Modulation of the Trafficking Efficiency and Functional Properties of ATP-sensitive Potassium Channels through a Single Amino Acid in the Sulfonylurea Receptor*

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Mutations in the sulfonylurea receptor 1 (SUR1), a subunit of ATP-sensitive potassium (K\text{ATP}) channels, cause familial hyperinsulinism. One such mutation, deletion of phenylalanine 1388 (ΔPhe-1388), leads to defects in both trafficking and MgADP response of K\text{ATP} channels. Here we investigated the biochemical features of Phe-1388 that control the proper trafficking and function of K\text{ATP} channels by substituting the residue with all other 19 amino acids. Whereas surface expression is largely dependent on hydrophobicity, channel response to MgADP is governed by multiple factors and involves the detailed architecture of the amino acid side chain. Thus, structural features in SUR1 required for proper channel function are distinct from those required for correct protein trafficking. Remarkably, replacing Phe-1388 by leucine profoundly alters the physiological and pharmacological properties of the channel. The F1388L-SUR1 channel has increased sensitivity to MgADP and metabolic inhibition, decreased sensitivity to glibenclamide, and responds to both diazoxide and pinacidil. Because this conservative amino acid substitution occurs in the SUR2A and SUR2B isoforms, the mutation provides a mechanism by which functional diversities in K\text{ATP} channels are generated.

ATP-sensitive potassium (K\text{ATP}) channels play a key role in linking metabolism to membrane excitability in muscle, neurons, and endocrine cells (1, 2). Each K\text{ATP} channel complex is composed of four subunits of a regulatory sulfonylurea receptor (SUR) and four subunits of a pore-forming inward rectifier potassium channel Kir6.2 (3–5). The activity of K\text{ATP} channels is regulated by intracellular ATP and ADP; ATP inhibits channel activity via nonhydrolytic binding to the channel, whereas ADP stimulates channel activity in the presence of Mg\textsuperscript{2+} in a nucleotide hydrolysis-dependent way (1, 2). Structure-function studies have led to the current view that the Kir6.2 subunit mediates channel inhibition by ATP (6, 7), and the SUR subunit mediates the stimulatory effects of MgADP as well as channel response to sulfonylureas and potassium channel openers (KCOs) (8–12). There are two known SUR genes, SUR1 and SUR2. SUR2 further gives rise to several splice variants, the major ones being SUR2A and -2B, which differ in the last 42 amino acids. SUR1, SUR2A, and SUR2B, when combined with Kir6.2, form the pancreatic, cardiac, and vascular smooth muscle subtypes of K\text{ATP} channels, respectively (10, 13–16). These subtypes of K\text{ATP} channels differ in their sensitivities to nucleotides, sulfonylureas, and KCOs (8, 17). SURs belong to the ATP Binding Cassette family of membrane proteins (ABC transporters); each SUR molecule contains two of the nucleotide binding domains (NBD) that are highly conserved in all ABC transporters. Biochemical evidence suggests that hydrolysis of MgATP at NBD2 stabilizes binding of ATP at NBD1 and thereby facilitates functional coupling between SUR1 and Kir6.2 and channel activation (18–20). An increase in ADP concentrations stimulates channel activity likely by slowing the rate of ATP hydrolysis at NBD2, locking NBD2 in a post-hydrolytic MgADP-bound state (19). Because the stimulatory effect of ADP requires Mg\textsuperscript{2+} and hydrolyzable nucleotides, we will refer to the effect as the MgADP response.

In the pancreas, K\text{ATP} channels regulate insulin secretion in response to changes in blood glucose levels. Loss of functional K\text{ATP} channels as a result of genetic mutations is a major cause of familial hyperinsulinism, a disease characterized by excessive insulin secretion and severe hypoglycemia (21, 22). In many cases, mutations in SUR1 or Kir6.2 attenuate or abolish K\text{ATP} channel function by causing protein truncations or by affecting the ability of the channel to respond to MgADP (11, 21–23). Recently, defective trafficking of K\text{ATP} channels has emerged as another important mechanism underlying the disease (24–27). Under normal conditions, trafficking of K\text{ATP} channel complexes out of the endoplasmic reticulum (ER) is controlled by a tripeptide Arg-Lys-Arg (RKR) retrieval signal present in each of the SUR and Kir6.2 subunits (28). Upon successful assembly of SUR and Kir into an octameric complex, the -RKR- motifs are concealed to allow the channel to translocate from the ER to the Golgi, where the sugar moiety on SUR is modified before further translocation to the plasma membrane (3, 25, 28–30). Thus, the -RKR- trafficking signal provides a quality control mechanism to prevent individual subunits as well as incompletely assembled channel complexes from trafficking to the cell surface.

One mutation that we have reported previously to cause defective channel trafficking is the deletion of phenylalanine at position 1388 of SUR1 (ΔPhe-1388). The ΔPhe-1388-SUR1 mutant channels were retained in the ER, unable to reach the cell surface (26). However, a small percentage of them could escape...
to the cell surface when the RKR retention signal in SUR1 was inactivated by mutation (26). The mutant channels thus expressed on the cell surface showed no response to MgADP and therefore would not be able to respond to metabolic changes. These observations indicate that Phe-1388 of SUR1 is critical for both correct trafficking and function of K<sub>ATP</sub> channels. In the present study, we investigated the biochemical properties of the phenylalanine residue that are important in each aspect of channel regulation by substituting Phe-1388 in SUR1 with other amino acids (collectively referred to as F1388X-SUR1 mutants). We show that the substitutions have differential effects on channel trafficking and channel response to MgADP (hence channel function). Whereas hydrophobicity determines the efficiency of channel trafficking, the detailed architecture of the amino acid side chain appears to be important in ensuring proper channel response to MgADP. Importantly, replacing Phe-1388 by leucine dramatically potentiated channel response to MgADP. Such a “gain of MgADP response” phenotype may be exploited for therapeutic purposes in managing insulin secretion. Moreover, F1388L-SUR1 mutant channels exhibited reduced sensitivities to glibenclamide and are stimulated by both ATP and ADP. These results demonstrate the role that the 1388 residue of SUR1 plays in modulating the physiological and pharmacological properties of K<sub>ATP</sub> channels. Because the SUR1-Phe-1388 equivalent position in SUR2A and SUR2B channels to express on the cell surface. To examine which chemical features of the phenylalanine residue are critical for proper trafficking of the channel, we substituted Phe-1388 in SUR1 with all other 19 amino acids and determined how the substitutions affected surface expression of K<sub>ATP</sub> channels. For all F1388X-SUR1 mutant constructs, a FLAG epitope tag was added to the N terminus to allow surface labeling of the protein. Each mutant SUR1 construct was cotransfected with wild-type (WT) Kir6.2 into COS cells, and surface expression of K<sub>ATP</sub> channels was quantified by a chemiluminescence assay (27). Results from chemiluminescence assays were further confirmed by immunofluorescent microscopy of surface-labeled FLAG-SUR1 (not shown). Based on surface expression levels, the mutants can be classified into two groups: those that give rise to expression levels above 50% of that of the WT, and those that give rise to levels below 50% of that of the WT (Fig. 1). Amino acids in the first group include Phe, Cys, Ala, Val, Leu, Ile, Met, and Trp; these amino acids are in general considered hydrophobic. By contrast, amino acids with polar and charged side chains dramatically reduced surface expression of the channel.

**Western Blot Analysis—**SUR1 protein that is expressed on the cell surface has traversed the Golgi apparatus where it becomes complex-glycosylated; this complex-glycosylated SUR1 has lower mobility than the core-glycosylated form on the SDS-PAGE and is referred to as the “upper band” (26–29). We examined the glycosylation pattern of F1388X-SUR1 mutants in cells coexpressing Kir6.2 by Western blots. All of the mutants in the first group of Fig. 1 (Cys, Ile, Leu, Met, Val, Ala, and Trp) have an upper band that corresponds to the complex-glycosylated form, similar to the WT protein. On the other hand, mutants in the second group of Fig. 1 (Phe, Cys, Ala, Val, Leu, Ile, Met, and Trp) have no upper band. In the third group of Fig. 1, the Phe, Cys, Ala, Val, Leu, Ile, Met, and Trp mutants that are located at the C terminus of SUR1 have a lower band corresponding to the core-glycosylated form of SUR1. This indicates that the core-glycosylated form of SUR1 is present in the Golgi apparatus before being transported to the cell surface.
hand, all of the mutants in the second group of Fig. 1 have none or have a barely detectable (when overexposed, not shown) upper band. These results are consistent with surface expression results obtained by the chemiluminescence assay. Previous work (26) has found that the ΔPhe-1388 mutation abolished surface expression of K\textsubscript{ATP} channels without significantly altering the steady-state SUR1 protein levels. We found that the steady-state total protein levels of F1388X-SUR1 mutants (the sum of the upper and the lower bands) in the first group are largely unaltered compared with the WT SUR1 (upper two panels of Fig. 2). However, mutants in the second group in general exhibited lower protein levels (lower two panels of Fig. 2), in particular, when Phe-1388 was substituted by Pro or Ser (marked with asterisks in Fig. 2). Similar results were obtained in cells expressing SUR1 mutant protein alone (not shown).

**MgADP Response**—Earlier studies have demonstrated that a small percentage of the ΔPhe-1388-SUR1 mutant channels retained in the ER could escape to the cell surface when the ΔRKR-ER retention/retrieval motif in SUR1 was inactivated by mutation to AAA (26). However, unlike the WT channels, the ΔPhe-1388\textsubscript{SUR1} channel expressed on the cell surface failed to respond to MgADP. The data presented above demonstrate that hydrophobicity of the amino acid at SUR1 position 1388 is a critical parameter in determining the ability of the channel to exit ER and express on the cell surface. To test if the same parameter governs channel response to MgADP, we performed inside-out patch clamp recordings of the F1388X-SUR1 mutant channels. Of the mutants that had poor surface expression, we managed to obtain a few patches from the F1388T, F1388V, F1388P, F1388S (n = 3–5), and F1388D (n = 2) mutants. No patches containing detectable currents were obtained for the F1388G, F1388E, F1388H, F1388K, F1388N, F1388Q, and F1388R mutants. Typical current traces from the WT and the mutant channels are shown in Fig. 3. The MgADP response was quantified as the current in a solution containing 0.1 mM ATP, 0.5 mM ADP, and 1 mM free Mg\textsuperscript{2+}, relative to the current in the absence of any nucleotides. This response in mutant channels was further normalized to the response obtained for the WT channel and is shown in Fig. 4.

Comparing Fig. 4 (MgADP response) to Fig. 1 (cell surface expression), it becomes immediately clear that the chemical properties of the amino acid at the SUR1 1388 site governing channel expression are distinct from those governing channel response to MgADP. Many of the amino acid substitutions that are tolerated for surface expression have very poor response to MgADP (for example, F1388C, F1388V, and F1388I), and vice versa, some of the amino acid substitutions that render poor surface expression of the channel still retain the ability to respond to MgADP (for example, F1388T and F1388S). Remarkably, the leucine and isoleucine substitutions, although both resulted in efficient channel expression, differed dramatically in their response to MgADP. Whereas leucine potentiated channel response to MgADP by nearly 1.7-fold (Fig. 4), isoleucine almost abolished channel response to MgADP (Figs. 3 and 4). The two amino acids have the exact same chemical compositions and similar physical chemical properties; the only difference between them is the architecture of the side chain. Therefore, the parameters required for conferring MgADP response appear to involve detailed side chain structures and not just hydrophobicity.

**Characterization of the Physiological and Pharmacological Properties of the F1388L-SUR1 Mutant Channel**—The observation that the F1388L-SUR1 mutant channels are better stimulated by MgADP prompted us to characterize these channels in greater detail. Sequence comparison between SUR1 and SUR2A and -2B reveals that the SUR1-Phe-1388 equivalent residue in SUR2A and -2B is a leucine (10). SUR1, SUR2A, and SUR2B, when combined with Kir6.2, form the pancreatic, cardiac, and vascular smooth muscle subtypes of K\textsubscript{ATP} channels, respectively. These K\textsubscript{ATP} channel subtypes display different sensitivities to ATP, MgADP, sulfonylureas, and potassium channel openers (8, 17, 31, 32). The SUR1/Kir6.2 recombinant channels have high sensitivities to inhibition by ATP (K\textsubscript{i} ≈ 10 μM) and glibenclamide (K\textsubscript{i} < 10 μM) and are stimulated only by the KCO diazoxide. Channels containing the SUR2 isoforms, on the other hand, have lower sensitivities to ATP and glibenclamide but are stimulated by potassium channel openers other than diazoxide, such as pinacidil and cromakalim (8, 10, 15, 17, 33–35). We wondered whether the conservative exchange between phenylalanine and leucine accounts for some of these physiological and pharmacological differences.

ATP dose-response measurements in the absence of Mg\textsuperscript{2+} yielded a K\textsubscript{i} of 13.8 ± 4.3 μM for the WT channels and 16.4 ± 3.5 μM for the F1388L-SUR1 channels (Fig. 5A). These values are not significantly different from one another (at p = 0.1), indicating that Phe-1388 is not a determinant of channel sensitivity to ATP. As shown in Figs. 3 and 4, F1388L-SUR1 channels are better stimulated than WT channels by 0.5 mM MgADP in the presence of 0.1 mM inhibitory ATP. We next examined the dose-response relationship of these two channels for MgADP activation in detail in the absence of ATP. Although MgADP stimulates channel activity via the SUR subunit, it...

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**Effects of SUR1 Mutations on K\textsubscript{ATP} Channels**

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**Fig. 1. Surface expression of the SUR1-F1388X mutant channels.** COS\textsubscript{7} cells transiently expressing Kir6.2 and either the FLAG-tagged wild-type SUR1 (WT) or the various FLAG-tagged F1388X-SUR1 mutants (ΔF1388X) were subjected to the chemiluminescence assay to monitor surface expression of the channel. Surface expression of all F1388X-SUR1 mutant channels was normalized to that of the WT channel (error bars represent standard error of the means of 3–10 independent experiments for each mutant). Channels with hydrophobic amino acids substituting Phe-1388 are grouped together and shown on the left side of the graph.
also inhibits channel activity by interacting with Kir6.2, with less potency than ATP. To remove the inhibitory effect of ADP from our analysis, we first constructed ADP inhibition dose-response curves for both WT and Phe-1388-SUR1 mutant channels. These curves were obtained in the absence of Mg$^{2+}$ (K-INT plus 1 mM EDTA) because the inhibitory effect of ADP on Kir6.2 does not require Mg$^{2+}$. Similar to ATP inhibition, no significant difference in ADP inhibition was observed between WT and mutant channels (not shown). Therefore, data from the two channels were pooled to generate one ADP inhibition dose-response curve (inset of Fig. 5B). The inhibitory effects of ADP were then compensated for in constructing the dose-response curves for the stimulatory effects of MgADP (Fig. 5B). The data for both WT and the F1388L-SUR1 mutant channels thus generated are well fitted by a modified Hill equation (see legends for Fig. 5B), giving rise to an EC$_{50}$ (half-maximal stimulation concentration) of 131 $\mu$M and 1.8-fold maximal stimulation for WT channels, and an EC$_{50}$ of 36 $\mu$M and 2.3-fold maximal stimulation for the F1388L-SUR1 mutant channels. These results clearly demonstrate that the F1388L-SUR1 channels are much more sensitive to a small increase in ADP concentrations than WT channels, and predict that the F1388L-SUR1 channels would open more readily when cells are challenged by metabolic inhibition. To confirm this, we subjected cells transfected with either WT or F1388L-SUR1 channels to different metabolic conditions and used the Rb$^+$ efflux assay to monitor channel activities in intact cells. In the absence of metabolic inhibitors, F1388L-SUR1 channels displayed higher basal activities than WT channels (Fig. 6). In cells treated with 0.1 to 1 mM 2-deoxy-D-glucose, the F1388L-SUR1 channel continued to show higher activities. These results are consistent with our prediction. When cell metabolism was maximally inhibited by 1 mM 2-deoxy-D-glucose plus 2.5 $\mu$g/ml oligomycin, the difference between F1388L-SUR1 and WT channels diminished, likely because both channels were now maximally activated, and the Rb$^+$ efflux rates were saturated.

Next, we examined glibenclamide sensitivities. WT channels were inhibited by ~60% at 10 nM glibenclamide (Fig. 7A), consistent with a $K_i$ of 10 nM from previous reports (8). The F1388L-SUR1 channels, however, were less sensitive to glibenclamide, showing only ~30% inhibition at 10 nM. Finally, we looked at how channel sensitivities to the potassium channel openers diazoxide, pinacidil, and cromakalim might be altered. Both WT and F1388L-SUR1 channels were stimulated by diazoxide. However, like MgADP, diazoxide is much more potent in stimulating F1388L-SUR1 than WT channels (Fig. 7B). Both cromakalim and pinacidil are effective KCOs for the SUR2A/Kir6.2 and SUR2B/Kir6.2 channels but not for the SUR1/Kir6.2 channels (8, 17, 31). By using the same protocol as shown in Fig. 7B (except that diazoxide was now substituted by cromakalim), neither WT nor F1388L-SUR1 channels were activated by cromakalim, even at 500 $\mu$M (data not shown). Interestingly, however, pinacidil stimulated the F1388L-SUR1 mutant channels at concentrations of 300 $\mu$M and above (Fig. 7C), although it remained ineffective on WT channels even above 500 $\mu$M. The overall physiological and pharmacological profile of the F1388L-SUR1 mutant channels led us to conclude that the F1388L mutation significantly shifts the functional properties of the channel toward those resembling subtypes containing the SUR2B isoform.

**DISCUSSION**

Many mutant proteins with trafficking defects are retained in the ER because they are unable to fold properly or efficiently. The same mutations that cause protein misfolding may also alter protein function. In these cases, the altered function may be due to direct involvement of the mutated residues in protein
function or may be an indirect consequence of protein misfolding. Our previous work on a disease-causing SUR1 mutation, ΔPhe-1388, has found that the mutation not only causes ER retention, and thereby defective trafficking, of the protein and its associated channel subunit Kir6.2, but also abolishes channel response to MgADP. By substituting Phe-1388 with different amino acids and analyzing the effect of each substitution on the trafficking and function of K<sub>ATP</sub> channels, we demonstrate here that the biochemical criteria for correct channel trafficking are separable from those for proper channel function, specifically channel response to MgADP. These results support the notion that Phe-1388 in SUR1 plays a direct role in channel function. This notion is further strengthened by the observations that conservative substitution of Phe-1388 by leucine, although with little effect on channel trafficking, markedly changed the sensitivities of the channel to MgADP, metabolic inhibition, glibenclamide, and potassium channel openers.

**Correlation between Structure and Function**—SUR is a member of the ABC transporter protein superfamily. The crystal structures of a number of bacterial ABC transporters or their
effects of SUR1 mutations on K\textsubscript{ATP} channels

\begin{figure}
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\caption{Nucleotide sensitivities of F1388L-SUR1 mutant channels. A, dose response of F1388L-SUR1 mutant channels to ATP, compared with that of WT channels. Currents in various concentrations of ATP are expressed as a fraction of the mean of currents obtained in the absence of ATP ($I_{\text{rel}}$). Both pipette and bath solutions contained K-INT plus 1 mM EDTA to minimize channel rundown. The curves are fitted to the Hill equation: $I_{\text{rel}}/I_{\text{max}} = 1/(1 + ([\text{ATP}] / K_{i})^n)$, where $K_i$ is the ATP concentration that gives half-maximal inhibition. For WT channels (black squares; $n = 4-7$ patches for each data point), $K_i = 13.8 \mu M$, and $H = 1.3$; for F1388L-SUR1 mutant channels (open squares; $n = 4-7$), $K_i = 16.4 \mu M$, and $H = 1.4$. B, dose response of WT and F1388L-SUR1 mutant channels to the stimulatory effect of MgADP. To correct for the inhibitory effect of ADP mediated by the Kir6.2 subunit, an ADP dose-response curve was first constructed in the absence of Mg\textsuperscript{2+} (see inset; because no significant difference was seen between WT and Phe-1388-SUR1 channels, the data were pooled to generate one curve fitted to the Hill equation described above, with $K_i = 110 \mu M$, and $H = 1.5$). To obtain the net stimulatory effects of MgADP, patches were excised into K-INT solutions and then exposed to various concentrations of MgADP (free Mg\textsuperscript{2+} concentration was kept at 1 mM). The relative current in each concentration of MgADP, calculated as a fraction of the mean of currents obtained before and after exposure to MgADP, was then added to the relative current inhibited by the same concentration of ADP (obtained from the ADP inhibition dose-response curve shown in the inset) to compensate for the inhibitory effect of ADP. The final $I_{\text{rel}}$ thus obtained was then plotted against MgADP concentrations. The curves are fitted to the modified Hill equation: $I_{\text{rel}}/I_{\text{max}} = 1 - (1 - I_{\text{max}}/[1 + ([\text{MgADP}] / EC_{50})^n])$, where $EC_{50}$ is the MgADP concentration that gives half-maximal stimulation and $I_{\text{max}}$ is the maximal stimulation. For WT channels (black squares; $n = 3-5$ patches), $I_{\text{max}} = 1.8$, $H = 2.1$, and $EC_{50} = 131 \mu M$; for F1388L-SUR1 mutant channels (open squares; $n = 3-5$ patches), $I_{\text{max}} = 2.3$, $H = 1.2$, and $EC_{50} = 36 \mu M$.

These studies show that the nucleotide binding domains from different ABC transporters share the same basic fold. Amino acid sequence alignment places Phe-1388 of SUR1 in the \textalpha-helix immediately following the Walker A motif. In terms of channel trafficking, most hydrophobic amino acid substitutions at the 1388 position are tolerated. It is not surprising that deletion of Phe-1388 or substitution of Phe-1388 by charged amino acids may disrupt the folding of the \textalpha-helix, thereby impeding subsequent trafficking of the protein. Interestingly, however, in the nucleotide binding domain of some ABC transporters, including MalK and the NBD1 of human glycoprotein and multidrug-resistant protein, the homologous position is occupied by a threonine (40, 42). This suggests that threonine should allow proper folding of the protein. However, in the case of SUR1, substitution of Phe-1388 by threonine significantly compromised the trafficking efficiency of K\textsubscript{ATP} channels. It is possible that this substitution affects events that are downstream of protein folding and that are relevant to channel trafficking. For example, it might affect the efficiency of assembly between SUR1 subunits and/or between SUR1 and Kir6.2 subunits or it might affect the shielding of the -RKR- ER retention/retrieval signals in the channel complex.

In some mutants, especially F1388P and F1388S, a lower steady-state protein level was seen (Fig. 2). One possible explanation is that these mutant proteins are rapidly degraded. Many proteins retained in the ER undergo ER-associated degradation via the ubiquitin-proteosome pathway (43). We found that treating cells with proteosome inhibitors led to a marked increase in the levels of the aforementioned SUR1 mutant proteins, as assessed by immunostaining. In Western blots, the same treatment led to the appearance of higher molecular weight complexes suggestive of polyubiquitinated forms of mutant SUR1. These findings provide preliminary evidence that the ΔF508 mutant of CFTR, some F1388X-SUR1 mutants are rapidly degraded by the proteosomes. They also raise an interesting future question of why some F1388X-SUR1 mutants are more prone to degradation than others.

How is Phe-1388 of SUR1 involved in channel response to MgADP? The side chain architecture of the amino acid appears important in conferring proper channel response to MgADP. This is most clearly demonstrated by comparing the leucine and isoleucine substitutions. Leucine and isoleucine have the same chemical composition, similar physical-chemical proper-
ties, and both are found in the Phe-1388 homologous position in other ABC transporters. Neither substitution leads to substantial reduction in surface expression of the channel. However, leucine greatly potentiates channel response to MgADP, whereas isoleucine nearly abolishes channel response to MgADP (assayed as currents stimulated by 0.5 mM MgADP in the presence of 0.1 mM ATP; Figs. 3 and 4). Given the proximity of this amino acid to the nucleotide binding Walker A motif, it is conceivable that the architecture of the side chain might directly influence nucleotide binding and its subsequent hydrolysis. Alternatively, it might play a role in the transduction step linking ATPase activity to channel opening. Recent work by Matsuo et al. (44) suggests that the linker motifs of both NBDs of SUR (at least SUR1 and 2B) participate in transducing ATP binding and hydrolysis at the NBDs to channel activity; mutations in the linker motifs reduced (NBD1) or abolished (NBD2) channel response to MgADP without altering nucleotide binding or hydrolysis. It is also evident that although in SUR, ATP hydrolysis occurs primarily at NBD2, both NBDs are required for transducing the enzymatic activity of SUR to channel activity (9, 12, 20). Thus, it is possible that the Phe-1388 residue may contact the linker or NBD1 during the transduction process. To distinguish these possibilities, future biochemical studies that compare nucleotide binding and hydrolysis between NBD2 of WT SUR1 and NBD2 containing the F1388L mutation will be required. Insight into the structural role of Phe-1388 in SUR1 might also be gained by comparing crystal structures of bacterial ABC transporters bearing Phe or Leu at the homologous site.

Physiological, Pharmacological, and Therapeutic Implications—The key to the function of K_{ATP} channels in sensing the energetic state of a cell is their ability to respond to changes in the concentrations of intracellular ATP and MgADP. In pancreatic β-cells, an increase in ADP/ATP ratio following glucose starvation activates the channel to stop insulin secretion. Many SUR1 mutations identified in patients with familial hyperinsulinism specifically abolish channel response to MgADP stimulation, causing persistent insulin secretion despite low blood glucose levels (11, 23). These findings underscore the importance of MgADP in determining K_{ATP} channel activity in physiological conditions. Significantly, substituting Phe-1388 with Leu substantially increases channel sensitivity to MgADP. Because in intact cells, the activity of K_{ATP} channels is dependent on the MgADP/ATP ratio, such a "gain of function" phenotype in MgADP response is expected to result in higher channel activity at a given metabolic condition. Indeed, our Rb^- efflux experiments using several metabolic conditions clearly demonstrate that the F1388L channels are more sensitive to metabolic stress than WT channels. These results and the observation that channel response to diazoxide is also enhanced by the F1388L mutation point to the potential of this site as a drug target in the management of familial hyperinsulinism. In this regard, it would be important to determine whether the F1388L mutation is able to compensate for the MgADP defects caused by other mutations in SUR1.

The realization that the leucine mutation occurs naturally in SUR2 suggests that this conservative amino acid substitution may be a mechanism for generating functional diversities in K_{ATP} channels. The SUR1/Kir6.2, SUR2A/Kir6.2, and SUR2B/Kir6.2 channels exhibit differential sensitivities to nucleotides and pharmacological agents. Several studies have examined the structural basis that underlies these differences. At the primary sequence level, SUR1 and SUR2A are 68% identical. SUR2B, a splice variant of the SUR2 gene, differs from SUR2A only in the last 42 amino acids. Interestingly, the C-terminal 42 amino acids of SUR2B share only ~30% homology to those of SUR2A but ~70% homology to SUR1 (31). The F1388L-SUR1 is therefore structurally closer to SUR2B than to SUR2A. This
FIG. 7. Pharmacological properties of the F1388L-SUR1 mutant channels. A, channel response to glibenclamide. The current in 10 nM or 10 μM glibenclamide was expressed as a fraction of that obtained in K-INT (Irel); n = 4–8 patches for each bar. B, channel response to diazoxide. Upper, representative current recordings from inside-out membrane patches containing WT or F1388L-SUR1 channels. Patches were excised into K-INT solutions and exposed to 1 mM ATP, 0.1 mM ATP, or 0.1 mM ATP plus 50 μM diazoxide. Free Mg²⁺ concentration was kept at 1 mM in all solutions. Lower, quantification of diazoxide response. Diazoxide response is expressed as currents obtained in K-INT solution containing diazoxide plus 0.1 mM ATP and 1 mM free Mg²⁺, relative to currents obtained in K-INT. Data points are mean ± S.E., n = 4–5 patches. C, same as B except the channels were now tested for pinacidil response. Data points are mean ± S.E., n = 3–6 patches. Note that the WT channel activity in 0.1 mM ATP is lower than that of the F1388L-SUR1 mutant channels in both B and C. This is because in the presence of Mg²⁺, ATP undergoes hydrolysis at SUR1 to generate MgADP, which stimulates channel activity. Because the mutant channel is more sensitive to MgADP stimulation, as demonstrated in Fig. 5, it exhibits higher activity than the WT channels.
fits our observations that the functional properties of the F1388L-SUR1 mutant channel are more similar to the SUR2B/Kir6.2 than to the SUR2A/Kir6.2 channel.

By using the chimeric protein approach, Bebenko et al. (58) found that the distal C terminus of SUR2A (–50 amino acids) is sufficient to confer the lower ATP sensitivity seen in the cardiac SUR2A/Kir6.2 channels. Consistent with this, we did not observe a significant difference between WT and F1388L-SUR1 channels in ATP sensitivity. The regions involved in MgADP response are more complex. Obviously, NBD2 is required for nucleotide binding, but NBD1 is also essential (9, 11, 12, 18, 20, 44). In addition, the C-terminal tail plays a role. First, a disease-associated point mutation located in the C terminus of SUR1, L1544P, diminishes channel response to MgADP (27). Second, analysis of chimeric SURs has led to the proposal that the C-terminal 42 amino acids of SUR2A imparts an inhibitory effect on MgADP-induced channel activation, explaining the lower sensitivity of the SUR2A/Kir6.2 channel to MgADP (45). Although SUR2A also has a leucine at the 1388-equivalent site, the potentiation effect of leucine is likely masked by the inhibitory effect imposed by the SUR2A C-terminal tail.

Pharmacologically, the F1388L-SUR1 channels show decreased glibenclamide sensitivity and altered KCO specificity. Sulfonylureas inhibit KATP channel activity by antagonizing the stimulatory effect of MgADP (46). The high affinity sulfonylurea-binding site has been localized to the C-terminal group of transmembrane helices (47–49). The decreased glibenclamide sensitivity of the F1388L-SUR1 channel therefore is probably not due to a change in the binding site peptide sequence but rather a consequence of increased channel sensitivity to MgADP. With regard to KCO response, the F1388L mutation caused increased sensitivity to diazoxide and rendered the channel sensitive to stimulation by pinacidil but not by cromakalim. Binding studies using a pinacidil analogue, P1075, showed that the KCO binds with high affinity to SUR2A and SUR2B but does not bind to SUR1 (50–52). The critical regions in SUR2 that confer high sensitivity to pinacidil and cromakalim have been mapped to TMs 16–17 and part of the cytosolic loop between TMs 13–14 (31, 33, 53, 54). Moreau et al. (34) further narrowed down the structural basis for the differential KCO sensitivities to two amino acids in the last transmembrane segment (TM 17) of SUR. The fact that the F1388L-SUR1 channels are stimulated by pinacidil at high concentrations suggests that pinacidil does bind F1388L-SUR1 channels with low affinity. Again, although it is possible that Phe-1388 in SUR1 is part of the pinacidil-binding site, a more likely explanation for the increased sensitivity to pinacidil is a result of the increased MgADP sensitivity. This would be in agreement with the idea that KCO responsiveness and even binding affinity is modulated by nucleotides (9, 12, 18, 20, 44).

In conclusion, our results show that the Phe-1388 residue in SUR1 plays an important role in the trafficking and function of KATP channels. The biochemical features of this residue that govern trafficking efficiency are distinct from those that govern functional properties. The F1388L mutation in SUR1 makes the overall physiological and pharmacological profile of the channel shift toward that of the SUR2B/Kir6.2 channel. Thus, genetic variation at this amino acid accounts for some of the functional differences seen in different subtypes of KATP channels, providing a mechanism for diversity.

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