 \times high affinity). A direct correlation between gating behavior of the channel and SU sensitivity has been previously demonstrated (17,18). The consequence of such a relation is that mutations in Kir6.2 that increase the stability of the open state not only reduce the apparent ATP sensitivity, but concomitantly decrease the high-affinity SU block. This is interpreted as a functional “uncoupling” of the regulatory SUR1 subunit from the Kir6.2 channel (17).

We thus predict that PND mutations that increase open-state stability (V59M, Q52R, and I296L) should also reduce SU sensitivity. This hypothesis is consistent with the previous observation that the block of whole-cell K\textsuperscript+ currents was not complete in 500 \text{\textmu mol/l} tolbutamide for PND mutant K\textsubscript{ATP} (15). Indeed, the gating mutations Q52R and V59M but not the ATP-binding mutant I182V exhibited the low-affinity block by tolbutamide.
a decrease in the fractional block by the high-affinity component. The Q52R and I296L mutants also exhibited a significant decrease in the sensitivity of the low-affinity component. The Q52R and I296L mutants also exhibited a decrease in the fractional block by the high-affinity component.

**A mechanistic model for nucleotide and SU sensitivity.** Diabetes-causing Kir6.2 mutations can be classified mechanistically as those that directly alter ATP binding and/or transduction of the ATP-mediated signal (I182V) versus those that indirectly affect ATP affinity by increasing the open-state stability (Q52R, V59M, I296L). The data are consistent with recent characterizations of other PND mutations that affect ATP binding (R201C, R201H, I182V) or alter gating behavior, leading to a secondary decrease in ATP sensitivity (V59G, Q52R) (10,15).

Models of $K_{ATP}$ channel gating and nucleotide and SU sensitivity have been developed. To have predictive use, such models should account for channel gating (in the absence of ATP), nucleotide sensitivity, and drug sensitivity, and by adjusting relevant parameters, should be able to account for the effects of mutations on channel function. The most complex models (20,28,31) account for the tetrameric nature of the channel and could potentially account for heteromeric channels consisting of wild-type and mutant subunits. However, a relatively simple three-state model (20) (see RESEARCH DESIGN AND METHODS, Fig. 1) can account for all of the pertinent features of macroscopic channel activity and has predictive qualities for the interpretation of nucleotide and drug sensitivities of neonatal diabetes mutants. We therefore postulated that mutational effects on SU sensitivity result directly from changes in open-state stability (17,18).

As shown in Fig. 8, the simplified three-state model quantitatively predicted the effects of neonatal diabetes mutations on each of the three features, $P_{o,\text{zero}},$ ATP sensitivity, and SU sensitivity, by adjusting only two parameters, $K_a$ and $K_{CO},$ which are the equilibrium constants that describe ATP affinity and open-state stability, respectively. By assuming that both the affinity and the fraction of high-affinity SU block are dependent on only $K_{CO},$ the steady-state SU sensitivities of wild-type and mutant channels can be predicted. As discussed below, this analytical approach may be a useful predictor of SU efficacy in human therapy trials.

**Implications for SU therapy of neonatal diabetes.** Recently, SUs have been shown to be effective, at least in the short-term, in maintaining control of blood glucose levels in several PND patients in which Kir6.2 mutations are causal (P331H, V59M, and R201H) (6,14). Given that blood glucose levels and HbA$_1c$ values were stable after discontinuation of insulin in these patients, it has been proposed that oral SUs can be an alternative therapy to subcutaneous insulin injections in patients with PND because of Kir6.2-activating mutations. The present results indicate, however, that the efficacy of SU therapy is likely to depend on the nature of the Kir6.2 mutation. Assuming that the SU sensitivity of reconstituted channels reflects the sensitivity of channels in vivo, carriers of the diabetes-causing I296L and similarly behaving Kir6.2 mutations may be less responsive to SU therapy. In one interesting case,

![Figure 6](https://example.com/figure6.png)
a PND patient with the syndromic V59M mutation was responsive to SU therapy and discontinued insulin injections (6), thereby suggesting that despite a significant decrease in sensitivity of homomeric V59M mutant channels to SU inhibition, sufficient high-affinity inhibition was still able to induce insulin release. In the original report by Gloyn et al. (7), it was observed that a parent of the R201H proband was diagnosed as a baby with diabetes and had been effectively treated with tolbutamide for 46 years. The mutation R201H causes a significant loss of ATP sensitivity (7), but it is predicted to be an ATP-binding site mutation (16,32,33) and thus may not show loss of SU sensitivity.

Functional characterization of additional PND-associated mutations in Kir6.2, as well as additional clinical studies, will provide essential data regarding the efficacy
of SU therapy in treating KATP-induced diabetes and the possibility of tailoring individual therapy based on the underlying Kir6.2 mutation. The present results suggest that careful attention should be paid to the degree to which neonatal diabetes mutations cause a loss of ATP sensitivity by an increase in open-state stability. The greater the mutational effect on open-state stability is, the higher the SU dose necessary to achieve a given degree of channel inhibition will be. Thus, to achieve the same therapeutic effect (i.e., to obtain sufficient closure of KATP under appropriate physiological conditions), proportionally higher doses of an SU may be clinically required.

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REFERENCES


28. Li L, Wang J, Drain P: The I182 region of k(ir)6.2 is closely associated with ligand binding in K(ATP) channel inhibition by ATP. *Biophys J* 79:841–852, 2000


