Effect of Two Amino Acids in TM17 of Sulfonlurea Receptor SUR1 on the Binding of ATP-Sensitive K+ Channel Modulators

Annette Hambrock, Tülay Kayar, Demet Stumpp, and Hartmut Osswald

The sulfonlurea receptor (SUR) is the important regulatory subunit of ATP-sensitive K+ channels. It is an ATP-binding cassette protein comprising 17 transmembrane helices. SUR is endowed with binding sites for channel blockers like the antidiabetic sulfonlurea glibenclamide and for the chemically very heterogeneous channel openers. SUR1, the typical pancreatic SUR isoform, shows much higher affinity for glibenclamide but considerably lower affinity for most openers than SUR2. In radioligand binding assays, we investigated the role of two amino acids, T1285 and M1289, located in transmembrane helix (TM)-17, in opener binding to SUR1. These amino acids were exchanged for the corresponding amino acids of SUR2. In competition experiments using [3H]glibenclamide as radioligand, SUR1(T1285L, M1289T) showed much higher affinity toward the cyanoguanidine openers pinacidil and P1075 than SUR1 wild type. The affinity for the thioformamide aprikalim was also markedly increased. In contrast, the affinity for the benzopyrans levcromakalim and rilmakalim was unaffected; however, the amount of displaced [3H]glibenclamide binding was nearly doubled. The binding properties of the opener diazoxide and the blocker glibenclamide were unchanged. In conclusion, mutation of two amino acids in TM17 of SUR1, especially of M1289, leads to class-specific effects on opener binding by increasing opener affinity or by changing allosteric coupling between opener and glibenclamide binding.

Diabetes 53 (Suppl. 3):S128–S134, 2004

ATP-sensitive K+ channels (KATP channels) are regulated by nucleotides and therefore form an important link between the metabolic status of a cell and its membrane potential (1). KATP channels are tetradimeric complexes of pore-forming Kir6.x subunits and regulatory sulfonlurea receptors (SURs). SUR comprises 17 transmembrane helices and two nucleotide-binding folds (NBFs) and is a member of the ATP-binding cassette (ABC) protein family (rev. in 2,3). It is also the target of pharmacologically important drugs. Among these substances are channel blockers like the sulfonlurea glibenclamide, which inhibits KATP channels in the pancreatic β-cell with high affinity and is commonly used in the treatment of patients with type 2 diabetes because of its stimulatory effect on insulin secretion. KATP channel openers (KCOs) on the other hand belong to a chemically heterogeneous group of substances that include the cyanoguanodines (-)pinacidil and P1075 [N-cyano-N′-(1,1-dimethylpropyl)-N″-3-pyridylguanidine], the benzopyrans levcromakalim and rilmakalim, thioformamides like aprikalim, and other substances like nicorandil (pyridyl nitrate), minoxidil sulfate (pyrimidine sulfate), or diazoxide (benzothiadiazine). By activating KATP channels, openers induce hyperpolarization and relaxation of smooth muscle. This promises a broad clinical potential (e.g., for the therapy of asthma, irritable bladder, and cardiac ischemia) (4–6). The clinical use of most openers, however, is impeded by insufficient tissue specificity. Between binding of openers and binding of glibenclamide, complex allosteric interactions exist (6–8) that are not completely understood so far.

KATP channels in various tissues show diverse pharmacological profiles due to their different subunit composition. SUR1 is the typical isoform of the pancreatic β-cell, SUR2A is predominantly found in heart and skeletal muscle, and SUR2B is found in smooth muscle (2). To some extent, SUR1 and SUR2 show inverse pharmacological profiles: SUR1 exhibits high affinity for glibenclamide and low affinity for most openers (with diazoxide as an exception), whereas SUR2 shows lower affinity for glibenclamide and high affinity for the openers (2,3,9). The development of β-cell selective (i.e., SUR1-specific) openers for treatment of inoperable insulinoma, some forms of persistent hypoglycemic hyperinsulinemia of infancy, or diabetes by inhibition of insulin secretion is the subject of many preclinical experiments (10,11).

So far, several efforts have been made to investigate binding sites and mechanisms of action of KCOs at SUR using either radioligand binding or electrophysiological experiments. Chimeric approaches took advantage of the different pharmacological properties of SUR1 and SUR2 and provided evidence for a central role of the “last” (i.e., COOH-terminal) domain of six helices (TMD2) in the
FIG. 1. Schematic representation illustrating the transmembrane topology of SUR (A) and the positions of the mutated amino acids in TM17 (B and C). A: The 17 TMs of SUR are arranged in three TMDs (TMD0, TMD1, and TMD2). The putative sites of drug binding are shown according to Ashfield et al. (16), Mikhailov et al. (17), and Uhde et al. (9). Binding of glibenclamide (GBC): broken lines and region comprising TM14–15. Binding of openers (KCOs): dotted lines and region comprising TM16–17. B: The amino acid sequences of rat SUR1 and rat SUR2 are compared within the predicted region of TM17 (horizontal boxes) (prediction of TM17 according to the NiceProtView of Swiss-Prot: Q09429 [GenBank X97279] and SwissProt: Q63563 [GenBank D83598], provided by the ExPASy Molecular Biology Server of the Swiss Institute of Bioinformatics). The five divergent amino acids (…), including the two mutated amino acids (—), are highlighted by the vertical boxes. C: Part of TM17 is displayed in the helical wheel projection ("Generunner" program; Hastings Software, Hastings on Hudson, NY). According to this secondary structure prediction, the amino acids T1285 and M1289 of SUR1 and L1249 and T1253 of SUR2 are located on the same side, separated by only one turn of the helix. Amino acids with polar side chains are displayed in italics.
FIG. 2. Inhibition of [3H]glibenclamide binding by different K\textsubscript{ATP} channel modulators. A: Binding of glibenclamide to wild-type SUR1, SUR1(M1289T), and SUR1(T1285L, M1289T) was tested in homologous competition experiments that were performed with membranes from stably transfected HEK 293 cells. Binding studies were done at 37°C in the presence of 1 mmol/l MgATP using [3H]glibenclamide as the radioligand ([SUR1, SUR1(T1285L, M1289T): \(L_0 = 0.7\) nmol/l; SUR1(M1289T): \(L_0 = 0.6\) nmol/l]. The data, expressed as percentage of specific [3H]glibenclamide binding (B\textsubscript{s}), are means from three to four independent experiments, and from these, data curves were fitted according to equation 1 in RESEARCH DESIGN AND METHODS for one inhibitory component and n\textsubscript{H} set to 1. B–G: Heterologous competition experiments were performed with SUR1 and SUR1(T1285L, M1289T) in the presence of 1 mmol/l MgATP using [3H]glibenclamide as the radioligand (L\textsubscript{0} = 0.6–0.8 nmol/l) and openers from different chemical classes as competitors (cyanoguanidines: P1075 [B] and pinacidil [C]; benzopyrans: rilmakalim [D] and levromakalim [E];...
binding or efficacy of several KCOs (9,12–15). In all these cases, TM17, the last COOH-terminal helix of SUR, was part of the possible binding region, except for a study suggesting that pinacidil bound within the region of TM13–16 (13). Uhde et al. (9) delimited the binding region for selected KCOs to two molecular segments: a short cytoplasmic segment between TM13 and TM14 and a longer region comprising TM16, TM17, and part of NBF2 (Fig. 1A). At first, an exclusively cytoplasmic localization of opener binding was assumed. Later, Moreau et al. (14) demonstrated in electrophysiological experiments that two amino acids in TM17 of SUR (T1286 and M1290 in hamster SUR1 and L1249 and T1253 in rat SUR2) are involved in activation of channels formed by SUR and Kir subunits. According to secondary structure predictions, both amino acids are located on the same side of TM17, separated only by one turn of the helix. Only these two amino acids and three other amino acids within TM17 are different between SUR1 and SUR2 (14) (Fig. 1B and C). In general, the sequence of the last carboxy-terminal helix is well conserved among ABC proteins (18).

Although the importance of the two amino acids in TM17 was shown clearly for channel activation by Moreau et al. (14), the question remained as to whether these two amino acids were directly involved in opener binding or whether they were only indirectly important for transducing the activating signal from SUR to Kir. Therefore, we investigated the binding of selected openers in radioligand binding assays using SUR1 mutants in which threonine and methionine at positions 1,285 and 1,289 or only methionine at 1,289 (rat clone numbering) were exchanged by leucine and threonine, the equivalent amino acids of SUR2. (Because we used rat clones in our experiments, numbering of the amino acids differs from that of the hamster SUR1 clone mentioned above). As it is of interest to see if there are any differences between various KCOs concerning opener affinity and interaction with glibenclamide binding, we tested openers representative for different chemical classes. The mutants and wild-type SUR1 were stably expressed in HEK 293 cells, and the binding of openers and glibenclamide was tested in competition assays.

RESEARCH DESIGN AND METHODS

Mutagenesis and mammalian expression. The mutants SUR1(M1286T) and SUR1(T1286L, M1289T) were constructed from rat SUR1 (GenBank X97279) using the QuickChange Site-Directed Mutagenesis System (Stratagene, Amsterdam, the Netherlands). Human embryonic kidney (HEK) 293 cells were cultured as described previously (19) and transfected with pcDNA 3.1 expression vector (Invitrogen, Karlsruhe, Germany) containing the coding sequences of wild-type or mutant SUR1. Cell lines stably expressing these proteins were isolated and cultured as described before (19).

Membrane preparation. Membranes from the different cell lines were prepared as described before (19) and frozen at a protein concentration of 0.7–2.0 mg protein/ml. Protein concentration was determined using Bio-Rad protein assay dye reagent (Bio-Rad, Munich, Germany) and bovine serum albumin as the standard.

Materials. [3H]glibenclamide (specific activity 1.60 TBq [43.3 Ci/nmol]) was purchased from Perkin Elmer Life Sciences (Wolznach, Germany). Glibenclamide, diazoxide, and pinacidil were from Sigma (Deisenhofen, Germany). Rilmakalim and aprikalim were kind gifts by Aventis Pharma (Frankfurt, Germany). Benzothiadiazine: diazoxide [3H]glibenclamide binding experiments. For competition experiments, membranes (final protein concentration: 20–60 μg protein/ml) were added to incubation buffer (139 mmol/l NaCl, 5 mmol/l KCl, and 5 mmol/l HEPES, pH 7.4, 4°C) supplemented with [3H]glibenclamide (0.6–0.8 nmol/l) and the substance of interest. Solutions of these drugs were prepared as described previously (19). Nonspecific binding was determined in the presence of 1 μmol/l glibenclamide. 1 μmol/l NaATP and 2.2 mmol/l MgCl2 were added to give a final MgATP concentration of 1 μmol/l (20). After incubation for 15 min at 37°C, three aliquots of 0.3 ml were rapidly filtrated under vacuum over GF/B filters (Whatman Scientific, Maidstone, U.K.). Each filter was immediately washed four times with 4 ml of cold quench solution [50 mmol/l Tris-(hydroxymethyl)-aminomethane, and 154 mmol/l NaCl, pH 7.4 at 0°C] and counted for [3H] in the presence of 5 ml Ultima Gold scintillant (Packard, Meriden, CT).

Data analysis. Equilibrium inhibition curves were analyzed according to the logistic equation for up to two components,

\[ y = 100 - \sum_{i=1}^{2} A_i \left(1 + 10^{(p_x - p_{IC50,i} - p_{K_p}) \cdot d_i}ight)^{-1} \tag{1} \]

Here \( A_i \) denotes the amount of inhibition or activation of component \( i \), \( p_{IC50,i} \) is the Hill coefficient, and \( p_{K_p} \) is the midpoint of component \( i \) with \( p_{IC50,i} = - \log_{10} p_{K_p} \). The concentration of the compound under study, with \( p_x = - \log C_x \), was considered as the extent of activation. IC_{50} values were converted into the respective constants \( K \) by correcting for the presence of the radioligand (L), according to the equation of Cheng and Prusoff (21):

\[ K = \frac{IC_{50}(1 + l/K_p)}{l} \tag{2} \]

where \( K_p \) is the equilibrium dissociation constant of the radioligand. In case of monophasic homologous competition, the inhibition constant \( K \) is identical to the \( K_p \) value. Curves were analyzed according to the method of least squares using the SigmaPlot program (SPSS, Chicago, IL). Errors in the parameters derived from the fit to a single curve were estimated using the univariate approximation (22) and assuming that \( A \) and \( p_x \) values are normally distributed. Two-tailed unpaired Student’s t test, P values were calculated on the level of the pK values.

Materials. [3H]glibenclamide (specific activity 1.60 TBq [43.3 Ci/nmol]) was purchased from Perkin Elmer Life Sciences (Wolznach, Germany). Glibenclamide, diazoxide, and pinacidil were from Sigma (Deisenhofen, Germany). Rilmakalim and aprikalim were kind gifts by Aventis Pharma (Frankfurt, Germany). NaATP and NaADP were from Roche Diagnostics (Mannheim, Germany), and the reagents used for cell culture and transfection were from Invitrogen (Karlsruhe, Germany).

RESULTS

Binding of glibenclamide. As we intended to compare binding properties of SUR1 with the mutants SUR1(T1286L, M1289L) and SUR1(M1289L), [3H]glibenclamide was chosen as the radioligand due to its high affinity to SUR1. It is possible to test opener binding properties in heterologous competition experiments because negative allosteric interactions exist between binding of openers and glibenclamide (7). Opener binding to SUR is supported by MgATP (19,23). Therefore, experiments were performed in the presence of 1 mmol/l MgATP.

Displacing [3H]glibenclamide by increasing concentrations of unlabeled glibenclamide (Fig. 2A) resulted in similar monophasic inhibition curves for SUR1, SUR1 (M1289L), and SUR1(T1286L, M1289L) with \( K_p \) values of
the benzopyran openers rilmakalim and levcromakalim, Binding of levcromakalim and rilmakalim. did not differ much from binding to SUR1(M1289L) (Table 1). In the following experiments, binding of openers representative of different chemical classes was systematically compared between SUR1 and SUR1(T1285L, M1289L); for P1075, levcromakalim, and diazoxide, the mutant SUR1 (M1289L) was also investigated.

**Binding of P1075 and pinacidil.** With the cyanoguanidine openers P1075 and pinacidil, monophasic inhibition curves were obtained (Fig. 2B and C; Table 1). For SUR1 (T1285L, M1289T), these curves were shifted toward higher opener concentrations with a 27-fold (P1075) or 14-fold (pinacidil) decrease in $K_i$ values. For wild-type SUR1, the amount of displaced specific $[^3H]$glibenclamide binding ($A \sim 70\%$) could not be determined precisely because the affinity for both openers was very low. In any case, for SUR1(T1285L, M1289T), $A$ (55\%) was not larger than for SUR1. Binding of P1075 to SUR1(T1285L, M1289T) did not differ much from binding to SUR1(M1289L) (Table 1).

**Binding of levcromakalim and rilmakalim.** In case of the benzopyran openers rilmakalim and levcromakalim, $K_i$ values for SUR1(T1285L, M1289T) were not significantly altered compared with those for the wild type ($\times 1.6$ and $\times 1.2$, respectively) (Fig. 2D and E; Table 1). The amount of displaced specific $[^3H]$glibenclamide binding, however, was markedly increased at SUR1(T1285L, M1289T) (rilmakalim: from 40 to 68\%; levcromakalim: from 27 to 52\%). The slight increase in $[^3H]$glibenclamide binding observed for wild-type SUR1 in the presence of low rilmakalim or levcromakalim concentrations was not seen with SUR1 (T1285L, M1289T). Binding of levcromakalim was also tested with SUR1(M1289T). Here, inhibition curves were very similar to those obtained for SUR1(T1285L, M1289T) (Table 1).

**Binding of aprikalim.** The thioformamide opener aprikalim did not displace any $[^3H]$glibenclamide binding at wild-type SUR1. Instead, there was a small increase in radioligand binding at higher aprikalim concentrations (Fig. 2F). Although an increase was also seen with SUR1 (T1285L, M1289T) at lower aprikalim concentrations, an inhibition of $[^3H]$glibenclamide binding was seen at higher concentrations (Table 1).

**Binding of diazoxide.** Diazoxide was the only opener tested for which no significant difference in binding properties was seen between SUR1, SUR1(T1285L, M1289T), and SUR1(M1289T) (Fig. 2G; Table 1). At high concentrations, an endogenous component had to be taken into account: In membranes from untransfected HEK cells, $[^3H]$glibenclamide binding was induced at diazoxide concentrations between $\sim 200$ and $500 \mu M$. This nonspecific component was also observed in the additional presence of $1 \mu M$ unlabeled glibenclamide.

**DISCUSSION**

In electrophysiological experiments performed at reconstituent *Xenopus* oocytes, Moreau et al. (14) demonstrated the importance of two single amino acids in TM17 of SUR for activation of K$_\text{ATP}$ channels by the cromakalim analog SR47063 and by rilmakalim and P1075. In our study, based on radioligand binding assays using a mammalian expression system, we show that at least one of these two amino acids is actually involved in binding of several openers to SUR1. This provides evidence against the possibility that the two amino acids are exclusively indirectly involved in signal transduction from SUR to Kir.

Exchanging both threonine and methionine at positions 1,285 and 1,289 in TM17 by the corresponding amino acids of SUR2 does not significantly affect binding of the blocker glibenclamide but markedly influences binding of the openers P1075, pinacidil, rilmakalim, levcromakalim, and aprikalim by changing opener affinity or allosteric coupling between opener and glibenclamide binding. On the other hand, the amino acids at these positions are not important for binding of diazoxide, which emphasizes the difference between this substance and the majority of other openers (e.g., reflected by its ability to activate SUR1-containing channels and to bind to SUR1 and SUR2B with similar affinities) (12,13,23–25). Moreau et al. (14) report only small effects of the two mutations in TM17 on the responsiveness of SUR1-containing channels to diazoxide compared with the larger effects induced by SR47063, rilmakalim, or P1075. Our data show no significant difference in diazoxide binding to wild-type SUR1 and the mutants SUR1(T1285L, M1289T) and SUR1(M1289T), and are in agreement with other publications that postulate that one or more regions of SUR (probably TM6–11 in

**TABLE 1**

Inhibition of $[^3H]$glibenclamide binding by different K$_\text{ATP}$ channel openers at SUR1, SUR1(T1285L, M1289T), and SUR1 (M1289T)

<table>
<thead>
<tr>
<th>Opener</th>
<th>SUR1</th>
<th>SUR1(T1285L, M1289T)</th>
<th>SUR1(M1289T)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_i$ (\mu M)</td>
<td>$A$ (%B$_0$)</td>
<td>$K_i$ (\mu M)</td>
</tr>
<tr>
<td>P1075</td>
<td>163 ± 48</td>
<td>71 ± 5*</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Pinacidil</td>
<td>1,023 ± 254</td>
<td>86 ± 8*</td>
<td>75 ± 20</td>
</tr>
<tr>
<td>Rilmakalim</td>
<td>16 ± 3</td>
<td>40 ± 4</td>
<td>10 ± 3</td>
</tr>
<tr>
<td>Levocromalim</td>
<td>30 ± 20</td>
<td>27 ± 3</td>
<td>26 ± 4</td>
</tr>
<tr>
<td>Aprikalim</td>
<td></td>
<td></td>
<td>90 ± 33</td>
</tr>
<tr>
<td>Diazoxide</td>
<td>54 ± 11</td>
<td>39 ± 2</td>
<td>34 ± 6</td>
</tr>
</tbody>
</table>

*Data are means ± SEM. Parameters for the inhibitory component of the binding curves ($K_i$, inhibition constant; $A$, amount of displaced specific $[^3H]$glibenclamide binding) were determined from individual experiments ($n = 3–6$) as described in the legend to Fig. 2. For correction of opener binding curves according to Cheng and Prusoff (21), the respective $K_i$ values for glibenclamide were taken into account. Comparison of $pK_i$ values gave a significant difference between SUR1(T1285L, M1289T) and wild type in case of P1075 and pinacidil ($P < 0.005$), but no significant difference in case of rilmakalim, levocromakalim, and diazoxide ($P > 0.05$). *A could not be determined precisely because affinity of SUR1 for the substance was very low. ND, not determined.

3.1 ± 0.8, 1.2 ± 0.4, and 1.8 ± 0.2 nmol/l, respectively (data are expressed as means ± SEM). Glibenclamide binding was not significantly changed by the mutations $[pK_i]$ SUR1 (M1289L) vs. SUR1: $P = 0.0868$; $pK_i$ SUR1(T1285L, M1289L) vs. SUR1: $P = 0.0825$.

In electrophysiological experiments performed at reconstituent *Xenopus* oocytes, Moreau et al. (14) demonstrated the importance of two single amino acids in TM17 of SUR for activation of K$_\text{ATP}$ channels by the cromakalim analog SR47063 and by rilmakalim and P1075. In our study, based on radioligand binding assays using a mammalian expression system, we show that at least one of these two amino acids is actually involved in binding of several openers to SUR1. This provides evidence against the possibility that the two amino acids are exclusively indirectly involved in signal transduction from SUR to Kir.

Exchanging both threonine and methionine at positions 1,285 and 1,289 in TM17 by the corresponding amino acids of SUR2 does not significantly affect binding of the blocker glibenclamide but markedly influences binding of the openers P1075, pinacidil, rilmakalim, levcromakalim, and aprikalim by changing opener affinity or allosteric coupling between opener and glibenclamide binding. On the other hand, the amino acids at these positions are not important for binding of diazoxide, which emphasizes the difference between this substance and the majority of other openers (e.g., reflected by its ability to activate SUR1-containing channels and to bind to SUR1 and SUR2B with similar affinities) (12,13,23–25). Moreau et al. (14) report only small effects of the two mutations in TM17 on the responsiveness of SUR1-containing channels to diazoxide compared with the larger effects induced by SR47063, rilmakalim, or P1075. Our data show no significant difference in diazoxide binding to wild-type SUR1 and the mutants SUR1(T1285L, M1289T) and SUR1(M1289T), and are in agreement with other publications that postulate that one or more regions of SUR (probably TM6–11 in
combination with NBF1) contribute to a diazoxide binding site distinct from the binding site(s) for other openers (10,12,26,27). For all other openers tested in our study, we find visible effects of the two mutations in TM17 on binding properties that are obviously specific for representatives of the different chemical classes.

In case of the cyanoguanidine openers P1075 and pinacidil, the mutations lead to marked decreases in \( K_i \) values that are probably due to large increases in affinity, although possible effects on allosteric coupling between opener and glibenclamide are difficult to assess. As the amount of displaced glibenclamide binding cannot be determined precisely for wild-type SUR1 due to the low affinity of these openers, it is either the same or maybe to some extent smaller at SUR1(T1285L, M1289T), where \([^{3}H]glibenclamide is only displaced to 50–55%.

For the thioformamide aprikalim, an increase in affinity is seen at SUR1(T1285L, M1289T): while aprikalim does not displace glibenclamide binding to wild-type SUR1 at concentrations up to 300 \( \mu \)mol/l, binding to mutant SUR1 is inhibited with \( K_i = 90 \) \( \mu \)mol/l.

Binding of the benzopyranes rilmakalim and levcromakalim is also markedly altered at SUR1(T1285L, M1289T), but in a different manner: Here, affinity is not significantly different between wild type and mutant. In contrast, the amount of displaced glibenclamide binding is extended by a factor of 1.7–1.9, suggesting a distinct increase in negative allosteric coupling between the binding of benzopyran openers and glibenclamide. In addition, the increase in \([^{3}H]glibenclamide binding seen with wild-type SUR1, indicating additional positive allosteric interactions at lower concentrations of these openers at SUR1, is missing at SUR1(T1285L, M1289T). One could speculate that the observed allosteric effects are due to molecular interactions within the SUR monomer or even between subunits of the SUR tetramer as already proposed for allosteric coupling between nucleotide, opener, and sulfonylurea binding to tetrameric SUR2 (8). According to such an assumption, the nature of allosteric coupling at SUR1 could be profoundly influenced by mutation in TM17. In general, questions remain concerning the coupling between opener and glibenclamide binding at SUR1. For all KCOs tested in this study, \([^{3}H]glibenclamide is not displaced to 100%. This is in contrast to data from Schwanstecher et al. (23), suggesting complete displacement of \([^{3}H]glibenclamide by pinacidil and diazoxide at SUR1, but is in accordance with previous results showing displacement only to \( \sim 80\% \) (\( \sim \) pinacidil) and \( \sim 50\% \) (diazoxide) at SUR1 or the splice variant SUR1\( \Delta \)17 (20). This suggests the existence of SUR1 complexes in which glibenclamide and openers are bound simultaneously (8).

Moreau et al. (14) describe the essential influence of two amino acids on opener action but indicate that this is mainly due to the second amino acid while mutation at the first position exhibited only slight effects. In our study, we also tested binding of P1075, levcromakalim, diazoxide, and glibenclamide to a mutant that was only altered at position 1,289 and received nearly the same results with this mutant, SUR1(M1289T), as with SUR1(T1285L, M1289T). This points to a prominent role of the amino acid at position 1,289, although it is of course necessary to take care in interpreting results from mutational analyses of single amino acids as the three-dimensional environment of the mutated region could also have important influence.

In conclusion, mutation(s) in TM17 leads to different effects on opener binding by either altering the affinity for these openers or the allosteric interaction between binding of openers and glibenclamide. This points to the existence of different binding sites for representatives of the various opener classes. As the affinity for P1075 and pinacidil is clearly altered at SUR1(T1285L, M1289T) without affecting the amount of displaced specific \([^{3}H]glibenclamide, this may suggest that the amino acids at positions 1,285 and especially 1,289 are located within the binding sites for cyanoguanidines. For many transporters from the ABC protein family, which show high homology to SUR within the last COOH-terminal helix (e.g., MRP1–3), it is assumed that binding of transport substrates takes place at this helix (18,28). In any case, final direct evidence for one or more amino acids in TM17 being part of the binding pocket for cyanoguanidines is still lacking and requires further experiments (e.g., via photoaffinity labeling). Binding of benzopyrans on the other hand seems to take place at another molecular region of SUR1, as can be seen by the altered allosteric effects. There is some evidence for a functional interaction between TM17 and TM14 at MRP1 (28) and for the existence of two interacting ligand binding sites at MRP2 (29). Zelcer et al. (29) also report that the transport of estradiol-17β-o-glucuronide, which binds to TM17 at MRP2 (18), is modulated by glibenclamide, probably via allosteric interaction. To some extent, this might be in parallel with an interaction between opener and glibenclamide binding sites at SUR. Binding of the opener diazoxide and of the blocker glibenclamide is not affected by the two mutations, which gives further evidence for a location of their binding sites being separate from the positions 1,285 and 1,289 at TM17 at SUR1. Comparative work at respective mutants of SUR2 will have to follow to see if the reverse is true for this isoform and to contribute to a better understanding of the complex allosteric interactions between binding of openers and blockers at SUR in general.

ACKNOWLEDGMENTS

The generous gift of aprikalim and rilmakalim by Aventis Pharma GmbH (Frankfurt, Germany) is gratefully acknowledged.

We thank Dr. Christian Derst (Jena, Germany) for kindly providing cDNA of rat SUR1. Claudia Müller is acknowledged for excellent technical assistance in molecular biology and cell culture, and we thank Dr. Yuan Ruan (Tübingen, Germany) for assistance in cell culture. We also thank Dr. Ulrich Quast (Tübingen, Germany) for helpful discussion.

REFERENCES

5. Coghlan MJ, Carroll WA, Gopalakrishnan M: Recent developments in the


19. Hambrock A, Loﬄer-Walz C, Kurachi Y, Quast U: Mg\textsuperscript{2+} and ATP dependence of *K* \textsubscript{ATP} channel modulator binding to the recombinant sulphonylurea receptor, SUR2B. *Br J Pharmacol* 125:577–583, 1998


21. Cheng Y, Prussow WH: Relationship between the inhibition constant (K\textsubscript{i}) and the concentration of inhibitor which causes 50% inhibition (IC\textsubscript{50}) of an enzymatic reaction. *Biochem Pharmacol* 22:3099–3108, 1973


