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The Size of a Single Residue of the Sulfonylurea Receptor Dictates the Effectiveness of K_{ATP} Channel Openers

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ABSTRACT

K_{ATP} channel openers are a diverse group of molecules able to activate ATP-sensitive K^+ channels in a tissue-dependent manner by binding to the channel regulatory subunit, the sulfonylurea receptor (SUR), an ATP-binding cassette protein. Residues crucial to this action were previously identified in the last transmembrane helix of SUR, transmembrane helix 17. This study examined the residue at the most important position, 1253 in the muscle isoform SUR2A and the matching 1290 in the pancreatic/neuronal isoform SUR1 (rat numbering). At this position in either isoform, a threonine enables action of openers, whereas a methionine prohibits it. Using single-point mutagenesis, we have examined the physicochemical basis of this phenomenon and discovered that it relied uniquely on side chain volume and not on shape, polarity, or hydrogen-bonding capacity of the residue. Moreover, the aromatic nature of neighboring residues conserved in SUR1 and SUR2A was found necessary for SUR2A to sustain the wild-type levels of channel activation by the openers tested, the cromakalim analog SR47063 [4-(2-cyanimino-1,2-dihydro-1-pyridyl)-2,2-dimethyl-6-nitrochromene] and the pinacidil analog P1075 [*N*-cyano-*N*′-(1,1-dimethylpropyl)-*N*′-3-pyridylguanidine]. These observations suggest that these residues can interact with openers via nonspecific stacking interactions provided that the adjacent 1253/1290 residue does not obstruct access. The smaller Thr1253 of SUR2A would permit activation, whereas the bulky Met1290 of SUR1 would not. This hypothesis is discussed in the context of a simple molecular model of transmembrane helix 17.

ATP-sensitive potassium (K_{ATP}) channels are inward-rectifying, potassium-selective channels gated by intracellular adenine nucleotides. Because their activity reflects the static and dynamic nature of cellular metabolism (Selivanov et al., 2004), they are thought to act as sensors of intracellular metabolism, tuning the potassium permeability, and in turn the electrical activity, of a cell to its energetic balance. K_{ATP} channels are present in most excitable cells. In pancreatic β cells, they play a key role in coupling insulin secretion to plasma glucose (Ashcroft, 2000). In heart and brain, their function is still discussed, but evidence supports an implication in the protective response to various metabolic insults

(Zingman et al., 2002; Seino and Miki, 2003; Suzuki et al., 2003).

The K_{ATP} channel results from the constitutive association of four pore-forming subunits Kir6.x and four regulatory subunits SUR (Inagaki et al., 1995; Shyng and Nichols, 1997). SUR, a member of the ABCC/MRP subfamily of ABC proteins, is the site of action of K_{ATP} channel openers, a collective term for a large set of chemically diverse, pharmacologically relevant molecules able to activate K_{ATP} channels (Terzic and Vivaudou, 2001; Mannhold, 2004; Moreau et al., 2005). These openers, which include pinacidil, cromakalim, and nicorandil, preferentially target the SUR2A and SUR2B isoforms predominantly expressed in cardiac muscle and smooth muscle, respectively. Except for the special case of diazoxide (D'hahan et al., 1999b), openers have little or no effects on SUR1-based channels found in pancreas and brain.

This clear phenotypic difference between SUR1 and SUR2 coupled with primary sequence homologies of ~80% permitted the development of chimeric approaches that led to the increasingly detailed identification of the determinants of action of nondiazoxide openers: first, the last transmembrane

ABBREVIATIONS: K_{ATP}, ATP-sensitive potassium; SUR, sulfonylurea receptor; ABCC, subfamily C of ATP-binding cassette proteins; MRP, multidrug resistance-associated protein; ABC, ATP-binding cassette; TM, transmembrane; SR47063, 4-(2-cyanimino-1,2-dihydro-1-pyridyl)-2,2-dimethyl-6-nitrochromene; P1075, *N*-cyano-*N*′-(1,1-dimethylpropyl)-*N*′-3-pyridylguanidine.

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domain TMD2 (D'hahan et al., 1999a; Fig. 1A); then, two smaller segments within TMD2, the cytoplasmic loop connecting TM helices 13 and 14, and a region encompassing TM helices 16 and 17 and a short segment of NBD2 (Uhde et al., 1999); finally, two residues within TM helix 17 (Leu1249 and Thr1253 in SUR2A, Thr1286 and Met1290 in SUR1) were shown to be necessary (when mutated in SUR2A, loss of opener action ensued) and sufficient since mutation of any of these residues could confer to SUR1 a sensitivity to openers that was either partial in the case of T1286L or equivalent to wild-type SUR2A in the case of M1290T (Moreau et al., 2000).

The broad specificity of SUR2 for openers; the role of TM helix 17, which has been implicated in binding of substrates in other polyspecific ABC transporters such as MRP1 (Daoud et al., 2001; Ito et al., 2001a; Zhang et al., 2001; Mao et al., 2002; Ren et al., 2002; Karwatsky et al., 2003), MRP3 (Oleschuk et al., 2003; Zhang et al., 2003), and multidrug resistance protein 1 (Loo and Clarke 1997, 2001, 2002; Demeule et al., 1998; Dey et al., 1999); and the link between openers and ATPase activity of SUR2A (Bienengraeber et al., 2000) led to the hypothesis that openers could act as pseudosubstrates of SUR by occupying a substrate-binding pocket structurally conserved in SUR and multidrug resistance proteins.

That single-point mutations in SUR1 can render SUR1/Kir6.2 channels sensitive to openers clearly indicates that SUR1 shares with SUR2A most of the elements of the opener binding site. Indeed, opener binding to SUR1 is detectable, although binding assays showed a 1000- to 10,000-fold difference in dissociation constants for pinacidil, P1075, or levcromakakim between SUR1 and SUR2 isoforms (Schwanstecher et al., 1998). Functional evidence of a low-affinity SUR1 site for nondiazoxide openers has also been presented by Cartier et al. (2003) who observed activation of SUR1-based channels by high concentrations of pinacidil after SUR1 had been mutated (F1388L) in NBD2, a region not thought to be directly involved in opener binding.

In the present work, we have used site-directed mutagenesis to investigate what features of the most important residue of helix 17, SUR2A-Thr1253 or SUR1-Met1290, are necessary for its effect on opener action. We further examine the involvement of neighboring aromatic residues shared by SUR1 and SUR2 in contributing the promiscuous interactions required to accommodate opener molecules of distinct structures.

Materials and Methods

Mouse Kir6.2 (Inagaki et al., 1995; GenBank accession no. D50581), hamster SUR1 (Aguilar-Bryan et al., 1995; GenBank accession no. L40623) and rat SUR2A (Inagaki et al., 1996; GenBank accession no. D83598) were subcloned in the *Xenopus laevis* oocyte expression vectors derived from pGEMHE (Liman et al., 1992). Site-directed mutagenesis was done by polymerase chain reaction amplification of both DNA strands of full-length plasmids with complementary primers mutated to produce the desired amino acid change (QuikChange site-directed mutagenesis kit; Stratagene, La Jolla, CA). Plasmid DNAs were amplified, confirmed by restriction analysis and by sequencing, linearized, and transcribed in vitro using the T7 mMessage mMachine kit (Ambion, Austin, TX). cRNAs were electrophoresed on formaldehyde gels, and concentrations were estimated from two dilutions using RNA marker as a standard.

cRNAs coding Kir6.2 (~2 ng) and wild-type or mutant SURs (~6 ng) were coinjected into defoliculated *X. laevis* oocytes. Injected oocytes were stored at 19°C in Barth's solution [1 mM KCl, 0.82 mM MgSO₄, 88 mM NaCl, 2.4 mM NaHCO₃, 0.41 mM CaCl₂, 0.3 mM Ca(NO₃)₂, and 16 mM HEPES, pH 7.4] with 100 U/ml penicillin, 100 μg/ml streptomycin, and 100 μg/ml gentamycin.

Electrophysiology. Three to 7 days after injection, oocytes were devitellinized, and recombinant K_{ATP} channels were characterized by the patch-clamp technique in the excised inside-out configuration (Hamill et al., 1981). Patch pipettes (2–10 MΩ) contained 154 mM K⁺, 146 mM Cl⁻, 5 mM Mg²⁺, and 10 mM PIPES, pH 7.1. The cytoplasmic face of the patch was bathed in solutions that all contained 174 mM K⁺, 40 mM Cl⁻, 1 mM EGTA, 1 mM Mg²⁺, and 10 mM PIPES, pH 7.1, and methanesulfonate⁻ as the remaining anions. ATP (potassium salt; Sigma-Aldrich, St. Louis, MO), SR47063 (20 mM stock in dimethyl sulfoxide; SANOFI Research Center, Montpellier, France) and P1075 (20 mM stock in dimethyl sulfoxide; Leo Pharmaceutical Products, Copenhagen, Denmark) were added as specified. The membrane potential was maintained at −50 mV. Experiments were conducted at room temperature (22–24°C).

Applications of the various solutions to the intracellular face of the patch was performed using an RSC-100 rapid-solution-changer (Bio-Logic, Claix, France) controlled by in-house software. Analog signals
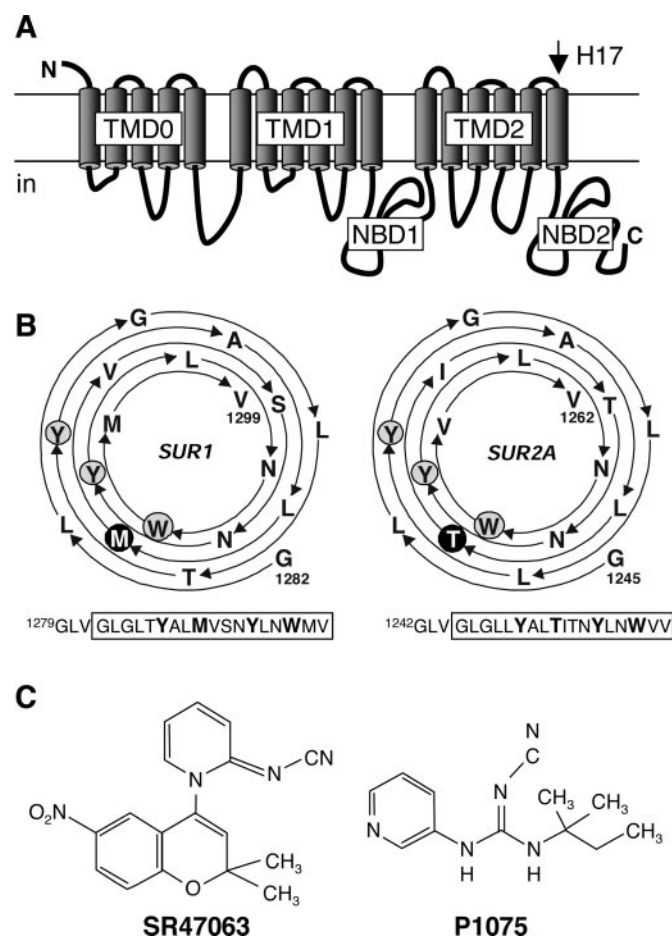

Fig. 1. A, predicted topology of SUR showing the three transmembrane domains (TMD) and two nucleotide-binding domains (NBD). TM helix 17 is indicated. B, helicoidal representation of helix 17 of SUR1 and SUR2A viewed from the extracellular side with key residues circled. SUR1-Met1290 and SUR2A-Thr1253 are white on a black background, whereas neighboring aromatic residues are black on a gray background (SUR1-Tyr1287, Tyr1294, and Trp1297 and SUR2A-Tyr1250, Tyr1257, and Trp1260). The linear sequences are shown at bottom. Numbering refers to the rat/hamster proteins. C, chemical structures of the K_{ATP} channel openers used in this study, the benzopyran SR47063 and the guanidine P1075.

were filtered at 300 Hz and sampled at 1 kHz. Slow fluctuations of the no-channel-open baseline of the signal were removed by interactive fitting of the baseline with a spline curve and subtraction of this fit from the signal. Acquisition, analysis, and presentation were performed with in-house software. The tracings shown in the illustrations represent continuous records with occasional short segments of extraneous data removed for display purposes. Results are displayed as mean ± S.E.M.

Molecular Modeling. The α-helices were built using the SYBYL software (Tripos, St. Louis, MO) and imported into the ViewerPro software (Accelrys, San Diego, CA) to set the display style. The surface was made in the ViewerPro software with a probe of 1.40 Å rolling over the residues located on the same face of TM helix 17 as residues Thr1253/Met1290. The potassium channel openers were drawn with ChemDraw software (CambridgeSoft, Cambridge, MA), imported in the ViewerPro software and transformed in three-dimensional structures.

Results

Pursuing a chimeric strategy, we previously identified residues in the last predicted transmembrane helix of SUR, TM helix 17 (Fig. 1A), as responsible for the isoform-specific pharmacological phenotype of K_{ATP} channels toward K_{ATP} channel openers (Moreau et al., 2000). In this work, we further explore the chemical basis of this finding through mutagenesis of key residues of TM helix 17 (Fig. 1B).

Threonine 1253 of SUR2A Is Not Involved in Specific Hydrogen Bonds with K_{ATP} Channel Openers. The threonine at position 1253 in SUR2A was previously reported to be necessary and sufficient to confer to SUR2A and SUR1 subunits the capacity to be activated by nondiazoxide openers (Moreau et al., 2000). The amino acid at the equivalent position in SUR1 is a methionine, SUR1-Met1290. The single substitution in SUR2A of threonine 1253 with a methionine (T1253M) is sufficient to abolish the sensitivity to openers such as SR47063 (a cromakalim analog) and P1075 (a pinacidil analog) (Fig. 1C). Likewise, the SUR1(M1290T) mutant is activated by openers, clearly highlighting the critical role of this threonine.

How does a single threonine in TM helix 17 confer sensitivity to openers in both SUR1 and SUR2A isoforms? Contrary to methionines, the threonine side chain has a hydroxyl group that can create intermolecular hydrogen bonds. Thus, threonine 1253 in SUR2A could create specific and critical hydrogen bonds with openers, which would not be possible with methionine 1290 in SUR1.

To test this hypothesis, we substituted SUR2A-Thr1253 with other amino acids of varying size and polarity: two with hydrogen bond-forming side chains of different sizes (serine and tyrosine), two with side chains of medium and small sizes but no capacity to form hydrogen bonds (valine and cysteine), and the last one (methionine) being the aligned residue in SUR1 (Fig. 1).

Each mutant was coexpressed with Kir6.2 in *X. laevis* oocytes, and the opener sensitivity of the resulting K_{ATP} channels was assessed by the patch-clamp technique in the excised inside-out configuration. The results shown in Fig. 2 demonstrate that mutations of SUR2A-Thr1253 to serine, cysteine, and valine did not significantly affect activation by SR47063 and P1075, whereas mutations to tyrosine and methionine drastically reduced activation.

Thus, even residues with short hydrogen bond-forming side chains permit activation by openers, whereas substitution with a bigger hydrogen bond-forming side chain (tyrosine) abolishes almost completely the sensitivity to the openers.

Most importantly, the valine mutant SUR2A(T1253V) was also activated by SR47063 and P1075 at the same level as the serine and cysteine mutants. This implies that the hydroxyl group of the 1253 residue is not a critical feature for activation by openers of SUR2A.

Although we did not quantify the on- and off-rates of activation because the speed of solution exchanges at the patch of membrane could not be strictly controlled by our gravity-fed, slow perfusion system, the off-rates of openers seemed somewhat faster for the T1253S and T1253C mutants than for the wild-type channel or for the T1253V mutant (Fig. 2). This was also true of on-rates, but the difference was not as significant.

Steric Hindrance of the Methionine 1290 Side Chain Prevents Activation of SUR1 by Openers. The substitution of the SUR2A threonine 1253 with bigger residues apparently induces a steric hindrance, leading to the lack of sensitivity to openers. To confirm that result, we mutated the aligned residue in SUR1, methionine 1290 to serine, cysteine, valine, tyrosine, and threonine and examined the phenotype of these mutants. Figure 3 shows that mutations of methionine 1290 to smaller side chain amino acids (threonine, serine, and cysteine) rendered SUR1 sensitive to SR47063 and P1075, whereas mutation to tyrosine did not. The valine mutant was also constructed, but an unusually low level of expression hampered its characterization. Thus, SUR1 becomes sensitive to openers when residue 1290 has a small side chain irrespective of its polar nature.

Role of TM Helix 17 Aromatic Residues in K_{ATP} Channel Activation by Openers. TM helix 17 of SUR possess three aromatic residues conserved in all SUR isoforms as well as in other members of the MRP/ABCC family. These residues, Tyr1250, Tyr1257, and Trp1260 in SUR2A, are in proximity to Thr1253 (Fig. 1B) and could interact directly with openers through stacking liaisons, because the only common motif among all opener molecules is the presence of at least one heterocycle ring.

To test this hypothesis, the three aromatic residues in SUR2A were replaced by a methionine to preserve the hydrophobicity and the steric hindrance of the side chain while removing the possibility of stacking interactions. Furthermore, the two tyrosines were also mutated to phenylalanines as a positive control for stacking interactions and to determine the possible implication of the tyrosine hydroxyl group in opener action. These mutations were characterized in the same manner as described above, and experimental results are presented in Fig. 4.

None of the aromatic residue mutations completely reduced the SR47063-induced activation (Fig. 4E). However, the sensitivity for this opener was drastically reduced for the Y1250M mutant (compare traces of Figs. 2A and 4B), whereas it was only slightly reduced for the Y1250F mutant compared with wild type. A similar, but smaller, difference toward SR47063 was seen between the Y1257F and Y1257M mutants. This difference is seen at 10 μM SR47063, which activated the Y1257F mutant less than wild type and more than the Y1257M mutant. In contrast, the W1260M

mutation, which reduces side chain size and eliminates aromaticity, had little effect on SR47063 sensitivity.

The responses to P1075 of the same mutants are summarized in Fig. 4F. The pattern of sensitivity to P1075 of the tyrosine mutants seems comparable with that observed with SR47063. Thus, the phenylalanine mutants of the residues Tyr1250 and Tyr1257 had the same phenotypes as wild-type SUR2A, whereas the methionine mutants displayed a reduced sensitivity to P1075 as evidenced by the much smaller activation elicited by the 10 μM concentration. Thus, the aromatic rings of both tyrosines seem to influence the acti-

vation threshold of both P1075 and SR47063. In contrast, the mutation of tryptophan 1260 to methionine reduced the level of activation by P1075 but not by SR47063 as evidenced in the patch-clamp record of Fig. 4D.

Discussion

Difference in Side Chain Size of SUR2A-Thr1253 and SUR1-Met1290 as a Critical Factor in the Effects of K_{ATP} Channel Openers. We have previously shown that the different pharmaceutical phenotypes of channels
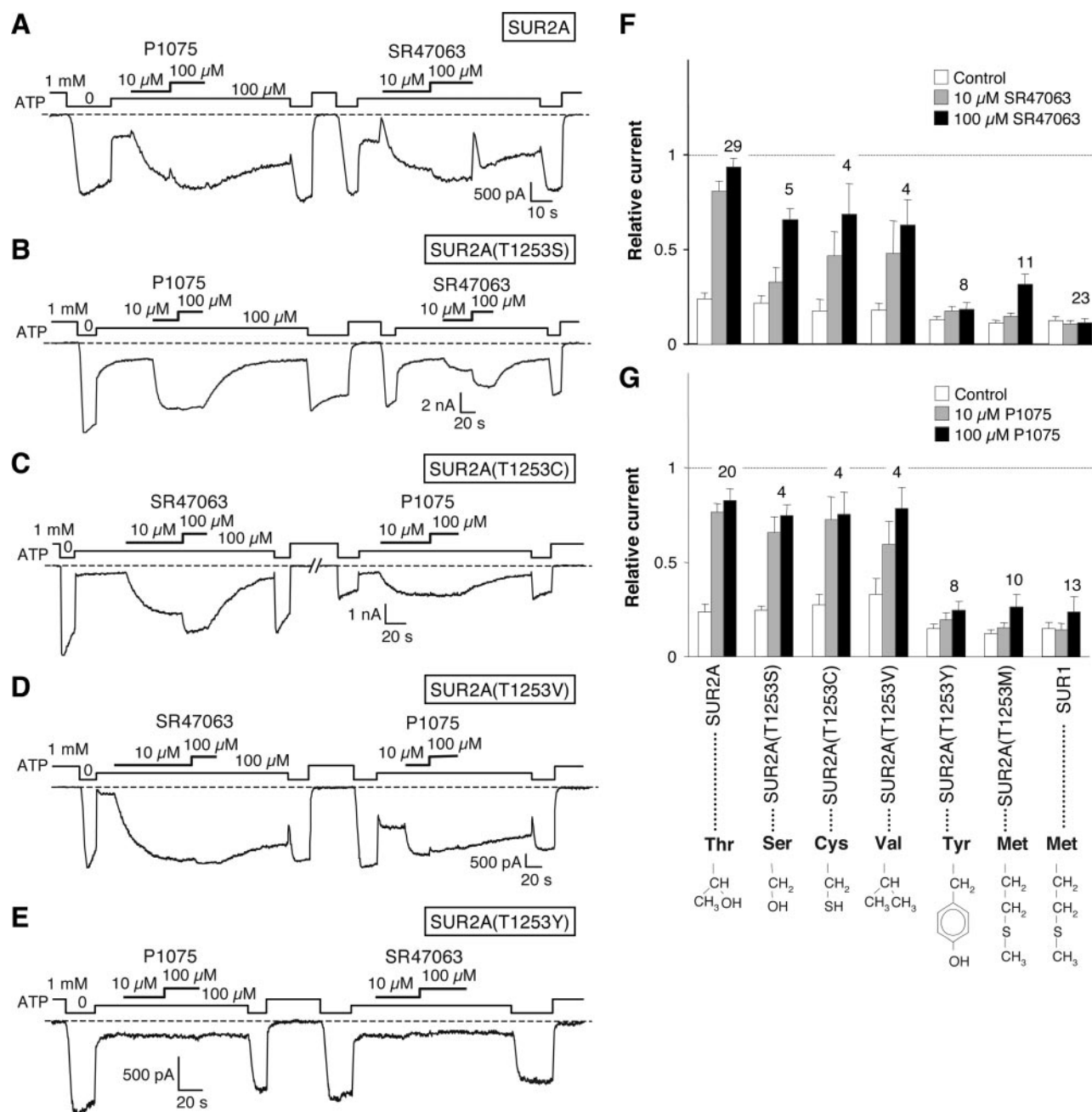

Fig. 2. Presence of large residues at position 1253 of SUR2A correlates with a reduction in activation by K_{ATP} channel openers. The responses of wild-type and mutated SUR2A to the openers SR47063 and P1075 were investigated by recording ATP-sensitive K^+ currents at -50 mV from inside-out patches of *X. laevis* oocytes expressing Kir6.2 and each SUR subunit. A to E, examples of individual records for the indicated SUR subunits. F and G, average relative currents measured before and during application of the specified openers. Currents were averaged after being normalized to the current measured in absence of ATP in each patch. The data were collected from the indicated number of patches where the protocols of A were used.

incorporating the isoforms SUR1 and SUR2A could be attributed in great part to the presence of distinct amino acids at two positions within the last postulated transmembrane helix of SUR. These were positions 1286 and 1290 in SUR1 (and the equivalent positions 1249 and 1253 in SUR2A). The former position, however, was of lesser importance because its mutation in SUR1 could confer a weak sensitivity to SR47063 but not to the other openers tested, P1075 and rilmakalim (Moreau et al., 2000); we have concentrated here on the latter position. A threonine at this position, as in wild-type SUR2A, greatly increases sensitivity to openers, whereas a methionine, as in wild-type SUR1, greatly decreases it. What specific physicochemical properties of threonine and methionine could be responsible for their effects? We have tried to answer that question by mutating Thr1253 in SUR2A and Met1290 in SUR1 to residues of various shapes, sizes, and polarities and by registering the responses

of the resulting mutant K_{ATP} channels to the openers SR47063 and P1075. The selected mutations were tested in two different contexts to fully validate their effects: in SUR1, where they caused no change or a gain of function, and in SUR2A, where they caused no change or a loss of function. The results for SUR1 and SUR2A were remarkably concordant and led to the conclusion that residue size mattered most. Thus, SUR1 became opener-activatable when the residue at position 1290 was more compact than methionine, and SUR2A lost its opener sensitivity when the residue at position 1253 was a methionine or a bulkier residue. Thus, opener-permissive residues included not only threonine, a hydrophilic residue able to form hydrogen bonds, but also serine, which has similar properties but is smaller, cysteine, which has comparable size but is unable to form hydrogen bonds, and valine, which has comparable size but is hydrophobic and unable to form hydrogen bonds. Nonpermissive
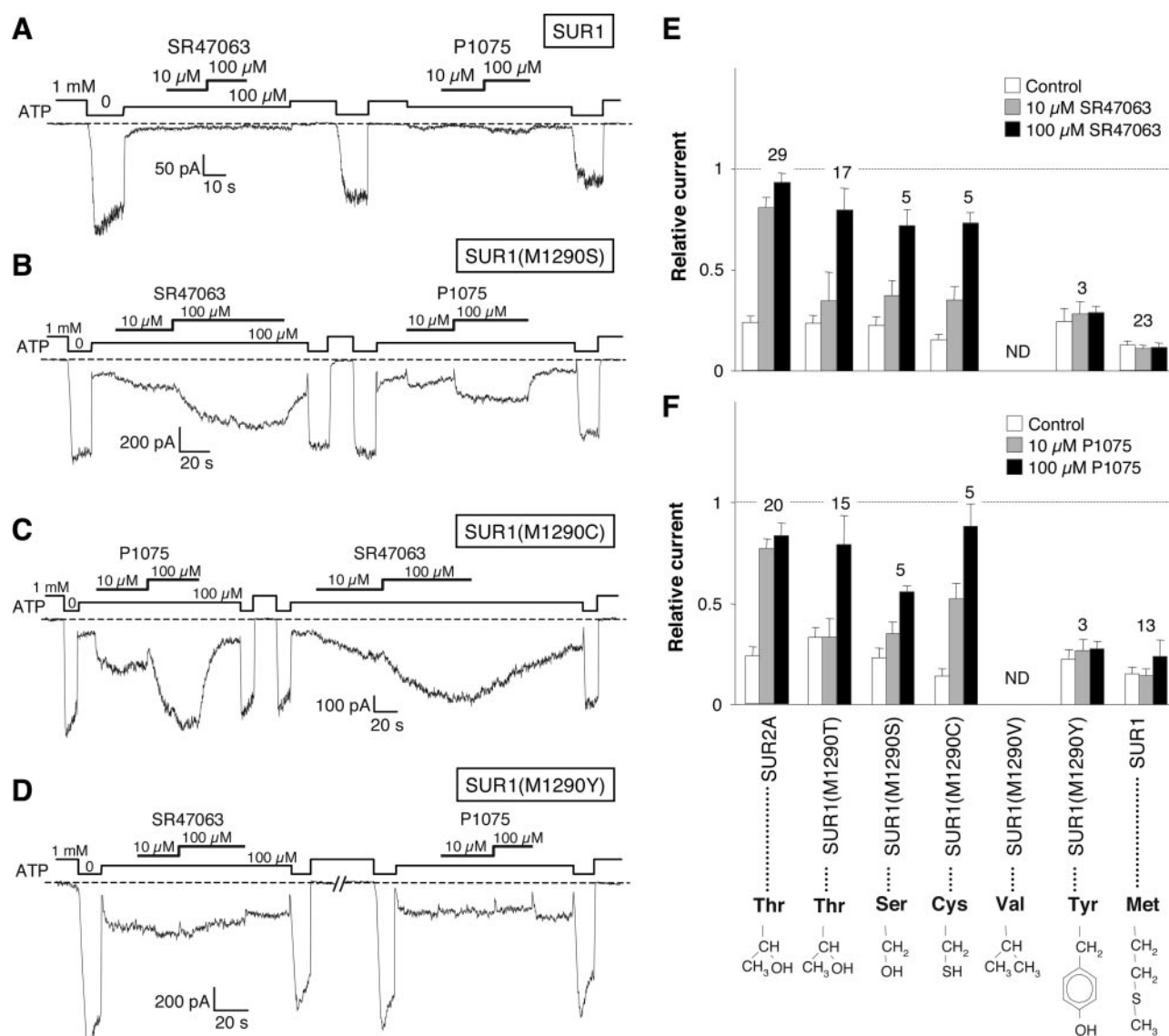

Fig. 3. Presence of small residues at position 1290 of SUR1 correlates with an increased sensitivity to activation by openers. The responses of wild-type and mutated SUR1 to SR47063 and P1075 were investigated by recording ATP-sensitive K^+ currents at -50 mV from inside-out patches of *X. laevis* oocytes expressing Kir6.2 and each SUR subunit. A to D, examples of individual records for the indicated SUR subunits. E and F, average relative currents measured before and during application of the specified openers. Currents were averaged after being normalized to the current measured in absence of ATP in each patch. The data were collected from the indicated number of patches where the protocols of A were used. Responses of the mutant SUR1(M1290V) were not determined (ND) because of low levels of expression.

residues included the large hydrophobic methionine and the large hydrophilic tyrosine.

These observations point to side chain size as the only determinant of opener sensitivity and argue against any intimate physical contact between SUR2A-Thr1253 and openers during binding. They suggest that this key residue could act as a gate toward the binding site, in agreement with the faster dissociation rates observed when Thr1253 was mutated to the smaller serine and cysteine residues, but not to the valine residue of similar volume.

Opener Action Is Modulated by TM Helix 17 Aromatic Residues. SUR1-Met1290 and SUR2A-Thr1253 are critical residues for the specificity of action of openers; however, the above-mentioned results clearly indicate that they do not participate in direct interactions with them. Nonetheless, binding and electrophysiological studies (D'hahan et al., 1999a; Uhde et al., 1999; Moreau et al., 2000) are in favor of a opener binding site including helix 17, with the likely involvement of other regions such as the intracellular loop between transmembrane

helices 13 and 14 (Uhde et al., 1999). Focusing our research on helix 17, we looked at three aromatic residues predicted to lie in space near SUR2A-Thr1253. These residues, SUR2A-Tyr1250, -Tyr1257, and -Trp1260, are absolutely conserved in all SUR isoforms and are also found in most ABC transporters of the ABCC/MRP subfamily. To examine whether they could create nonspecific stacking interactions with the aromatic rings present in opener molecules, we substituted them with a methionine, a nonaromatic hydrophobic residue of similar size. The effects of these mutations were not as clear-cut as the all-or-nothing effects of the mutations of SUR1-Met1290 and SUR2A-Thr1253. All mutants displayed sensitivity to the two openers tested, but there was a definite reduction in sensitivity when either tyrosine 1250 or 1257 was changed to methionine. This was not seen when tyrosines were changed to phenylalanine, suggesting that aromaticity of these residues is crucial. This was not the case for tryptophan 1260, which could be mutated to methionine without affecting activation by SR47063.
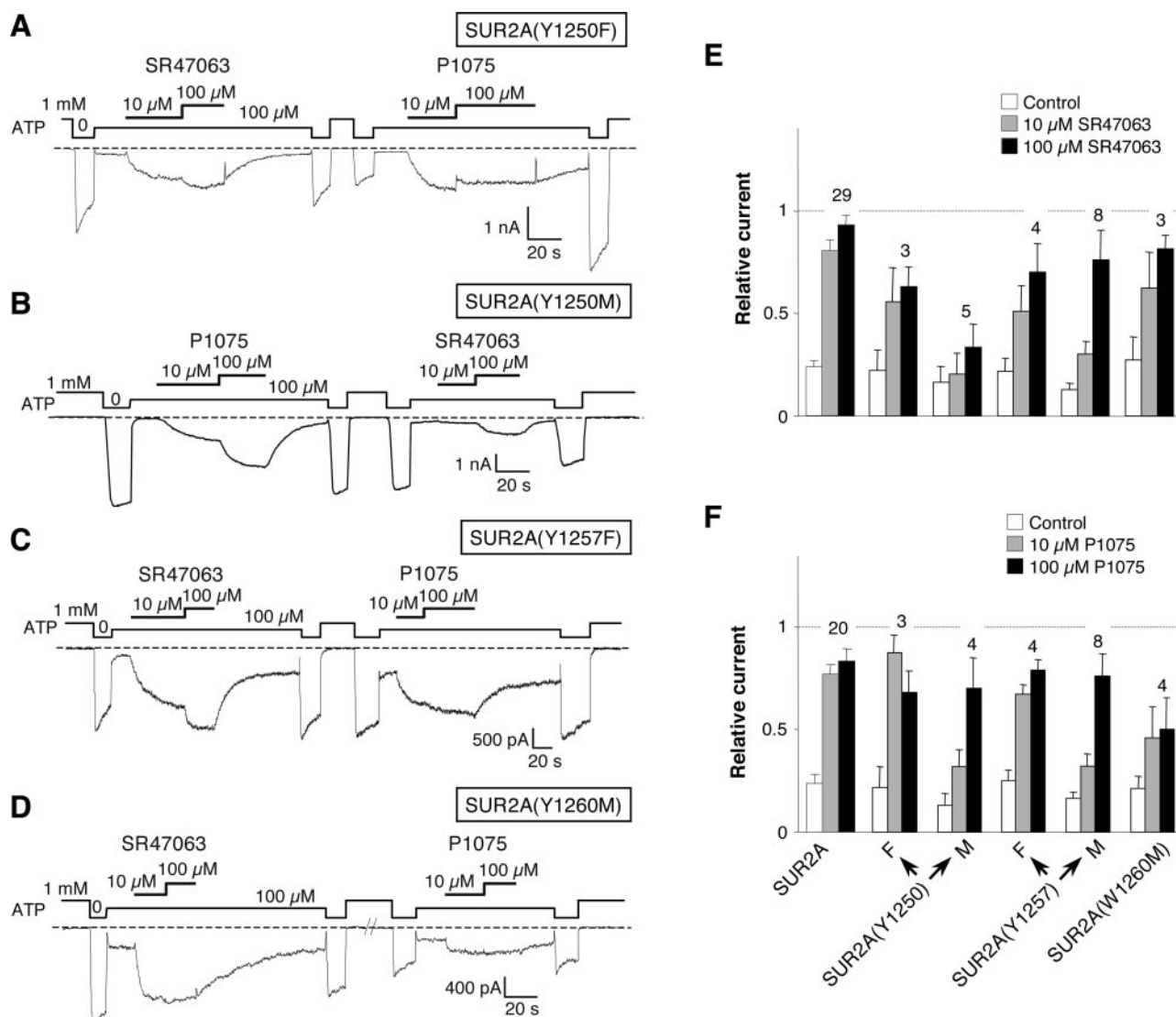

Fig. 4. Effects of mutating conserved aromatic residues of SUR2A helix 17 on the sensitivity to K_ATP channel openers. A to D, patch-clamp records illustrating the responses to the openers SR47063 and P1075 of channels formed with Kir6.2 and the specified SUR2A mutant. E and F, average relative currents measured before and during application of the specified openers. Currents were averaged after being normalized to the current measured in absence of ATP in each patch. The data were collected from the indicated number of patches where the protocols of A were used.

However, it clearly seems that mutations of the aromatic residues induce differential effect on activation by the two openers. The SUR2A-Y1250M mutation reduced the activation by SR47063 much more than that by P1075, whereas the SUR2A-W1260M affected only the activation by P1075. These effects are concordant with the substrate-specific phenotypes observed with MRP1 and MRP2 when the residues aligned to SUR2A-Trp1260, MRP1-Trp1246, and MRP2-Trp1254 were mutated (Ito et al., 2001a,b).

These observations indicate that aromatic residues of helix 17 interact with openers and that these interactions depend on the structure of the openers, as if these residues were part of a binding pocket accessible to various openers.

Modeling the Interaction of Openers with Helix 17. The evidence presented asserts the fundamental role of TM helix 17 in controlling the activation of K_{ATP} channels by openers. One residue, SUR1-Met1290 or SUR2A-Thr1253, acts as a size-dependent on/off switch, whereas neighboring aromatic residues modulate that activation.

How can the size, but not the shape or polarity, of the side chain of residues at the equivalent position in the last SUR1 and SUR2A transmembrane helix affect so drastically the sensitivity to openers? Besides a complex allosteric effect

linking this residue to a distant site shared by SUR1 and SUR2A, the simplest hypothesis may be that the steric hindrance of the SUR1-Met1290 side chain directly prevents openers from reaching a nearby binding site. This site could be partly constituted of aromatic residues contributing π-π stacking interactions, which would be sufficiently aspecific to confer a broad tolerance for molecules of diverse structures (Pawagi et al., 1994). A hypothetical molecular representation of TM helix 17 (Fig. 5) reveals that such a scheme would fit well within the constraints imposed by the primary sequence and the sizes of residue side chains as well as of openers.

To date, high-resolution structural information on the transmembrane spanning domains of eukaryotic ABC transporters is not available, and consequently, the orientation of SUR1-Met1290 and SUR2A-Thr1253 is unknown. However, models can be created using the published crystal coordinates of the bacterial ABC transporters MsbA and BtuCD (Chang and Roth, 2001; Löcher et al., 2002; Chang, 2003). An MsbA-based model of the transmembrane domains of MRP1, an ABC transporter highly homologous to SUR, was recently developed (Campbell et al., 2004). In that study, several aromatic residues were predicted to face the substrate
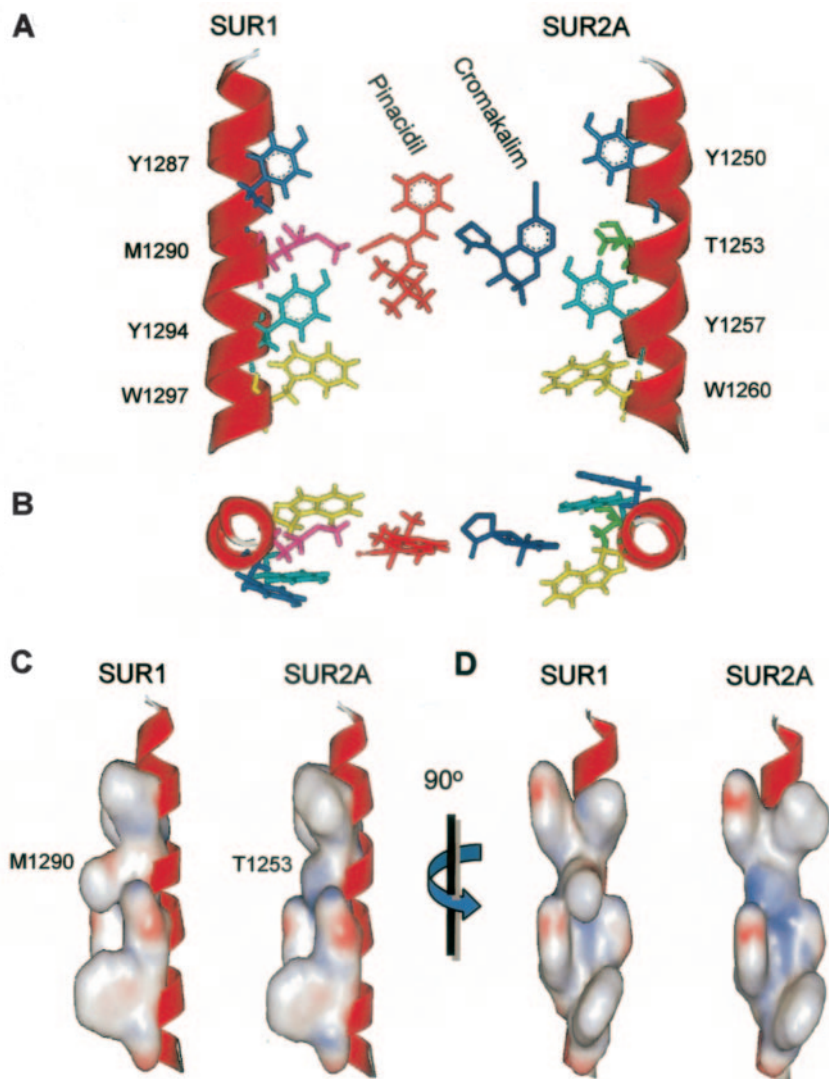

Fig. 5. Hypothetical model of the molecular interactions between helix 17 with openers. A, side view of TM helix 17 of SUR1 and SUR2A with stick representation of salient residues and of the model openers cromakalim (in dark blue) and pinacidil (in red), from which SR47063 and P1075 were derived. B, top view from the extracellular side of the same. C and D, molecular surface rendering of helix 17 of SUR1 and SUR2A from 2 orthogonal points of view. Key residues Met1290 and Thr1253 are labeled. Surface colors reflect the electrostatic potential of calculated Gasteiger charges.

translocation pathway, and experimental assays confirmed that prediction by demonstrating their critical role in substrate transport. Among these residues, MRP1-Trp1246 matches SUR1-Trp1297 and SUR2A-Trp1260. Thus, according to this MRP1 model, because these tryptophan residues are located on the same face of helix 17 as SUR1-Met1290 and SUR2A-Thr1253 (Fig. 1), their side chains would all be facing inward within a potential central substrate pocket. This would be consistent with a role for SUR1-Met1290 in obstructing access of openers to interacting residues common to SUR1 and SUR2A such as the aromatic residues tested.

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