and insensitive to the mutation S1237Y. We have examined the pancreatic channel, whereas B-ligands are nonselective grouped accordingly. A-ligands are highly selective in closing the KATP channel in pancreatic β-cells and stimulate insulin secretion. The insulinotrope binding site of the pancreatic channel (Kir6.2/SUR1) consists of two overlapping (sub)sites, site A, located on SUR1 and containing Ser1237 (which in SUR2 is replaced by Tyr1206), and site B, formed by SUR1 and Kir6.2. Insulinotropes bind to the A-, B-, or A + B-site(s) and are grouped accordingly. A-ligands are highly selective in closing the pancreatic channel, whereas B-ligands are nonselective and insensitive to the mutation S1237Y. We have examined the binding of insulinotropes representative of the three groups in [3H]glibenclamide competition experiments to determine the contribution of Kir6.6 to binding affinity, the effect of the mutation Y1206S in site A of SUR2, and the subtype selectivity of the compounds. The results show that the bipartite nature of the SUR1 binding site applies also to SUR2. Kir6.2 as part of the B-site may interact directly or allosterically with structural elements common to all insulinotropes, i.e., the negative charge and/or the adjacent phenyl ring. The B-site confers a moderate subtype selectivity on B-ligands. The affinity of B-ligands is altered by the mutation SUR2(Y1206S), suggesting that the mutation affects the binding chamber of SUR2 as a whole or subsite A, including the region where the subsites overlap.

Sulfonylureas and glinides (termed here insulinotropes) are used in the therapy of type 2 diabetes. They act by closing the ATP-sensitive K+ (KATP) channel in pancreatic β-cells, thereby inducing depolarization, Ca2+ entry, and insulin secretion (Sturgess et al., 1985; for review, see Proks et al., 2002). KATP channels are closed by intracellular ATP and opened by MgADP. They are composed of two types of subunits, inwardly rectifying K+ channels (Kir6.x), which form the pore of the channel, and sulfonylurea receptors (SURx), which serve as regulatory subunits (Fig. 1). SURs are members of the ATP-binding cassette protein superfamily and carry binding sites for nucleotides, sulfonylureas, and the KATP channel openers. Both Kir6.x and SURx are encoded by two genes, giving rise to two subtypes each; in addition, alternative splicing of SUR2 results in two major isoforms, SUR2A and SUR2B, which differ in the last 42 amino acids. The KATP channels in pancreatic β-cells have the composition Kir6.2/SUR1, in cardiomyocytes Kir6.2/SUR2A, and in vascular myocytes Kir6.1/SUR2B (Aguilar-Bryan and Bryan, 1999; Gribble and Reimann, 2003; Seino and Miki, 2003). KATP channels subserve important functions not only in the pancreatic β-cells (in which they link the secretion of insulin to the plasma glucose level) but also in other tissues (Seino and Miki, 2003). Hence, the selectivity of the insulinotropes for the pancreatic KATP channel is an important clinical issue (for review, see Quast et al., 2004).

With the advent of the long-chain sulfonylureas such as glibenclamide, structure-activity relationships suggested that the binding site of the pancreatic channel for sulfonylureas had two attachment points, the “A-site” for the sulfonylurea part and the “B-site” for the new carboxamido part (Fig. 2) (Rufer and Losert, 1979; Brown and Foubister, 1984), and a major task has been to identify the parts of the pancreatic KATP channel that represent the hypothetical (sub)sites A and B (for review, see Bryan et al., 2005). The actual state of knowledge is summarized in Fig. 1.

The short sulfonylureas bind to the A-site. A single amino acid in SUR1, Ser1237, which corresponds to Tyr1206 in SUR2 (mouse numbering), affords to these compounds an up

**ABBREVIATIONS:**

KATP, ATP-sensitive K⁺; Kir, inwardly rectifying K⁺ channel; SUR, sulfonylurea receptor; UL-DF 9, 4-[2-(3,4-dihydro-7-methoxy-4,4-dimethyl-1,3-dioxo-2(1H)-isoquinoliny1)-ethyl]benzoic acid; AZ-DF 265, 4-[[N-(α-phenyl-2-piperidino-benzyl)carbamoyl[methyl]]-benzoic acid; P1075, N-cyano-N’-(1,1-dimethylpropyl)-N’-3-pyridylguanidine; HEK, human embryonic kidney.
to 1000-fold selectivity for the pancreatic over the cardiovascular $K_{ATP}$ channels (Ashfield et al., 1999; Proks et al., 2002). Nateglinide also belongs to the A-ligands (Hansen et al., 2002; Chachin et al., 2003). Glibenclamide and the other long sulfonylureas occupy subcompartments A + B; these compounds exhibit a more limited selectivity (for review, see Proks et al., 2002). The B-ligands are those glinides that are developed from the carboxamido-part of glibenclamide (Fig. 2). They show little if any selectivity for the pancreatic channel (Ashfield et al., 1999; Dabrowski et al., 2001; but see Stephan et al., 2006), suggesting that the B-site is very similar in all SURs. In addition, their potency in closing the pancreatic channel was not affected by the mutation S1237Y in SUR1 (Ashfield et al., 1999; Hansen et al., 2002). Regarding the subdivision in A- and B-ligands, Fig. 2 shows that all insulinotropes have in common a negative charge (which is provided by either the sulfonylurea group or an isosteric carboxylic acid function), and the central phenyl ring, suggesting that the A- and the B-sites overlap to accommodate these parts (Brown and Foubister, 1984).

The selectivity and the classification of the insulinotropes were established for the pancreatic channel, Kir6.2/SUR1, and mostly by studying channel inhibition (Ashfield et al., 1999; Proks et al., 2002). Here, we sought to investigate the binding process and to evaluate to which degree the bipartite model of the insulinotrope binding site of the pancreatic channel extends to the cardiovascular channels. Therefore, the binding properties of 10 insulinotropes were characterized in [3H]glibenclamide competition assays. The compounds comprised three A-ligands (including nateglinide), three A + B-ligands, and four B-ligands [meglitinide and UL-DF 9 (i.e., the “B-parts” derived from glibenclamide and glipizide), i.e., two piperidino-glinides, repaglinide and AZ-DF 265, with a piperidino substituent attached to the carboxamido part; Fig. 2]. We evaluated the effect of coexpression with Kir6.x on the affinity of SURx for the insulinotropes, the effect of the mutation SUR2(Y1206S), i.e., the reverse of the mutation SUR1(S1237Y), and the selectivity in binding to the recombinant pancreatic, myocardial, and vascular $K_{ATP}$ channels. The results show that the bipartite organization of the insulinotrope binding site can be extended to Kir6.x/SUR2 with minor modifications.

Materials and Methods

Materials. [3H]Glibenclamide (specific activity, 1.85 TBq/mmol) was purchased from PerkinElmer Life Sciences (Bad Homburg, Germany). The reagents and media used for cell culture and transfection were from Invitrogen (Karlsruhe, Germany). Glibenclamide was purchased from Sigma (Deisenhofen, Germany). Repaglinide was a gift from Novo Nordisk (Bagsvaerd, Denmark) or purchased from Toronto Research Chemicals (Toronto, ON, Canada). The following compounds were gifts from the respective companies: (−)AZ-DF 265 and UL-DF 9 (Boehringer Ingelheim GmbH, Ingelheim, Germany), glibornuride (Roche, Basel, Switzerland), glipizide (Servier, Paris, France), glimepiride and meglitinide (Sanofi-Aventis, Frankfurt, Germany), gliclazide (Yamanouchi Pharmaceuticals, Heidelberg, Germany), and nateglinide (Novartis Pharma, Basel, Switzerland). The opener, P1075, was from Leo Pharmaceuticals (Ballerup, Denmark) or Tocris/Biozol (Eching, Germany). The $K_{ATP}$ channel modulators were dissolved in dimethyl sulfoxide/ethanol (50/50, v/v) and further diluted with the same solvent or with incubation buffer (final solvent concentration < 1%).

Cell Culture and Transfection. HEK 293 cells were cultured in minimum essential medium containing glutamine and supplemented with 10% fetal bovine serum and 20 µg/ml gentamicin as described previously (Hambröck et al., 1998, 2001). Cells were transfected with rat SUR1 (GenBank accession no. X97279) or the murine clones of SUR2A (D86037), SUR2B (D86038), Kir6.1 (D88159), or Kir6.2 (D90581) using the pcDNA 3.1 (+) vector, Lipofectamine 2000, and Opti-MEM (Invitrogen) according to the manufacturer’s instructions (Hambröck et al., 1998, 2001). The mutants SUR2A(Y1206S) and SUR2B(Y1206S) were prepared as described previously (Hambröck et al., 2001; Stephan et al., 2005). For transient coexpression, cells were transfected with Kir and SUR at a molar plasmid ratio of 1:1. To improve the signal/noise ratio in the [3H]glibenclamide binding assays to Kir6.1/SUR2B channels, an
After an incubation time of 30 min, the reaction was stopped by diluting 0.3-ml aliquots (in triplicate) in 10 ml of ice-cold quench solution [50 mM Tris-(hydroxymethyl)-aminomethane, 154 mM NaCl, pH 7.4]. Bound and free ligands were separated by rapid filtration over Whatman GF/C filters (Whatman International, Maidstone, UK). Filters were washed twice with quench solution and counted for [3H] in the presence of 4.5 ml of scintillant (Ultima Gold; Packard, Meriden, CT). At higher concentrations (e.g., glibenclamide > 100 nM), the inhibitors interfered with [3H]glibenclamide binding to intrinsic binding sites in HEK cells, and this was particularly evident in experiments with SUR2. Complete binding curves were either dissected by two component analysis with the high-affinity component representing binding to SUR2 or by defining specific [3H]glibenclamide binding as the binding displaced by P1075 (100 μM). Both methods gave identical results (see e.g., Russ et al., 1999; Hambrock et al., 2001; Löfler-Walz et al., 2002). Nonspecific binding ranged from 40 to 60% of total binding (SUR2); in the case of SUR1, nonspecific binding was determined in the presence of 100 nM glibenclamide and was approximately 10%. The maximal number of [3H]glibenclamide sites (B_max values) were between 0.4 and 1.5 pmol/mg protein according to the transfectant and the method used (permanent versus transient transfection).

**Data Analysis.** Binding inhibition curves were analyzed according to the equation:

\[ y = 100 \left(1 + \frac{10^{\log K_i - px}}{10^{\log K_i}}\right)^{-1} \]  

with y denoting specific binding, n as the Hill coefficient, and x as the inhibitor concentration with \( p_x = -\log x \) and \( pIC_{50} = -\log IC_{50} \). Curve analysis gave Hill coefficients \(-1\) and the respective IC_{50} values with their 95% confidence intervals. IC_{50} values were corrected for the presence of the radioligand according to the Cheng-Prusoff equation (Cheng and Prusoff, 1973) giving the inhibition constants, \( K_i \):

\[ K_i = IC_{50}(1 + L/K_i)^{-1} \]

Here, \( L \) denotes the concentration and \( K_i \) denotes the equilibrium dissociation constant of the radioligand; in the case of homogeneous displacement, this reduces to \( K_i = K_i = IC_{50} - L \).

Data are shown as means ± S.E.M. Fits of eq. 1 to the data were performed according to the method of least-squares using the program SigmaPlot 9.0 (SPSS Science, Chicago, IL). Individual experiments were analyzed, and the parameters were averaged assuming that amplitudes and pIC_{50} values were normally distributed (Christopoulos, 1998). In the text, \( K_i/K_i \) values are given followed by the 95% confidence interval in parentheses. Linear correlation analysis was performed using the pK_i values. In calculations involving two mean values with S.E.S, propagation of errors was taken into account according to Bevington (1969). Significance of differences between two normally distributed parameters with equal variance was assessed using the two-tailed unpaired Student’s t test.

**Results**

**Binding to SURx and Effect of Coexpression.** Figure 3 illustrates the inhibition of [3H]glibenclamide binding to SUR and K_{ATP} channel subtypes by glibenclamide, repaglinide, and nateglinide, i.e., by A + B, B-, and A-ligands, respectively. Experiments were performed at 37°C in intact cells to have an intact cell interior present; in particular, the cytoskeleton (Löfler-Walz and Quast, 1998) and physiological nucleotide concentrations (Hambrock et al., 2002). Inhibition curves were regular with a Hill coefficient of 1 and gave the pK_i values listed in Tables 1 and 2. pK_i values are directly related to binding affinity, with an increase by 1 log unit corresponding to a binding energy of 1.4 kcal/mol (Berg et al., 2002).
Figure 3 (top panels) presents the curves of the three ligands binding to SUR1 and Kir6.2/SUR1. The large leftward shift of the repaglinide curve upon coexpression is striking, and Tables 1 and 2 show that, in the presence of Kir6.2, the $K_i$ for binding of repaglinide to SUR1 was decreased (i.e., affinity increased) by a factor of $10^{2.1}/H^{1011}$ (see Hansen et al., 2005; Stephan et al., 2006). An even larger shift was determined for the other piperidino-glucinide, AZ-DF265 ($370/H^{1003}$; Tables 1 and 2). The $pK_i$ values of all insulinotropes are compared in Fig. 4a. With the exception of the two piperidino-glucinides, all values fell on a straight line with slope 1 and at a distance of 0.52 from the line of identity (Table 3). This means that coexpression with Kir6.2 decreased $K_i$ for binding of insulinotropes to SUR1 by the factor of $10^{0.52}/H^{1005}$, the exception being the piperidino-glucinides.

The extension of these observations to the SUR2 subtypes is hampered by their low affinity for glibenclamide, which leads to high (about 80%) levels of nonspecific binding in the binding experiments. Therefore, we used the mutation Y1206S, which increases the affinity of SUR2 for glibenclamide sufficiently to allow quantitative work (Hambrock et al., 2001; Stephan et al., 2005). Figure 4b compares the $pK_i$...
TABLE 2
Inhibition of [3H]glibenclamide binding to recombinant KATP channel subtypes by insulinotropes

<table>
<thead>
<tr>
<th>Insulinotrope</th>
<th>pK&lt;sub&gt;i&lt;/sub&gt; (Kir6.2/SUR1)</th>
<th>pK&lt;sub&gt;i&lt;/sub&gt; (Kir6.2/SUR2A)</th>
<th>pK&lt;sub&gt;i&lt;/sub&gt; (Kir6.1/SUR2B)</th>
<th>pK&lt;sub&gt;i&lt;/sub&gt; (Kir6.2/SUR2A(Y5))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glibenclamide</td>
<td>9.35 ± 0.08</td>
<td>8.21 ± 0.02</td>
<td>7.49 ± 0.04</td>
<td>9.28 ± 0.06</td>
</tr>
<tr>
<td>Glimepiride</td>
<td>9.24 ± 0.02</td>
<td>7.98 ± 0.02</td>
<td>7.15 ± 0.02</td>
<td>8.16 ± 0.05</td>
</tr>
<tr>
<td>Gliclazide</td>
<td>8.51 ± 0.05</td>
<td>6.93 ± 0.02</td>
<td>6.06 ± 0.04</td>
<td>8.19 ± 0.02</td>
</tr>
<tr>
<td>Glibornuride</td>
<td>6.16 ± 0.06</td>
<td>5.13 ± 0.01</td>
<td>4.14 ± 0.04</td>
<td>6.20 ± 0.07</td>
</tr>
<tr>
<td>Nateglinide</td>
<td>6.44 ± 0.02</td>
<td>5.17 ± 0.02</td>
<td>5.11 ± 10</td>
<td>6.28 ± 0.04</td>
</tr>
<tr>
<td>Meglitinide</td>
<td>5.50 ± 0.03</td>
<td>6.08 ± 0.05</td>
<td>5.67 ± 0.06</td>
<td>5.53 ± 0.05</td>
</tr>
<tr>
<td>UL-DF 9</td>
<td>4.38 ± 0.01</td>
<td>5.52 ± 0.11</td>
<td>5.06 ± 0.03</td>
<td>4.67 ± 0.05</td>
</tr>
<tr>
<td>Repaglinide</td>
<td>9.14 ± 0.04</td>
<td>8.81 ± 0.02</td>
<td>7.81 ± 0.03</td>
<td>9.08 ± 0.02</td>
</tr>
<tr>
<td>AZ-DF 265</td>
<td>8.43 ± 0.06</td>
<td>7.60 ± 0.02</td>
<td>6.55 ± 0.05</td>
<td>7.37 ± 0.05</td>
</tr>
</tbody>
</table>

For repaglinide and AZ-DF, the decrease was 56- and 30-fold (Fig. 3, middle; Tables 1 and 2), respectively, i.e., 2 and 12× less than in the case of SUR1. The affinity of UL-DF was little affected by coexpression. For SUR2B(Y5), coexpression with Kir6.1 decreased the K<sub>i</sub> values of all compounds 2.6×, and the special effect of coexpression on the piperidino-glucoses was no longer observed (Fig. 3, bottom; Table 3).

In addition, the selectivity of the insulinotropes in binding to the SUR subtypes expressed alone was examined. Comparing SUR1 and SUR2B(Y5), it was found that the A- and A + B-ligands were, in the mean, 3.5-fold selective for SUR1 (Table 3). In contrast, UL-DF showed a 3-fold selectivity for SUR2B(Y5), whereas meglitinide and the piperidino-glucoses showed no preference (Table 1). Very similar results were obtained for the comparison of SUR1 with SUR2B(Y5). Table 3 also shows that there was no selectivity in binding of the insulinotropes to mutants SUR2A and 2B.

The Mutation Y1206S in SUR2. Figure 5a compares binding to the wild-type and the mutant myocardial channel. The pK<sub>i</sub> values of A- and A + B-ligands were on a line with slope 1 shifted leftwards from the line of identity by 1.12 U. This showed that the mutation decreased the K<sub>i</sub> values of the A- and A + B-ligands ~13-fold (Table 3). Of the B-ligands, the K<sub>i</sub> values of the piperidino-glucoses remained essentially unchanged; in contrast, the K<sub>i</sub> values of meglitinide and UL-DF were increased (i.e., affinity decreased) 3.5- and 7.1-fold, respectively (Fig. 5a; Table 2). Similar results were obtained for the mutation of the vascular channel. In the mean, K<sub>i</sub> values of the A- and A + B-ligands were decreased ~19× (Table 3), whereas those of the B-ligands (with the exception of repaglinide) were slightly increased by the mutation (~4×; Table 2).

It was also examined whether the insulinotropes discriminated between the mutant cardiovascular channels, Kir6.2/SUR2A(Y5) and Kir6.1/SUR2B(Y5). Correlation analysis showed that the two piperidino-glucoses were ~20-fold, and the other compounds 2.6-fold selective for the myocardial channel (Table 3; P < 0.05).

Subtype-Selective Binding to Wild-Type Channels. Figure 5b compares binding to the pancreatic and myocardial channels. The pK<sub>i</sub> values of the A- and A + B-ligands were on a line shifted rightwards from the line of identity by 1.29 U (Table 3), showing that these compounds were ~7× pancreas- and UL-DF ~14× cardioselective.
The B-ligands were differentially affected by coexpression; meglitinide and UL-DF resembled the A- and A + B-ligands, whereas for the piperidino-glinides, the effect was large for Kir6.2/SUR1 (also see Hansen et al., 2005) and smaller for the other combinations. This peculiar behavior depends on the piperidino-substituent, and it makes a major contribution to the selectivity of repaglinide (and AZ-DF) for the pancreatic over the cardiovascular KATP channels (Stephan et al., 2006). With the amino terminus of Kir6.2 being part of the B-site, one might speculate that it either interacts directly with the piperidino group or that it induces a conformation in a subcompartment of the B-site that is able to interact with this group.

It is also of interest to consider the differences between coexpression of SURx with Kir6.1 versus Kir6.2. There was no difference in insulino trope binding to SUR2A(YS) and SUR2B(YS); however, with the respective mutant channels, Kir6.2/SUR2A(YS) versus Kir6.1/SUR2B(YS), the insulino tropes were 2.6-fold selective for the cardiac channel with the exception of the piperidino-glulides, which were ~20-fold selective (see above). This shows that with respect to insu linotrope binding, Kir6.2 couples more efficiently to SUR2 than Kir6.1 and that the piperidino-glulides are exquisitely sensitive to this difference.

The Mutation SUR2(Y1206S). The mutation of Ser1237 in the A-site of SUR1 to Tyr (the corresponding amino acid in SUR2) abolishes both high-affinity binding of glibenclamide to SUR1 and high-affinity channel closure by the A-ligands, tolbutamide and nateglinide (Ashfield et al., 1999; Chachin et al., 2003); however, channel closure by the B-ligands, meglitinide and repaglinide, remained unaffected (Ashfield et al., 1999; Hansen et al., 2002). The dramatic effect of this mutation in SUR1 contrasts with the moderate effects of the inverse mutation Y1206S in SUR2, which increases the binding affinity of the myocardial and vascular channels for A- and A + B-ligands 13 and 19×, respectively, and the sensitivity of the cardiovascular channels to block by gliben clamide ~30× (Stephan et al., 2005). Obviously, in SUR1, Ser1237 is essential for the ability of the A-site to interact with A-ligands, whereas these ligands bind to the SUR2 containing channels at still reasonable concentrations (Table 2). Regarding the A-site of SUR2, amino acids other than Tyr1206 (which weakens the interaction with the A-ligands) contribute to the site to allow binding, and, for all SURs,
binding to the A-site is enhanced by the presence of the amino terminus of Kir6.2 (see above).

Surprisingly, the mutation Y1206S produced also (moderate) effects on the binding of B-ligands. The most striking case was UL-DF, whose affinity for the myocardial channel was decreased seven times. As another example, the mutation reduced the preference of most insulinotropes (including UL-DF and meglitinide) for the myocardial over the vascular channel from $\sim 6$ to $2.6 \times$ but increased that of the piperidino-glulindines to $20 \times$. Two conclusions may be drawn. First, the mutation in the A-site of SUR2 seemingly affects the conformation of the binding pocket as a whole (i.e., A + B) or the A-site including the region where the two sites overlap. Second, and as noted above for the effect of coexpression, the heterogeneous response of the B-ligands to the mutation shows that the B-site has two subcompartments, one occupied by all B-ligands and an additional subcompartment for the piperidino moiety of repaglinide and AZ-DF. This is in contrast to the A- and A + B-ligands, which were homogeneous in all aspects. Collectively, the results show that the bipartite nature of the insulinotrope binding site, established for SUR1, extends also to SUR2.

**Subtype Selectivity.** Previous electrophysiological, radioligand binding, and photoaffinity labeling studies suggest that the interaction of an insulinotrope with the A-site of SURx determines the subtype specificity, whereas the B-site is similar in all Kir6.2/SURx subtypes and is, therefore, permissive (Ashfield et al., 1999; Proks et al., 2002; Vila-Carriles et al., 2007). In this study, the dominant role of the A-site for selectivity is highlighted by the comparison of A + B-ligands with their cognate B-ligands, i.e., the pairs glibenclamide-meglitinide and gliquidone-UL-DF. The $K_i$ ratios of the two pairs were $\sim 10,000$ at the pancreatic but $<100$ at the cardiovascular channels. Table 2 shows that this decrease is caused mainly by high selectivity of the A + B-ligand for pancreatic channel, i.e., by the differential interaction of the long sulfonylureas with the A-site of SURx, which, in turn, hinges mostly on the change from Ser1237 in SUR1 to Tyr1206 in SUR2 (Ashfield et al., 1999). Regarding the permissive nature of the B-site, the results of this study show that the B-site can afford (moderate) subtype selectivity as shown by the following examples: comparing binding to the pancreatic and myocardial channels, selectivity ranged from $\sim 14 \times$ cardio- (UL-DF) to $\sim 7 \times$ pancreas-selective (AZ-DF), and AZ-DF bound with $70 \times$ higher affinity to the pancreatic than to the vascular channel (Table 2).

**Binding and Effect.** The A- (and A + B-ligands) were $\sim 19\text{-fold}$ selective for binding to the pancreatic as compared with the myocardial and $\sim 76\text{-fold}$ with the vascular $K_{ATP}$ channel. These values are much lower than those determined in electrophysiological studies, in particular for the A-ligands. The concentrations of e.g., tolbutamide (Gribble et al., 1998), gliclazide (Gribble and Ashcroft, 1999), and nateglinide (Chachin et al., 2003) required to inhibit the recombinant cardiac and/or smooth muscle channels are very similar to

![Fig. 5. Correlation analyses. a, comparison of insulinotrope binding to the wild-type and the mutant myocardial channel to show the effect of the mutation SUR2(Y1206S). b and c, comparison of the binding affinities of insulinotropes for $K_{ATP}$ channel subtypes. b, Kir6.2/SUR2A on Kir6.2/ SUR1; c, Kir6.1/SUR2B on Kir6.2/SUR1. $pK_i$ values are given in Table 2, numbering of compounds in Table 1; the results of correlation analysis are listed in Table 3.](https://jpet.aspetjournals.org)
that those block the channel by binding to the pore (which is formed by Kir6.2). Hence, it is impossible to decide whether it is the binding of these A-ligands to SUR2 or to Kir6.2 that closes the channel, and it may well be that binding to SUR2 is not transduced into channel closure, i.e., that it is “non-productive.” A major difference in signal transduction between SUR1- and SUR2-containing channels lies in the differential effect of Mg$^{2+}$ salts of nucleotides, in particular MgADP, on the inhibitory action of sulfonamides; inhibition of Kir6.2/SUR1 channels is enhanced by nucleotides but that of Kir6.2/SUR2 channels unchanged or reduced (Gribble et al., 1998; Reimann et al., 2003). Conspicuously, this difference is paralleled by a 2× higher ATPase activity of the second nucleotide-binding domain of SUR1 as compared with that of SUR2A (Masia et al., 2005).

In conclusion, the results of this binding study show that the bipartite model of the sulfonylurea binding site established for Kir6.2/SUR1 extends also to Kir6.x/SUR2. The A-site of SUR dominates the subtype selectivity of sulfonamide binding, but the B-site can confer moderate selectivity to B-ligands. The B-site in SUR2 senses the mutation Y1206S in the A-site and the amino terminus of Kir6.2 (which is part of the B-site) may accept in part the functional groups present in all active sulfonamides, i.e., the central phenyl ring and the negative charge.

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