Neonatal diabetes and congenital hyperinsulinism caused by mutations in ABCC8/SUR1 are associated with altered and opposite affinities for ATP and ADP

David Ortiz1 and Joseph Bryan2*

1 Department of Medicinal Chemistry, University of Washington, Seattle, WA, USA
2 Pacific Northwest Diabetes Research Institute, Seattle, WA, USA

INTRODUCTION

Neuroendocrine ATP-sensitive K\(^+\) (K\(_{ATP}\)) channels composed of potassium inward-rectifier type 6.2 and sulfonylurea receptor type 1 subunits (Kir6.2/SUR1)\(_4\) are expressed in various cells in the brain and endocrine pancreas where they couple metabolic status to membrane potential. In β-cells, increases in cytosolic [ATP/ADP]\(_c\) inhibit K\(_{ATP}\) channel activity, leading to membrane depolarization and exocytosis of insulin granules. Mutations in ABCC8 (SUR1) or KCNJ11 (Kir6.2) can result in gain or loss of channel activity and cause neonatal diabetes (ND) or congenital hyperinsulinism (CHI), respectively. SUR1 is reported to be a Mg\(^{2+}\)-dependent ATPase. A prevailing model posits that ATP hydrolysis at SUR1 is required to stimulate openings of the pore. However, recent work shows nucleotide binding, without hydrolysis, is sufficient to switch SUR1 to stimulatory conformations. The actions of nucleotides, ATP and ADP, on ND (SUR1\(_{E1506D}\)) and CHI (SUR1\(_{E1506K}\)) mutants, without Kir6.2, were compared to assess both models. Both substitutions significantly impair hydrolysis in SUR1 homologs. SUR1\(_{E1506D}\) has greater affinity for MgATP than wildtype; SUR1\(_{E1506K}\) has reduced affinity. Without Mg\(^{2+}\), SUR1\(_{E1506K}\) has a greater affinity for ATP\(^{4-}\) consistent with electrostatic attraction between ATP\(^{4-}\), unshielded by Mg\(^{2+}\), and the basic lysine. Further analysis of ND and CHI ABCC8 mutants in the second transmembrane and nucleotide-binding domains (TMD2 and NBD2) found a relation between their affinities for ATP (±Mg\(^{2+}\)) and their clinical phenotype. Increased affinity for ATP is associated with ND; decreased affinity with CHI. In contrast, MgADP showed a weaker relationship. Diazoxide, known to reduce insulin release in some CHI cases, potentiates switching of CHI mutants from non-stimulatory to stimulatory states consistent with diazoxide stabilizing a nucleotide-bound conformation. The results emphasize the greater importance of nucleotide binding vs. hydrolysis in the regulation of K\(_{ATP}\) channels in vivo.

Keywords: neonatal diabetes, congenital hyperinsulinism, sulfonylurea receptor, K\(_{ATP}\) channels
SUR1 catalytic rate and/or reduce the off-rate of the MgADP-Pi products, thereby augmenting the population of post-hydrolytic stimulatory intermediates (4, 5). A similar argument has been made for the S1369A polymorphism in SUR1, which is reported to increase ATPase activity and thus to increase the risk for type 2 diabetes (7). In contrast to the conventional regulatory model, we have reported that ATP binding, without hydrolysis, switches SUR1 from conformations with high affinity for the channel antagonist, glibenclamide (GBC, glyburide in the United States), to conformations with lower affinity for GBC and higher affinity for the channel agonist, diazoxide, i.e., from non-stimulatory to stimulatory states (8, 9). This was demonstrated by removing the Mg^{2+} cofactor required for hydrolysis and by mutating the highly conserved catalytic glutamate, E1506, to glutamine (SUR1E1506Q), a substitution that strongly impairs hydrolysis in SUR1 homologs [see, for example, Ref. (10–13)]. Structural studies of ABC proteins indicate that ATP drives dimerization of the cytosolic NBDs upon binding at two sites at the dimer interface. NBD dimerization reconfigures the transmembrane helix bundles (TMD1 and TMD2) from inward-facing to outward-facing orientations [see Ref. (1–4) for review]. Thus, we proposed that nucleotide-bound (MgATP and/or MgADP) outward-facing conformations of SURs stimulate K^{+} bound (MgATP and/or MgADP) outward-facing conformational switching thus leading to underactive channels and CHI. However, a recent report indicates that two catalytic glutamate substitutions that impair hydrolysis should impede conformational switching thus leading to opposite disease phenotypes; SUR1E1506D produces hyperactive channels and mutant SUR1 in the absence of added nucleotide, was assessed using saturation binding. Membranes containing WT or mutant SUR1 (~150 pM) were suspended in a Mg^{2+}-free physiological salt solution (139 mM NaCl, 5 mM KCl, 1 mM EDTA, 50 mM HEPES, pH 7.4) with increasing concentrations of [3H]-labeled GBC. Following incubation for 30 min at 37°C, unbound ligand was removed by rapid filtration as described (17) to determine total bound ligand. Non-specific binding was determined in the presence of 1 μM unlabeled GBC. Specific GBC binding is defined as: 

\[ \text{Total Bound} = \frac{B_{\text{MAX}} \times G}{K_G + G} + \text{Non Specific} \]

Where \( B_{\text{MAX}} \) is the total amount of receptor, G is the concentration of free [3H]-labeled GBC in the reaction, \( K_G \) is the equilibrium dissociation constant of GBC, and Non-Specific is the amount of non-specific binding typically 10–15% of total bound [3H]GBC.

**Nucleotide inhibition assays**

Nucleotide-induced conformational changes in SUR1 were assessed by measuring [3H]GBC binding at varying nucleotide concentrations. [3H]GBC was held fixed at 1 nM and included the indicated concentrations of nucleotides with or without added Mg^{2+}. A creatine phosphokinase-based ATP-regenerating system was used to maintain constant ATP concentrations over a 30 min incubation in experiments with MgATP (free [Mg^{2+}] > 1 mM) (18). The stability of ATP levels was verified by luciferase assays (Sigma Chemical Co.). MgADP assays included 10 mM AMP to inhibit endogenous adenylyl kinase activity and thus minimize ATP production. The free Mg^{2+} level was 1 mM in experiments with MgADP and MgATP analogs where the ATP-regenerating system was not used. Mg^{2+}-free experiments included 1 mM EDTA. Non-specific binding, typically ~10–15% of total bound, was

**PLASMIDS, CELLS, AND MEMBRANES**

The methods used to generate plasmids, to express wildtype (WT) and mutant SUR1 in *Pichia pastoris* and to isolate membranes were described previously (9) and modified to reduce carryover of endogenous nucleotides, which might affect mutant receptors with highest affinities for nucleotides (8). Amino acid numbering is based on Reference Sequence NM_000352.4. It is worth noting that there are two transcript variants of SUR1/ABCC8 with lengths of 1581 and 1582 amino acids, respectively. Reference sequences NM_000352.2, NM_000352.3, and NM_000352.4 all specify the 1581 variant referred to as “variant 2” in the NCBI data base. “Variant 1,” with 1582 amino acids, is found in NCBI Reference Sequence: NM_001287174.1. Both variants are expressed in islet cells, with variant 1 being somewhat more frequent (16). It is thus likely that K_{ATP} channels contain a random assortment of both variants. Our unpublished electrophysiological data found no difference between channels of either variant alone (Andrey Babenko and Joseph Bryan). We have used Reference Sequence NM_000352.4 in keeping with most recent reports on ABCC8 mutations producing ND and/or CHI.

**[3H] GBC BINDING STUDIES**

Saturation binding assays

Values for \( K_G \), the dissociation constant for [3H]GBC from WT and mutant SUR1 in the absence of added nucleotide, was assessed using saturation binding. Membranes containing WT or mutant SUR1 (~150 pM) were suspended in a Mg^{2+}-free physiological salt solution (139 mM NaCl, 5 mM KCl, 1 mM EDTA, 50 mM HEPES, pH 7.4) with increasing concentrations of [3H]-labeled GBC. Following incubation for 30 min at 37°C, unbound ligand was removed by rapid filtration as described (17) to determine total bound ligand. Non-specific binding was determined in the presence of 1 μM unlabeled GBC. Specific GBC binding is defined as:

\[ \text{Total Bound} = \frac{B_{\text{MAX}} \times G}{K_G + G} + \text{Non Specific} \]

Where \( B_{\text{MAX}} \) is the total amount of receptor, G is the concentration of free [3H]-labeled GBC in the reaction, \( K_G \) is the equilibrium dissociation constant of GBC, and Non-Specific is the amount of non-specific binding typically 10–15% of total bound [3H]GBC.

**Nucleotide inhibition assays**

Nucleotide-induced conformational changes in SUR1 were assessed by measuring [3H]GBC binding at varying nucleotide concentrations. [3H]GBC was held fixed at 1 nM and included the indicated concentrations of nucleotides with or without added Mg^{2+}. A creatine phosphokinase-based ATP-regenerating system was used to maintain constant ATP concentrations over a 30 min incubation in experiments with MgATP (free [Mg^{2+}] > 1 mM) (18). The stability of ATP levels was verified by luciferase assays (Sigma Chemical Co.). MgADP assays included 10 mM AMP to inhibit endogenous adenylyl kinase activity and thus minimize ATP production. The free Mg^{2+} level was 1 mM in experiments with MgADP and MgATP analogs where the ATP-regenerating system was not used. Mg^{2+}-free experiments included 1 mM EDTA. Non-specific binding, typically ~10–15% of total bound, was

**MATERIALS AND METHODS**

**REAGENTS**

[3H]glibenclamide, 5-chloro-N-(4-[(N-cyclohexylcarbamoyl)sulfamoyl]phenethyl)-2-methoxybenzamide, NET0124250UC at 40–70 Ci/mmol was purchased from Perkin-Elmer, Inc., Waltham, MA, USA.
Adenine nucleotides have a negative allosteric action on the binding of GBC to SUR1 (17, 19, 20). This action is ascribed to nucleotide-dependent switching between a nucleotide-free conformation with highest affinity for $[^3]$H]GBC and a nucleotide-bound state with reduced affinity (9). We hypothesize that these states are the inward vs. outward-facing conformations of SURs, respectively. A four-state equilibrium model, Figure 1, has been used to analyze the effects of nucleotides on GBC binding. SUR1, like several other ABCC subfamily members, is an asymmetric ABC protein. NBD1, the degenerate site, has a greater affinity for ATP and is presumed to have little or no enzymatic activity, while NBD2 has a lower affinity and ATPase activity. The four-state model presumes NBD1 is nearly saturated at the nucleotide concentrations needed to switch conformations. This is a reasonable assumption for WT SUR1 and the CHI mutants, but may be problematic for ND mutations with higher affinity for ATP.

**Allosteric analysis**

Adenine nucleotides have a negative allosteric action on the binding of GBC to SUR1 (17, 19, 20). This action is ascribed to nucleotide-dependent switching between a nucleotide-free conformation with highest affinity for $[^3]$H]GBC and a nucleotide-bound state with reduced affinity (9). We hypothesize that these states are the inward vs. outward-facing conformations of SURs, respectively. A four-state equilibrium model, Figure 1, has been used to analyze the effects of nucleotides on GBC binding. SUR1, like several other ABCC subfamily members, is an asymmetric ABC protein. NBD1, the degenerate site, has a greater affinity for ATP and is presumed to have little or no enzymatic activity, while NBD2 has a lower affinity and ATPase activity. The four-state model presumes NBD1 is nearly saturated at the nucleotide concentrations needed to switch conformations. This is a reasonable assumption for WT SUR1 and the CHI mutants, but may be problematic for ND mutations with higher affinity for ATP.

Derivation of the equations for the four-state equilibrium model followed Wyman and Gill (21) as described (9). The binding equation was obtained from the binding partition function,

$$P = \frac{[R] + [RG] + [RT] + [RTG]}{R},$$

the sum of the contributions of the different species relative to one reference species, taken here as the unliganded receptor, R. Substituting the dissociation constants gives a binding polynomial:

$$P = 1 + \frac{[G]}{K_G} + \frac{[T]}{K_T} + \frac{[G][T]}{\beta K_G K_T}.$$

$\bar{G}$, the amount of $[^3]$H]GBC specifically bound per mole of SUR1, dependent on both [G] and [ATP], is given by:

$$\bar{G} = \frac{\delta \ln P}{\delta \ln G} = \frac{[G]}{P} \frac{\partial P}{\partial G}.$$

This can be expressed as a binding isotherm:

$$\bar{G} = \frac{G \left( \frac{1}{K_G} + \frac{T}{K_T \gamma_{G,T}} \right)}{1 + \frac{[G]}{K_G} + \frac{[T]}{K_T} + \frac{[G][T]}{\beta K_G K_T}},$$

where $K_G$ and $K_T$ are the equilibrium dissociation constants for GBC and ATP (T), respectively. $\beta$ is an allosteric constant; values of $\beta > 0$ indicate a reduced affinity of nucleotide-bound SUR for $[^3]$H]GBC. The experimental data, Specific Bound GBC, are modeled by:

$$\text{Specific Bound GBC} = \frac{\bar{G}}{G_{T=0}}.$$

Values for $K_T$ and $\beta$ were estimated by fitting the four-state model to plots of Specific Bound GBC vs. [nucleotide (T)] with fixed $K_G$ values that were determined independently using saturation binding assays as described above.

**STATISTICS**

Where indicated the IC$_{50}$ values were estimated by fitting a logistic equation using Origin 2015 (OriginLab Corp., Northampton, MA, USA). The means ±SE are plotted; the number of replicate experiments varies, but $n \geq 3$ in all cases. ND mutation data are given in red, CHI mutation data are in blue, and WT data are in black throughout. The fitting parameters and SE derived using a four-state model were estimated using Mathematica (Wolfram Research Inc., Champaign, IL, USA). Figures were prepared using Origin 2015.

**RESULTS**

**SPECIFIC MUTATIONS HAVE LITTLE TO NO EFFECT ON THE AFFINITY OF INWARD FACING, NUCLEOTIDE-FREE CONFORMATIONS OF SUR1 FOR $[^3]$H]GBC**

To assess the effect of the ABCC8/SUR1 mutations used in this study on GBC binding the dissociation constants, $K_G$, were determined in saturation binding assays in the absence of nucleotides.
and Mg$^{2+}$. SUR1, like other ABC proteins, is presumably in the inward-facing conformation under this condition. These mutations are not at the putative sulfonylurea binding site [reviewed in Ref. (22, 23)] and have only small effects on GBC binding (see $K_D$ values in Table 1).

Two substitutions, E1506D and E1506K, causes of ND and CHI, respectively, have opposite effects on the affinity for MgATP. Several ND mutations in SUR1 increase the apparent affinity for ATP (8, 9). To extend these observations two SUR1 substitutions, I1424V (24) and G1479R (26) and C1174F S1185A, respectively, have opposite effects on the affinity for MgATP (see K$_D$ values in Table 1).

Table 1: Comparison of binding parameters for neonatal diabetes and congenital hyperinsulinism mutations.

| Mutation | Reference | $K_G$ (nM) | $K_T$ (+Mg$^{2+}$) (µM) | $K_T$ (−Mg$^{2+}$) (µM) | $K_D$ (+Mg$^{2+}$) (µM) | $eta$ |
|----------|-----------|------------|------------------------|------------------------|------------------------|------|
| E1506D$^a$ | (8) | 0.6 ± 0.2 | 9.0 ± 0.2 | 40 ± 20 | 94 ± 9 | 40 ± 11 | 211 ± 34 | 76 ± 2.2 |
| E1506D$^a$ | (15) | 0.4 ± 0.04 | 3.2 ± 1 | 8.6 ± 1.5 | 5570 ± 1200 | 72 ± 1.5 | 289 ± 122 | 4.7 ± 2.2 |
| G1178R$^b$ | (24) | 1.0 ± 0.1 | 9.2 ± 1.3 | 10 ± 1 | 1030 ± 200 | 9.1 ± 1.7 | 13.9 ± 2.0 | 20.7 ± 8.9 |
| I1424V | (24) | 0.5 ± 0.03 | 7.1 ± 2.2 | 5.6 ± 0.7 | 2840 ± 700 | 7.6 ± 1.5 | 12.1 ± 3.7 | 14.8 ± 6.5 |
| R1182Q$^a$ | (24) | 0.5 ± 0.15 | 13.1 ± 2.3 | 10.3 ± 1.4 | 11100 ± 1600 | 4.1 ± 0.4 | 13.1 ± 2.2 | 16.4 ± 4.6 |
| WT | | 0.25 ± 0.02 | 200 ± 18 | 13 ± 1 | 10900 ± 3400 | 16 ± 11 | 60 ± 16 | 14 ± 6.6 |
| S1185A$^c$ | (9) | 0.3 ± 0.05 | 416 ± 75 | 4.9 ± 0.5 | 19100 ± 3800 | 6.4 ± 1.5 | 36.6 ± 8 | 10.4 ± 2.5 |
| C1174F$^c$ | (9) | 0.5 ± 0.04 | 2690 ± 725 | 5.9 ± 2.3 | >20000 | 13 ± 6 | 66 ± 13 | 76 ± 1.7 |
| E1506K | (25) | 0.3 ± 0.03 | 8450 ± 1200 | 5.5 ± 0.6 | 256 ± 55 | 5.3 ± 0.4 | >10000 | n.d. |
| G1479R | (25) | 0.5 ± 0.04 | >10000 | n.d. | >20000 | n.d. | >10000 | n.d. |

$^a$Includes data from Ref. (6).

$^b$Includes data from Ref. (9).

$^c$Personal communication, Dr. Lydia Aguilar-Bryan.

ND mutations in red; CHI in blue.

$K_G$, $K_T$, and $K_D$ are the dissociation constants for GBC, ADP, and ATP, respectively.

$\beta$ is an allosteric constant defined in Section “Materials and Methods.”

Patients with E1506K (27) and G1479R (26, 28) mutations are responsive to diazoxide.

**SUR1$^{E1506D}$ and SUR1$^{E1506K}$ both have increased affinity for ATP**

While eliminating Mg$^{2+}$ counterions generally reduces SUR1 affinity for ATP (8, 9), Figure 2C shows that the rank order of the mutations is changed; SUR1$^{E1506K}$ now has a greater affinity for ATP$^+$ than SUR1$^{E1506D}$, the reverse of the result for MgATP. The results imply that without Mg$^{2+}$, electrostatic interactions between lysine and phosphate favor ATP binding to SUR1$^{E1506K}$. The data support the argument that the Mg$^{2+}$ counterion, chelated by the ATP $\beta$, $\gamma$-phosphates, shields the catalytic glutamate, and that the shorter aspartate and neutral glutamine side chains reduce electrostatic repulsion with the $\gamma$-phosphate to improve ATP binding. The results (Table 1) are consistent with the hypothesis that at physiologic concentrations of MgATP SUR1$^{E1506D}$ would spend more time in conformations that stimulate K$_{ATP}$ channel activity vs. WT SUR1, whereas SUR1$^{E1506K}$ would spend less time in stimulatory conformations.

**SUR1$^{E1506D}$ and SUR1$^{E1506K}$ both have reduced affinity for MgADP**

The current regulatory model suggests that ND and CHI mutations would have opposite effects on either the rate of catalysis or the affinity for MgADP. Since both E1506 substitutions are predicted to impair hydrolysis, we tested their effects on MgADP-induced conformational switching. Contrary to
to the current regulatory model, both E1506 substitutions have reduced affinity for MgADP (Figure 4), consistent with electrophysiological data demonstrating that SUR1E1506D/Kir6.2 and SUR1E1506K/Kir6.2 channels are less sensitive to MgADP stimulation (15). The neutral E1506Q substitution leads to a similar reduction in MgADP affinity (8).

The affinity of SUR1 for ATP correlates with clinical phenotype

The results for SUR1E1506D vs. SUR1E1506K and prior data on SUR1 ND mutations (8, 9) suggested a possible correlation between the affinity of a mutant SUR1 for ATP and its clinical phenotype, i.e., ND vs. CHI. To support this hypothesis we analyzed additional mutations including I1424V (ND) and G1479R (CHI) in NBD2 and a cluster of disease causing mutations in TMD2: C1174F (CHI), S1185A (CHI), Q1178R (ND), and R1182Q (ND). We previously showed that SUR1Q1178R and SUR1R1182Q, which are on transmembrane helix 15, have an increased affinity for ATP (9). Figure 3A shows that there is a strong correlation of ND (shown...
in red) and CHI mutations (shown in blue) with their respective clinical phenotype. An increased affinity for MgATP vs. WT SUR1 associates with ND, a decreased affinity with CHI. In other words, the ND mutants switch to a stimulatory conformation, with reduced affinity for glibenclamide, at lower concentrations of nucleotide.

In terms of the conventional model, this correlation could reflect differences in ATPase activity that result, for example, in an increase in the stimulatory MgATP-bound form of SUR1. To assess this possibility directly, we reduced the concentration of Mg$^{2+}$ to submicromolar levels to eliminate ATP hydrolysis and reassessed conformational switching with ATP$^{4-}$. Figure 3B shows that, with the exception of SUR1E1506K discussed above, the ND substitutions all have greater affinities for MgATP$^{4-}$ than WT SUR1; the CHI mutations all have reduced affinity. The data for SUR1E1506Q are provided for comparison. For quantitative comparison hydrolysis, in the absence of Mg$^{2+}$, was assumed to be negligible and a four-state equilibrium model was fit to the data. The estimates for $K_T$, the ATP dissociation constant and the allosteric constant $\beta$ are given in Table 1; the curves in Figure 3 were calculated using the four-state model.

**MgADP affinity is weakly correlated with clinical phenotype**

Based on the conventional regulatory model, in which the post-hydrolytic, MgADP-bound conformation is presumed to be stimulatory, one would anticipate the ND mutants might display greater affinities for MgADP vs. CHI mutants. Figure 4 shows that this is not the case. While three of four ND mutants have a higher affinity for MgADP, SUR1E1506D has a significantly weaker affinity than WT SUR1; two of four CHI mutants have lower affinities for MgADP vs. WT, but the affinities of SUR1S1185A and SUR1C1174F are nearly indistinguishable from WT. It is more difficult, however, to quantify differences in ADP affinity. Membrane preparations have significant endogenous adenylate kinase activity that generates ATP. This activity is partially suppressed by the addition of 10 mM AMP, which alone has no effect on conformational switching. At the higher MgADP levels required to switch conformations of some of the mutant receptors the generated MgATP becomes a significant factor. At 300 $\mu$M MgADP, ~4 $\mu$M MgATP is generated in our assays (9) and is expected to have significant effects, for example, on SUR1E1506Q and SUR1E1506D, which have higher affinities for MgATP, $K_T$ values of ~1 and 3 $\mu$M, respectively. The results for these mutants suggest MgADP may bind, but not switch conformations and thus may act as a competitive inhibitor. Thus, we have not attempted to compare the actions of MgADP quantitatively.

**Diazoxide sensitivity of CHI mutants**

Some CHI patients respond to diazoxide therapy and we showed previously that diazoxide, which binds at a site distinct from the NBDs and the GBC binding site, has a positive allosteric effect on MgATP binding to SUR1, i.e., diazoxide interacts preferentially with the nucleotide-bound conformation of SUR1 to stabilize an outward-facing configuration with reduced affinity for GBC (9). We assessed whether diazoxide had a similar action on these CHI mutants by measuring GBC binding at a constant concentration of MgATP (1 mM). Figure 5 shows that diazoxide potentiates the action of 1 mM MgATP on these CHI mutants. The inset shows the relative levels of conformational switching of three CHI mutants by 1 mM MgATP vs. WT SUR1 and indicates the action of diazoxide is effectively the same on each mutant. The result implies these mutations are at residues distinct from the diazoxide binding site and do not significantly affect either the SUR1/diazoxide interaction or the allosteric linkage between the GBC, ATP, and diazoxide.
While we cannot “grade” the clinical phenotype, especially since 2 diabetes. The exception to the rule is SUR1 insulating how to manipulate these allosteric properties would have clear for ATP correlates with ND, weaker affinities correlate with CHI. While we cannot “grade” the clinical phenotype, especially since phenotypes and siblings carrying the same mutation can differ in disease severity, these results establish a relationship between the allosteric properties of SUR1 and clinical phenotype. Knowing how to manipulate these allosteric properties would have clear translational relevance for treating these genetic disorders and type 2 diabetes. The exception to the rule is SUR1<sub>E1506K</sub>, which exhibits the expected reduced affinity for MgATP, the physiologic ligand, and a significantly increased affinity for ATP<sup>4−</sup>. We assume, on simple electrostatic grounds, that without Mg<sup>2+</sup>, the positively charged lysine must attract the phosphates of ATP. The results support earlier studies showing that ATP binding, not hydrolysis, is essential for switching SUR1 from a configuration with highest affinity for sulfonylureas, K<sub>ATP</sub> Channel antagonists, to a “stimulatory” conformation with weaker GBC affinity, but a stronger interaction with diazoxide, a K<sub>ATP</sub> channel agonist.

The results for MgADP are more complex. Models that assume hydrolysis is required to switch SUR1 to a stimulatory conformation suggest ND mutants would have a greater affinity for MgADP. While this is correct for some ND mutations, e.g., SUR1Q1178R, SUR1<sub>E1506D</sub> has a significantly weaker affinity. As pointed out above, determining affinities for MgADP is difficult as ATP, generated by adenylyl kinase, becomes significant at higher ATP concentrations. It is notable that the E1506D, and particularly the E1506Q, substitutions with higher affinities for ATP are not switched appropriately by endogenously generated ATP. A possible interpretation is that MgADP binds, but does not switch these mutant SURs and thus acts as a competitive inhibitor of ATP. It is not clear from the analysis of this limited number of mutants whether this effect is restricted to substitutions for the catalytic glutamate. It is worth emphasizing that based on the relative affinities of WT SUR1 for MgATP vs. MgADP (200 vs. 60 µM) that MgADP generated by hydrolysis could function as a “product inhibitor” to slow turnover.

Early studies (17, 19, 20) on the action of K<sub>ATP</sub> channel agonists, including diazoxide suggested ATP hydrolysis was required for these compounds to stimulate channel activity. A re-evaluation of these results using both ND and WT SUR1 showed that diazoxide binds preferentially to the ATP-bound form of SUR1 to potentiate ATP-driven receptor switching. The ATP, diazoxide, and GBC binding sites are allosterically linked; added diazoxide effectively increases the affinity of SUR1 for ATP; the ATP-bound conformation of SUR1 has a reduced affinity for GBC.

Some patients with dominant CHI mutations in ABCC8/SUR1 are responsive to diazoxide therapy (27, 30). However, usage of diazoxide is limited by low affinity and poor selectivity, resulting in side effects including fluid retention and hypertrichosis. Although CHI SUR1 mutants in this study exhibit reduced sensitivity to switching by stimulatory MgATP, our results indicate that diazoxide potentiates the switching action of MgATP on mutant SURs; specifically, high concentrations of diazoxide (≥ 100 µM) enhance the switching of millimolar MgATP. The data indicate these CHI mutations neither significantly impair diazoxide binding nor the positive allosteric linkage between diazoxide and MgATP suggesting that more potent, SUR1 selective diazoxide analogs such as NN414 could be more effective at switching SUR1 mutants at lower pharmacological concentrations, thus benefitting CHI patients.

ACKNOWLEDGMENTS

This work was funded by American Diabetes Association grant ADA 1-10-BS-21 and by National Institutes of Health grant DK098647 to JB.

REFERENCES

1. Carrier EA, Shen S, Shyng SL. Modulation of the trafficking efficiency and functional properties of ATP-sensitive potassium channels through a single amino acid in the sulfonylurea receptor. J Biol Chem (2003) 278:7081–90. doi:10.1074/jbc.M211959200
2. Sharma N, Crane A, Clement JP IV, Gonzalez G, Bahenken AP, Bryan J, et al. The C terminus of SUR1 is required for trafficking of K<sub>ATP</sub> channels. J Biol Chem (1999) 274:20628–32. doi:10.1074/jbc.274.20628
3. Yan FF, Lin YW, MacMullen C, Ganguly A, Stanley CA, Shyng SL. Congenital hyperinsulinism associated ABCC8 mutations that cause defective trafficking of ATP-sensitive K<sup>+</sup> channels: identification and rescue. Diabetes (2007) 56:2339–48. doi:10.2337/db07-0150
4. de Wet H, Mikhailov MV, Potinouc C, Dreger M, Craig TJ, Venien-Bryan C, et al. Studies of the ATPase activity of the ABC protein SUR1. FEBS J (2007) 274:5552–44. doi:10.1111/j.1742-4658.2007.05879.x
5. Mikhailov MV, Campbell JD, de Wet H, Shimomura K, Zadek B, Collins RE, et al. 3-D structural and functional characterization of the purified K<sub>ATP</sub> channel complex Kir6.2-SUR1. EMBO J (2005) 24:4166–75. doi:10.1038/sj.emboj.7600877
6. Proks P, Ashcroft FM. Modeling K<sub>ATP</sub> channel gating and its regulation. Prog Biophys Mol Biol (2009) 99:7–19. doi:10.1016/j.pbiomolbio.2008.10.002
7. Fatehi M, Raja M, Carter C, Soliman D, Holt A, Light PE. The ATP-sensitive K<sup>+</sup> channel ABCC8 S1369A type 2 diabetes risk variant increases MgATPase activity. Diabetes (2012) 61:241–9. doi:10.2337/db11-0371
8. Ortiz D, Gossack L, Quast U, Bryan J. Reinterpreting the action of ATP analogs on K<sub>ATP</sub> channels. J Biol Chem (2013) 288:18984–902. doi:10.1074/jbc.M113.476887
9. Ortiz D, Voryvodic P, Gossack L, Quast U, Bryan J. Two neonatal diabetes mutations on transmembrane helix 15 of SUR1 increase affinity for ATP and ADP at nucleotide binding domain 2. J Biol Chem (2012) 287:17985–95. doi:10.1074/jbc.M112.349019
10. Urbatsch JL, Julen M, Carrier I, Rousseau ME, Cayrol R, Gros P. Mutational analysis of conserved carbohydrate residues in the nucleotide binding sites of P-glycoprotein. Biochemistry (2000) 39:14138–49. doi:10.1021/bi001128w
11. Carrier I, Julen M, Gros P. Analysis of catalytic carbohydrate mutants E552Q and E1197Q suggests asymmetric ATP hydrolysis by the two-nucleotide-binding domains of P-glycoprotein. Biochemistry (2003) 42:12875–85. doi:10.1021/bi023425w
12. Stratford PL, Ramjesingh M, Cheung JC, Huan LJ, Bear CE. The Walker B motif residue adjacent to the Walker-B motif is the catalytic base for ATP hydrolysis in the ABC protein SUR1. FEBS J (2007) 274:7081–90. doi:10.1111/j.1742-4658.2007.05879.x
13. Orelle C, Dalmas O, Gros P, Di Pietro A, Iault JM. The conserved glutamate residue adjacent to the Walker-B motif is the catalytic base for ATP hydrolysis in the ATP-binding cassette transporter BmTmR. J Biol Chem (2003) 278:47002–8. doi:10.1074/jbc.M306826200
14. Rees DC, Johnson E, Lemonis O. ABC transporters: the power to change. Nat Rev Mol Cell Biol (2009) 10:218–27. doi:10.1038/nrm2646

www.frontiersin.org April 2015 | Volume 6 | Article 48 | 7
Clinical phenotype correlates with nucleotide-binding to ABCC8/SUR1

15. Mannikko R, Flanagan SE, Sim X, Segal D, Hussain K, Ellard S, et al. Mutations of the same conserved glutamate residue in NBD2 of the sulfonylurea receptor 1 subunit of the KATP channel can result in either hyperinsulinism or neonatal diabetes. Diabetes (2011) 60:1813–22. doi:10.2337/db10-1583
16. Flanagan SE, Clauin S, Bellanne-Chantelot C, de Lonlay P, Harries LW, Gloyn AL, et al. Update of mutations in the genes encoding the pancreatic beta-cell KATP channel subunits Kir6.2 (KCNJ11) and sulfonylurea receptor 1 (ABCC8) in diabetes mellitus and hyperinsulinism. Hum Mutat (2009) 30:170–80. doi:10.1002/humu.20838
17. Hambrock A, Löffler-Walz C, Quast U. Glibenclamide binding to sulphonylurea receptor subtypes: dependence on adenine nucleotides. Br J Pharmacol (2002) 136:995–1004. doi:10.1038/sj.bjp.0704801
18. Löffler-Walz C, Quast U. Binding of KATP channel modulators in rat cardiac membranes. Br J Pharmacol (1998) 123:1395–402. doi:10.1038/sj.bjp.0701756
19. Schwanzel-Mécher M, Loser S, Rietze I, Panten U. Phosphate and thiophosphate group donating adenine and guanine nucleotides inhibit glibenclamide binding to membranes from pancreatic islets. Naunyn Schmiedebergs Arch Pharmacol (1991) 343:83–9. doi:10.1007/BF00180681
20. Schwanzel-Mécher M, Loser S, Brandt C, Scheffer K, Rosenberger F, Panten U. Adenine nucleotide-induced inhibition of binding of sulphonylureas to their receptor in pancreatic islets. Br J Pharmacol (1992) 105:531–4. doi:10.1111/j.1476-5381.1992.tb0914.x
21. Wyman J, Gill SJ. Binding and Linkage: Functional Chemistry of Biological Macromolecules. Mill Valley, CA: University Science Books (1990).
22. Ashfield R, Gribble FM, Ashcroft SJ, Ashcroft FM. Identification of the high-affinity tolbutamide site on the SUR1 subunit of the KATP channel. Diabetes (1998) 48:1341–7. doi:10.2337/diabetes.48.6.1341
23. Vila-Carriles WH, Zhao G, Bryan J. Defining a binding pocket for sulfonylureas in ATP-sensitive potassium channels. FASEB J (2007) 21:18–25. doi:10.1096/fj.06-6730fbyp
24. Babenko AP, Polak M, Cave H, Busiah K, Czarnecki P, Scharfmann R, et al. Activating mutations in the ABCB3 gene in neonatal diabetes mellitus. N Engl J Med (2006) 355:456–66. doi:10.1056/NEJMoa050968
25. Huopio H, Otonkoski T, Vauhkonen I, Reimann F, Ashcroft FM, Laakso M. A new subtype of autosomal dominant diabetes attributable to a mutation in the gene for sulfonylurea receptor 1. Lancet (2003) 361:301–7. doi:10.1016/S0140-6736(03)12325-2
26. Nichols CG, Shyng SL, Nestorowicz A, Glaser B, Clement JP IV, Gonzalez G, et al. Adenosine diphasophate as an intracellular regulator of insulin secretion. Science (1996) 272:1785–7. doi:10.1126/science.272.5269.1785
27. Snider KE, Becker S, Boyajian L, Shyng SL, MacMullen C, Hughes N, et al. Genotype and phenotype correlations in 417 children with congenital hyperinsulinism. J Clin Endocrinol Metab (2013) 98:E355–63. doi:10.1210/jc.2012-2169
28. Kapoor RR, Flanagan SE, James CT, McKiernan J, Thomas AM, Harmer SC, et al. Hyperinsulinaemic hypoglycaemia and diabetes mellitus due to dominant ABCB8/KCNJ11 mutations. Diabetologia (2011) 54:2575–83. doi:10.1007/s00125-011-2207-4
29. Pinney SE, MacMullen C, Becker S, Lin YW, Hanna C, Thornton P, et al. Clinical characteristics and biochemical mechanisms of congenital hyperinsulinism associated with dominant KATP channel mutations. J Clin Invest (2008) 118:2877–86. doi:10.1172/JCI35414
30. Macmullen CM, Zhou Q, Snider KE, Tewson PH, Becker SA, Aziz AR, et al. Diazoceine-unresponsive congenital hyperinsulinism in children with dominant mutations of the beta-cell sulfonylurea receptor SUR1. Diabetes (2011) 60:1797–804. doi:10.2337/db10-1631
31. Dawson RJ, Locher KP. Structure of a bacterial multidrug ABC transporter. Nature (2006) 443:180–5. doi:10.1038/nature05155
32. Dawson RJ, Locher KP. Structure of the multidrug ABC transporter Sav1866 from Staphylococcus aureus in complex with AMP-PNP. FEBS Lett (2007) 581:935–8. doi:10.1016/j.febslet.2007.01.073
33. Chen YJ, Pornillos O, Liu S, Ma C, Chen AP, Chang G. X-ray structure of EmrE supports dual topology model. Proc Natl Acad Sci U S A (2007) 104:18999–9004. doi:10.1073/pnas.0709387104
34. Ward A, Reyes CL, Yu J, Roth CB, Chang G. Flexibility in the ABC transporter MdrA: alternating access with a twist. Proc Natl Acad Sci U S A (2007) 104:19005–10. doi:10.1073/pnas.0709388104

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 27 October 2014; accepted: 21 March 2015; published online: 15 April 2015. Citation: Ortiz D and Bryan J (2015) Neonatal diabetes and congenital hyperinsulinism caused by mutations in ABCB8/SUR1 are associated with altered and opposite affinities for ATP and ADP. Front. Endocrinol. 6:48. doi: 10.3389/fendo.2015.00048
This article was submitted to Genomic Endocrinology, a section of the journal Frontiers in Endocrinology.
Copyright © 2015 Ortiz and Bryan. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.