Reinterpreting the Action of ATP Analogs on K<sub>ATP</sub> Channels*  

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**Background:** Stimulation by post-hydrolytic, ADP-bound conformations of SUR1 underlies current models of K<sub>ATP</sub> channel activation; ATP analogs are assumed to lower activity by reducing hydrolysis.

**Results:** ATPγS switches conformations with lowered affinity; AMP-PxP selectively bind NBD1, preventing switching.

**Conclusion:** The actions of ATP analogs on K<sub>ATP</sub> channels require reinterpretation.

**Significance:** Reduced affinities of SUR1 NBDs for ATP analogs limit conformational switching and channel activity.

Neuroendocrine-type K<sub>ATP</sub> channels, (SUR1/Kir6.2)<sub>4</sub>, couple the transmembrane flux of K<sup>+</sup>, and thus membrane potential, with cellular metabolism in various cell types including insulin-secreting β-cells. Mutant channels with reduced activity are a cause of congenital hyperinsulinism, whereas hyperactive channels are a cause of neonatal diabetes. A current regulatory model proposes that ATP hydrolysis is required to switch SUR1 into post-hydrolytic conformations able to antagonize the inhibitory action of nucleotide binding at the Kir6.2 pore, thus coupling enzymatic and channel activities. Alterations in SUR1 ATPase activity are proposed to contribute to neonatal diabetes and type 2 diabetes risk. The regulatory model is partly based on the reduced ability of ATP analogs such as adenosine 5′-O-(β,γ-imino)triphosphate (AMP-PNP) and adenosine 5′-O-[(thiotriphosphate) (ATPγS) to stimulate channel activity, presumably by reducing hydrolysis. This study uses a substitution at the catalytic glutamate, SUR1<sub>E1507Q</sub>, with a significantly increased affinity for ATP, to probe the action of these ATP analogs on conformational switching. ATPγS, a slowly hydrolyzable analog, switches SUR1 conformations, albeit with reduced affinity. Nonhydrolyzable AMP-PNP and adenosine 5′-O-(β,γ-methylene)triphosphate (AMP-PCP) alone fail to switch SUR1, but do reverse ATP-induced switching. AMP-PCP displaces 8-azido-[125]ATP from the noncanonical NBD1 of SUR1. This is consistent with structural data on an asymmetric bacterial ABC protein that shows that AMP-PNP binds selectively to the noncanonical NBD to prevent conformational switching. The results imply that MgAMP-PNP and MgAMP-PCP (AMP-PxP) fail to activate K<sub>ATP</sub> channels because they do not support NBD dimerization and conformational switching, rather than by limiting enzymatic activity.

Neuroendocrine ATP-sensitive K<sup>+</sup> channels, (SUR1/Kir6.2)<sub>4</sub>, couple membrane electrical activity with cell metabolism in neurons and many endocrine cells, including pancreatic islet α-, β-, and δ-cells (see Ref. 1 for review). These channels respond to changes in the levels of MgATP and ADP, which have both inhibitory and stimulatory actions. Nucleotides bind to Kir6.2 to reduce the channel open probability, whereas ATP interactions with SUR1 antagonize this inhibitory effect. These nucleotide effects are modulated by other factors including phosphoinositides (2–9) and long-chain acyl-CoAs (10–16). SUR1, the channel regulatory subunit, is an enzyme, a member of the ATP-binding cassette (ABC) family of proteins that utilize the energy of ATP binding and hydrolysis to transport substrates across cell membranes (17, 18). A current model of K<sub>ATP</sub> channel regulation assumes that a post-hydrolytic, ADP-bound enzymatic intermediate or conformation of SUR1 stimulates channel openings, i.e. that hydrolysis is essential for activation (Ref. 19 and reviewed in (20–22). This model is based in part on studies demonstrating that nonhydrolyzable ATP analogs such as AMP-PNP and AMP-PCP fail to stimulate channel activity, whereas the slowly hydrolyzable ATPγS stimulates less efficiently than ATP (23–30).

Contrary to the current model, we observed that the binding of ATP, without hydrolysis, is sufficient to switch SUR1 from conformations with high affinity for glibenclamide (GBC), a K<sub>ATP</sub> channel antagonist, to conformations with reduced antagonist affinity, but a markedly enhanced affinity for the channel agonist diazoxide, i.e. from nonstimulatory to stimulatory states (31). ATP<sup>4</sup>, in the absence of Mg<sup>2+</sup> required for enzymatic activity (17), efficiently switched the conformations of wild-type (WT) SUR1 and mutant receptors known to hyperactivate Kir6.2 pores and thus cause neonatal diabetes (ND).

The ND mutant receptors, SUR1<sub>Q1178R</sub> and SUR1<sub>R1182Q</sub>, substitutions outside the nucleotide-binding domains (NBDs), have increased affinities for MgATP and ATP<sup>4</sup>, consistent with their spending more time in stimulatory conformations able to increase channel activity. Structural studies on multiple

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The abbreviations used are: Kir6.2, potassium inward rectifier type 6.2; SUR1, sulfonylurea receptor type 1; ABC, ATP-binding cassette; GBC, glibenclamide; ND, neonatal diabetes; NBD, nucleotide-binding domain; ATPγS, adenosine 5′-O-(thiotriphosphate); AMP-PxP, adenosine 5′-(β,γ-methylene)triphosphate; AMP-PCP, adenosine 5′-(β,γ-methylenephosphorothioate); MgAMP-PNP, Mg-5′-adenosylimidodiphosphate; MgAMP-PCP, Mg-adenylyl (β,γ-methylene) diphosphonic acid; AMP-PxP, MgAMP-PNP and MgAMP-PCP.
ABC proteins show that ATP binding to the NBDs induces the formation of an NBD dimer with two ATP molecules sandwiched in the dimer interface. Dimerization drives reconfiguration of the transmembrane helix bundles from inward-to-outward-facing conformations (reviewed in Refs. 32 and 33). Therefore we proposed that ATP binding and NBD dimerization, without hydrolysis, drive SUR1 from a nonstimulatory, inward-facing conformation with highest affinity for GBC to an outward-facing stimulatory state with >10-fold reduced affinity for GBC and >100-fold increased affinity for channel agonists (31).

AMP-PNP stabilizes NBD dimers of symmetric ABC proteins in outward-facing configurations with two bound nucleotides (34, 35). On the other hand, the structure of an asymmetric ABC protein, TM287/288 from Thermotoga maritima, shows a single AMP-PNP bound to an inward-facing conformation (36). Therefore the action of AMP-PNP and AMP-PCP on ATP-induced switching of SUR1, which like several other ABC proteins (31), was not required, included 1 mM free Mg2+ with MgADP and MgATP analogs, where a regenerating system of adenylate kinases to reduce ATP production. Experiments with MgADP included 10 mM AMP to inhibit endogenous adenylyl kinases to reduce ATP production. Experiments with MgADP and MgATP analogs, where a regenerating system was not required, included 1 mM free Mg2+. Mg2+-free experiments included 1 mM EDTA. Nonspecific binding was determined in the presence of 1 μM unlabeled GBC and was typically 15% of total binding. The results are plotted as

\[
\text{Specific bound GBC} = \frac{\text{specific bound (+X)}}{\text{specific bound (−X)}} \tag{1}
\]

where X is the reagent whose effect is being assayed, e.g. ± ATP2+, etc.

Photolabeling—8-azido-[32P]ATP photolabeling and densitometry were done as described previously (31).

Allosteric Analysis—The equations for the equilibrium models were derived following Wyman and Gill (51); the algebraic manipulations were done using Mathematica (Wolfram Research, Champaign, IL). The binding equations were derived from the binding partition functions, the sum of the contributions of the different species relative to one reference species, taken here as the unliganded receptor, R. The eight-state allosteric model shown in Fig. 4C is used as an example. The model describes the linkage between the equilibrium binding of MgAMP-PNP (A), 3H-labeled GBC (G), and MgATP (T) on SUR1(E1507Q) (R). \( K_A \) is the equilibrium dissociation constant for binding of A, \( K_G \) is the constant for G, and \( K_1 \) and \( K_2 \) are the constants for the binding of T to NBD1 and NBD2, respectively. \( \alpha \), \( \beta \), and \( \gamma \) are allosteric constants. \( \alpha = \gamma = 1 \) because previous data indicate that binding of nucleotide to NBD1 alone does not alter GBC binding (31). \( \beta \) is the allosteric constant reflecting the linkage between GBC binding and structural changes associated with occupation of both NBDs. For \( \beta > 1 \), nucleotide binding reduces the affinity of SUR1 for sulfonylureas. The partition function for the eight-state model is

\[
P = \frac{[R][RA][RG] + [\Gamma R][\Gamma RG] + [\Gamma R][\Gamma RG] + [\Gamma R][\Gamma RG]}{[R]} \tag{2}
\]

where \( \Gamma R \) and \( \Gamma T \) indicate binding at NBD1 and both NBD1 and NBD2, respectively. Substituting dissociation constants gives the binding polynomial.

\[
P = 1 + \frac{A}{K_A} + \frac{G}{K_G} + \frac{T}{K_1} + \frac{T^2}{K_1K_2} + \frac{GT}{\alpha K_1K_G} + \frac{GT^2}{\beta K_1K_2K_G} + \frac{AG}{\gamma K_1K_2K_G} \tag{3}
\]
**Action of ATP Analogs on SUR1**

\( G \), the amount of \(^3\)H-labeled GBC specifically bound per mole of SUR1, which is dependent on \([G]\), \([T]\), and \([A]\), is given by

\[
G = \frac{\partial P}{\partial \ln G} = \frac{[G]}{P} \frac{\partial P}{\partial G} \tag{Eq. 4}
\]

where

\[
G = \frac{1}{\alpha K_G + \beta K_T + \gamma K_A} \tag{Eq. 5}
\]

The plotted experimental variable, specific bound GBC, is defined as \( G / G_{so,ATP} \). Parameters were estimated using non-linear fit models in the Mathematica environment with \( \alpha = 1 \) and predetermined values of \( \beta, K_{C_G} \) and \( G \).

The equation for the four-state equilibrium model is

\[
G = \frac{1}{\alpha K_G + \beta K_T \gamma K_A} \tag{Eq. 6}
\]

Fittings were estimated using predetermined values for \( K_C \).

**Statistics**—Where indicated, the IC\(_{50}\) values were estimated by fitting a logistic equation. The means ± S.E. are plotted; the number of replicate experiments varies, but \( n \geq 3 \) in all cases.

**RESULTS**

**ATP-induced Conformational Switching of WT and Mutant SUR1**—The effect of ATP on SUR1 conformation was determined using GBC as a conformational probe. Fig. 1 shows that ATP, with or without Mg\(^{2+}\), has a strong negative effect on GBC binding. ATP effectively switches WT and substituted SUs from inward-facing conformations with highest affinities for GBC to outward-facing states with reduced affinities as shown schematically. The apparent differences in affinity, judged for example by their IC\(_{50}\) values (panel A and Table 1), is ~430-fold for WT versus SUR1\(_{E1507Q}\); the value for SUR1\(_{Q1178R}\), characterized previously, is intermediate (31). Comparison of panels A and B shows that Mg\(^{2+}\) significantly increases the apparent affinity of all three receptors for ATP.

The E1507Q and Q1178R substitutions do not have large effects on the affinities of the apo-receptors for GBC, given by the dissociation constants (\( K_C \)), which are determined in independent experiments (Table 1). To characterize the negative allosteric linkage between binding sites in SUR1\(_{E1507Q}\) we assume that hydrolysis is negligible both in the presence and in the absence of Mg\(^{2+}\) (43, 44). A four-state equilibrium model (Fig. 1, inset) was used to estimate the affinities for ATP (\( K_T \)) and the allosteric constants (\( \beta \)). The results are tabulated in Table 1 along with the IC\(_{50}\) values. The four-state model has not been used to analyze the MgATP effects on WT and SUR1\(_{Q1178R}\), which are potentially in an enzymatic steady state. In Fig. 1, the solid lines are the best-fit curves derived from the
four-state model. The fits were constrained using the values for $K_G$, the dissociation constants for GBC binding to the apo-receptors (Table 1). The product of the allosteric constant, $\beta$, and $K_G$ determines the plateau values at saturating concentrations of nucleotide and is the dissociation constant for GBC binding to the fully ATP-ligated receptor. The estimates of $\beta$ for WT and SUR1Q1178R are consistent with fully ATP-ligated, outward-facing receptors having approximately a 10-fold lower affinity for GBC (31). The value for SUR1E1507Q, 40, is significantly greater, implying that the affinity of fully ATP-ligated SUR1E1507Q for GBC is considerably reduced, i.e. 0.63 versus 25 nM, unliganded versus liganded, respectively.

ATP$^4^-$ switches the conformation of WT and mutant receptors. Fig. 1B shows a significant difference, ~27-fold, in the affinities of SUR1E1507Q versus SUR1Q1178R for ATP$^4^-$.

The effect of nucleotide on WT is not saturated, but the estimated affinity of SUR1E1507Q is ~150-fold greater than WT (Table 1). The structural difference between SUR1E1507Q and WT (Glu-1507) is the elimination of the negative charge at position 1507. The results imply that electrostatic interactions between phosphate residues and the carboxyl group at position 1507 is a significant determinant of nucleotide affinity. Neutralizing the charge significantly increases the binding energy by ~3 kcal/mol at 37°C, SUR1E1507 versus WT. The general finding that added Mg$^{2+}$, bound to the $\gamma$ phosphates, increases the apparent affinity supports this idea.

Comparing the affinities of SUR1E1507Q for MgATP versus ATP$^4^-$ shows that the receptor binds MgATP ~100-fold more tightly than ATP$^4^-$.

Thus the addition of Mg$^{2+}$ contributes ~2.8 kcal/mol to the binding energy at 37°C. The WT and SUR1Q1178R receptors show a semiquantitatively similar Mg$^{2+}$ effect when comparing the $IC_{50}$ values for MgATP versus $K_G$ for ATP$^4^-$ (Table 1). It is worth noting that nucleotide binding and GBC binding are negatively linked allosteric functions; thus the GBC (1 nM) used to assess conformational changes reduces the apparent affinity for nucleotide, i.e. "right shifts" the response curves. This is apparent, for example, for SUR1E1507Q where the $IC_{50}$ is ~3-fold greater than the estimated $K_G$ value.

**MgADP-induced Switching**—Fig. 2 shows that SUR1E1507Q has a reduced affinity for MgADP when compared with WT SUR1. The membrane preparations have significant endogenous adenylate kinase activity able to convert MgADP to ATP. This activity is suppressed by the addition of AMP (10 mM). ATP measurements using luciferase show that the concentration of ATP is ~4 μM when the concentration of MgADP is 300 μM (31). Thus at the highest concentration, 1 mM MgADP, a fraction of the switching is due to ATP.

It is worth noting that although the E1507Q substitution has not been associated with disease, the E1507D substitution, also expected to have impaired ATPase activity, is a cause of ND. The SUR1E1507D/Kir6.2 channels exhibit a comparable reduced sensitivity to stimulation by MgADP (52).

Interestingly, although the SUR1E1507Q substitution has a reduced affinity for MgADP, SUR1Q1178R, which also hyperactivates Kir6.2 pores to produce ND, has a significantly higher apparent affinity for MgADP (31) (Fig. 2).

**ATP$^S$, a Slowly Hydrolyzable ATP Analog, Switches SUR1 Conformations with Reduced Affinity**—ATP$^S$, an analog of ATP with one oxygen of the $\gamma$-phosphate replaced by a sulfur atom, is slowly utilized by a variety of kinases and ATPases (53). In the current regulatory model, reduced enzymatic activity would slow transition to a post-hydrolytic stimulatory conformation. This idea was tested by assessing the action of ATP$^S$ on GBC binding under steady state and equilibrium conditions (~Mg$^{2+}$). Fig. 3A compares the action of MgATP versus MgATP$^S$ on conformational switching. SUR1E1507Q has a higher affinity for MgATP versus MgATP$^S$, estimated using a four-state equilibrium model. The dissociation constants ($K_G$) for ATP are 1.0 ± 0.2 versus 29 ± 7 μM, MgATP versus MgATP$^S$, respectively. To support the use of the four-state model and to assess the binding of ATP$^S$ directly, i.e. without Mg$^{2+}$ present, the affinities of SUR1E1507Q for ATP$^S^-$ and ATP$^S$$^4^-$ were compared (Fig. 3B). The estimated $K_G$ values were 94 ± 8 versus 234 ± 31 μM, for ATP$^S^-$ versus ATP$^S$$^4^-$, respectively. It is worth noting that the allosteric constants, $\beta$ values, for the two nucleotides are significantly different. The final plateau at saturating concentrations of nucleotide determines this parameter. The $\beta$ values used to specify the curves in Fig. 3B were 3.2 ± 0.1 versus 40 ± 1, for ATP$^S^-$ versus ATP$^S$$^4^-$, respectively. The results show that ATP$^S$ supports conformational switching of SUR1 and that the affinity for this analog is less than for ATP, or without Mg$^{2+}$.

**AMP-PNP and AMP-PCP Reverse the ATP-induced Conformational Switching of SUR1**—Nonhydrolyzable ATP analogs, specifically MgAMP-PNP and MgAMP-PCP, are unable to activate $K_{ATP}$ channels (19, 25, 54–56) and will reduce channel activity when MgATP is present (Ref. 19, but see Ref. 57). MgAMP-PNP and MgAMP-PCP are assumed not to hydrolyze
and thus prevent the transition of SUR to post-hydrolytic, stimulatory conformations (for review, see Refs. 20 and 21). MgAMP-PNP does dimerize the NBDs of symmetric ABC proteins (34, 35); thus we anticipated that it could switch SUR1. However, Fig. 4 shows that MgAMP-PNP at concentrations 100 times the IC50 values for MgATP (Fig. 1) has no significant effect on GBC binding, i.e. AMP-PNP alone does not support conformational switching of either SUR1Q1178R (Fig. 4A) or SUR1E1507Q (Fig. 4B). AMP-PCP alone also has no effect. This could imply that AMP-PNP does not interact with SUR1 as suggested by others (57). To test this possibility, receptors were incubated with 30 μM MgATP (to “preswitch” them) and increasing concentrations of AMP-PNP or AMP-PCP. AMP-PNP concentration-dependently reverses the effect of MgATP on GBC binding, similar to an early observation by Schwanstecher et al. (58). Fig. 4 (A and B) shows the results for SUR1Q1178R and SUR1E1507Q, respectively. As an additional control, these experiments were performed with and without the ATP-regenerating system present with similar results. Fig. 4B suggests that the affinity of SUR1E1507Q for AMP-PCP is ~10-fold less than for AMP-PNP. The reversal effect of AMP-PNP is not dependent on Mg2+. AMP-PNP4− effectively reverses the action of 500 μM ATP4− (Fig. 4B).

FIGURE 3. SURs have reduced affinity for ATP versus ATP. A, comparison of MgATP-S versus MgATP effects. The curves are best fits to a four-state model (Fig. 1B, inset). The best-fit dissociation constants are Kt = 1 and 29 μM for MgATP and MgATP-S, respectively. The β values are 40 and 11.6 for MgATP and MgATP-S, respectively. The dissociation constant, Kd = 0.63 nm for GBC, was determined independently. B, comparison of the affinities of SUR1E1507Q for ATP− versus ATP−. The solid curves are the best fits to the four-state model; the parameters are Kt = 94 versus 234 μM; β = 40 versus 3.2, ATP− versus ATP−, respectively. The dissociation constant for GBC, Kd = 0.63 nm, was fixed during fitting.

FIGURE 4. AMP-PxP reverse ATP-induced conformational switching. A and B, MgAMP-PNP alone does not affect the conformational state of SUR1Q1178R (A) or SUR1E1507Q (B). MgAMP-PNP reverses the conformational shift induced by MgATP (30 μM) in both SURs. MgAMP-PCP and AMP-PNP4− also reverse, but the apparent affinities are lower. The dashed lines are logistic curves through the data points; the parameters are S0.5 = 530 ± 124 μM for SUR1Q1178R (A). The SUR1E1507Q S0.5 values in B are 573 ± 103, 3360 ± 410, and 31,100 ± 2240 μM for MgAMP-PNP, MgAMP-PCP, and AMP-PNP4−, respectively. C, the solid curve in B for SUR1E1507Q is a fit to an eight-state model with the following parameters: α = γ = 1, β = 40, Kd = 0.63 nm, K1 = 0.7 ± 0.2 μM, K2 = 1.5 ± 0.1 μM, Kα = 1.2 ± 0.2 μM.
TABLE 2

| SUR1    | MgAMP-PNP | MgAMP-PCP | AMP-PNP | $S_{0.5}$ | $K_1$ | $K_2$ | $K_A$ |
|---------|-----------|-----------|---------|-----------|-------|-------|-------|
| Q1178R  | 530 ± 124 | 3360 ± 410| 31100 ± 2240|  —       | 0.7 ± 0.2 | 1.5 ± 0.1 | 1.2 ± 0.2 |
| E1507Q  | 573 ± 103 |  —       |  —       |  —       |  —       |  —       |  —       |

An eight-state equilibrium model was used to estimate the linkage between the binding of AMP-PNP, ATP, and GBC on SUR1 (Fig. 4C, graphic). The model includes the binding of two ATP (T) molecules at the NBDs of SUR1 and the binding of a single GBC molecule (G). Based on the reversal experiments, and the observation of Hohl et al. (36) that AMP-PNP interacts with a single NBD in an asymmetric ABC protein to stabilize the inward-facing conformation, we assume that AMP-PNP (A) binds to NBD1 to stabilize inward-facing conformations of SUR1 with the highest affinity for GBC. The estimated binding parameters are reasonably consistent with the results in Fig. 1. The dissociation constant ($K_2$) for MgATP at NBD2 is 1.5 μM, in agreement with ~1 μM determined in Fig. 1B. The dissociation constant ($K_1$) for MgATP at NBD1 is estimated at 0.7 μM, broadly consistent with estimates of IC50, 10–40 μM, for 8-azido-[32P]ATP4− binding at NBD1 (31) and the observation that adding Mg2+ increases the affinity of nucleotide binding at NBD2 by ~100-fold. The fitting suggests that the dissociation constant ($K_1$) for MgAMP-PNP at NBD1 is similar to that for MgATP. The allosteric constant, $\beta$ = 40, agrees with the estimates in Fig. 1. In Fig. 4 (A and B), the dashed lines are curves drawn through the points; no attempt has been made to fit an eight-state model to the partial saturation data for AMP-PCP and AMP-PNP4− or for SUR1-Q1178R, which is potentially in steady state. The binding parameters are summarized in Tables 1 and 2.

**DISCUSSION**

ATP has inhibitory and stimulatory actions on $K_{ATP}$ channels. Nucleotide binding to the Kir pore reduces the probability of channel openings, whereas interactions with SUR1 antagonize this inhibition to stimulate channel activity. SUR1 is an ABC protein, and like other ABC proteins, is reported to have Mg2+−dependent ATPase activity (17, 18). An ADP-bound, post-hydrolytic conformation of SUR1 is proposed to be the enzymatic intermediate that stimulates openings of ATP-inhibited Kir6.2 pores. By contrast, we observe that ATP binding, in the absence of Mg2+ needed for hydrolysis, is sufficient to switch the conformations of WT SUR1 and SUR1 ND mutants. The ATP-bound states are presumed to be stimulatory conformations based on pharmacologic criteria, i.e. their reduced affinity for GBC, a channel antagonist, and increased affinity for diazoxide, a channel agonist (31). The structures of multiple apo- and ATP-bound ABC proteins (reviewed in Refs. 33 and 35) imply that ATP binding switches SUR1 from inward-facing, nonstimulatory apo states to ATP-liganded, outward-facing stimulatory configurations. This is shown as a graphic in Fig. 1. The increased affinity of the ND mutant receptors for ATP implies that they will spend more time in stimulatory conformations and thus produce the hyperactive $K_{ATP}$ channels characteristic of neonatal diabetes.

The inhibitory actions of ATP analogs have been used to support the current regulatory model by assuming that reduction of the rate of transition to a post-hydrolytic stimulatory conformation would reduce $K_{ATP}$ channel openings. To test this assumption, we analyzed the mechanism(s) by which ATPγS and AMP-PxP affect conformation switching. Substi-
tuted receptors with increased affinities for ATP were used to facilitate the analysis. Substitution of the SUR1 catalytic glutamate, Glu-1507, with glutamine, expected to drastically reduce enzymatic activity and thus conversion to ADP-bound intermediates, significantly increased the affinity for ATP, which enhanced, rather than impaired, conformational switching (Fig. 1).

The results show that ATPγS can efficiently switch the conformation of substituted SUR1, albeit the affinities for this analog are weaker than for ATP. The affinity of SUR1E1507Q for MgATPγS is ~30-fold weaker than for MgATP. Mg2+ is not required for switching, and thus reduced rates of hydrolysis are not a factor. The ATP γ-phosphate has multiple interactions with the NBDs, and thus the reduced affinities for ATPγS versus ATP imply that substitution of a sulfur atom for oxygen weakens one or more of these interactions. We suggest that the reduced stimulatory action of MgATPγS is a reflection of the reduced affinity of SUR1 for this analog rather than a slow rate of hydrolysis.

The interactions with AMP-PNP and AMP-PCP are more complex. Several studies showed that these nonhydrolyzable analogs alone fail to stimulate KATP channels, will inhibit ATP stimulated channels, and fail to support the action of channel agonists (23–30). The usual interpretation has been that these stimulated channels, and fail to support the action of channel factors affecting ATP binding. Mg2+ pare the affinities of SUR1E1507Q for MgATP

matic activity, a four-state equilibrium model was used to com-}

properties of TM287-TM288 with a single AMP-PNP bound to the noncanonical NBD, which supports dimerization, thus leading to outward-facing conformations (36) and with data on cystic fibrosis transmembrane conductance regulator showing preferential binding at NBD1 (48, 49). An eight-state model (Fig. 4C) was used to make a semiquantitative estimate of the affinity of AMP-PNP for SUR1E1507Q. Based on the structure of TM287-TM288 with a single AMP-PNP bound to the noncanonical NBD, the eight-state model assumes binding only at NBD1 at concentrations below 10 mM. Equilibrium conditions are assumed to hold for SUR1E1507Q. A dissociation constant (Kd) ~1.2 μM for MgAMP-PNP binding to NBD1 was obtained.

The analysis of switching in SUR1E1507Q provides insight into factors affecting ATP binding. Mg2+ significantly increases the apparent affinity of SUR1 for nucleotides (see Ref. 31) (compare Fig. 1A with Fig. 1B), consistent with Mg2+ interacting with Glu-1507 and the γ-phosphate directly or via a bridging water molecule as observed in other ABC proteins (35, 62). To quantify the effect of Mg2+ on ATP binding in the absence of enzymatic activity, a four-state equilibrium model was used to compare the affinities of SUR1E1507Q for MgATP versus ATP4-.

The estimated dissociation constants (Kd) are 1 versus 94 μM, ± Mg2+, respectively, i.e. Mg2+ increases the affinity ~100-fold, adding ~2.8 kcal/mol of binding energy.

A second observation is worth noting. The allosteric constant, β, in the four-state model is significantly greater for SUR1E1507Q than WT or any of the other mutant receptors. Typical values range from ~7 to 15, but the value for SUR1E1507Q is ~40 with or without Mg2+ present. The product, βKd, reflects the affinity of the fully ATP-ligated receptor for GBC. Previous estimates for the affinities for GBC of WT, SUR1Q1178R, and SUR1E1182Q in the ATP-bound outward-facing state showed that they were 10–12 times weaker than for the inward-facing conformation, i.e. ~10 nm versus 1 nm for the apo-receptors (31). Thus the affinity of ATP-bound SUR1E1507Q is ~25 nm (40 × 0.63 nm), reflected in the lower plateau values at saturating concentrations of ATP. The structural difference(s) responsible for this change are not clear, but we speculate that the SUR1E1507Q NBD dimer may be more compact, allowing a greater “twist” of the transmembrane helical domains, TM1D and TM2D, that determine the GBC-binding pocket.

The E1507Q substitution has not been identified with disease to date, but its high affinity for ATP predicts that it would produce hyperactive channels. SUR1E1507Q has a somewhat reduced affinity for MgADP versus WT, but a more precise estimate is difficult because its affinity for ATP is high and it is hard to suppress the endogenous adenylyl kinase in our membrane preparations. The results are consistent with electro- physiologic data on the E1507D substitution that, when assembled with Kir6.2, produces hyperactive KATP channels that are less sensitive to stimulation by MgADP (52).

In summary, the present results demonstrate the need to reinterpret early results that suggested that ATP analogs regulate KATP channel activity by affecting ATP hydrolysis. The present data suggest that ATPγS is less effective than ATP because SUR1 binds it less tightly, whereas AMP-PNP and AMP-PCP interact with asymmetric SUR1 via selective binding to NBD1 that prevents NBD dimerization and thus conformational switching.

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