Interaction of the Sulfonylthiourea HMR 1833 with Sulfonylurea Receptors and Recombinant ATP-Sensitive K⁺ Channels: Comparison with Glibenclamide

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ABSTRACT

The novel sulfonylthiourea 1-[5-[2-(5-chloro-o-anisamido)ethyl]-2-methoxyphenyl]sulfonyl]-3-methyli thiourea (HMR 1883), a blocker of ATP-sensitive K⁺ channels (K_ATP channels), has potential against ischemia-induced arrhythmias. Here, the interaction of HMR 1883 with sulfonylurea receptor (SUR) subtypes and recombinant K_ATP channels is compared with that of the standard sulfonylurea, glibenclamide, in radioligand receptor binding and electrophysiological experiments. HMR 1883 and glibenclamide inhibited [³H]glibenclamide binding to SUR1 with Kᵢ values of 63 μM and 1.5 nM, and [³H]opener binding to SUR2A/2B with Kᵢ values of 14/44 μM and 0.5/2.8 μM, respectively (values at 1 mM MgATP). The interaction of HMR 1883 with the SUR2 subtypes was more sensitive to inhibition by MgATP and MgADP than that of glibenclamide. In inside-out patches and in the absence of nucleotides, HMR 1883 inhibited the recombinant K_ATP channels from heart (Kir6.2/SUR2A) and nonvascular smooth muscle (Kir6.2/SUR2B) with IC₅₀ values of 0.38 and 1.2 μM, respectively; glibenclamide did not discriminate between these channels (IC₅₀ ~ 0.026 μM). In whole cells, the recombinant vascular K_ATP channel, Kir6.1/SUR2B, was inhibited by HMR 1883 and glibenclamide with IC₅₀ values of 5.3 and 0.043 μM, respectively. The data show that the sulfonylthiourea exhibits a selectivity profile quite different from that of glibenclamide with a major loss of affinity toward SUR1 and slight preference for SUR2A. The stronger inhibition by nucleotides of HMR 1883 binding to SUR2 (as compared with glibenclamide) makes the sulfonylthiourea an interesting tool for further investigation.

In severe ischemia and hypoxia, the ATP/ADP ratio decreases, thereby triggering the opening of K⁺ channels that are closed by intracellular ATP and opened by MgADP (Noma, 1983; Venkatesh et al., 1991; for review, see Gross and Auchampach, 1992). The opening of these ATP-sensitive K⁺ channels (K_ATP channels) clamps the cardiocyte at the potassium equilibrium potential and renders it nonexcitable. Whereas this may salvage ATP and preserve the structural integrity of the cell (Noma, 1983; Gross and Auchampach, 1992), it also increases the electrical heterogeneity of the heart and promotes reentry arrhythmias (Janse and Wit, 1989). In addition, the opening of K_ATP channels leads to the accumulation of extracellular K⁺ in the ischemic zone, depolarizes the cell, and induces cytotoxic Ca²⁺ entry (Wilde et al., 1990; Venkatesh et al., 1991). In accordance with these ideas, block of plasmalemmal K_ATP channels in the cardiac myocyte has been shown to protect against ischemia-induced ventricular fibrillation (for reviews, see Wilde, 1994; Gögelein et al., 1999).

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ABBREVIATIONS: HMR 1883, 1-[5-[2-(5-chloro-o-anisamido)ethyl]-2-methoxyphenyl]sulfonyl]-3-methyli thiourea; K_ATP channel, ATP-sensitive K⁺ channel; Kir, inwardly rectifying K⁺ channel; SUR, sulfonylurea receptor.
thiazolidinediones, glibenclamide, and the sulfonylurea blocker of the pancreatic β-cell channel, in several aspects (Fig. 1; see also Gögelein et al., 1999). Experiments in isolated tissues and in animals in vivo have shown that these modifications lead to an unusual pharmacological profile (for review, see Gögelein et al., 1999). In contrast to glibenclamide, HMR 1883 has only a weak potency at pancreatic β-cells but inhibits the K<sub>ATP</sub> channel in the sarcolemma of the cardiocyte with micromolar potency; at 10 μM, the compound does not affect coronary flow (Gögelein et al., 1998), and it does not inhibit the mitochondrial K<sub>ATP</sub> channel at 30 μM (Sato et al., 2000). However, little is known about the interaction of HMR 1883 with recombinant surface K<sub>ATP</sub> channels and sulfonylurea receptors (SURs).

Surface K<sub>ATP</sub> channels are composed of two types of subunits, inwardly rectifying K<sup>+</sup> channels (Kir6.x) and SURs (for reviews, see Ashcroft and Gribble, 1998; Aguilar-Bryan and Bryan, 1999). The Kir6.x subunits form the pore of the channel. SUR is a member of the ATP-binding cassette protein superfamily (Ashcroft and Gribble, 1998; Aguilar-Bryan and Bryan, 1999) and carries binding sites for nucleotides (Ueda et al., 1998), for the sulfonylureas (Aguilar-Bryan et al., 1995), and for the K<sub>ATP</sub> channel openers like pinacidil (Hambrock et al., 1998; Schwanstecher et al., 1999), and for the sulfonylurea blocker of the pancreatic β-cell channel, in several aspects (Fig. 1; see also Gögelein et al., 1999). Two genes code for the SUR subtypes. SUR1 is mainly found in pancreatic β-cells and in neurons (Aguilar-Bryan et al., 1995); SUR2 is in myocytes with one isoform (SUR2A) in skeletal and cardiac and the other (SUR2B) in smooth muscle (Inagaki et al., 1996; Isomoto et al., 1996). SUR1 has high affinity for the sulfonylureas and a low affinity for the SUR2A channel opens; the converse is true for the SUR2A isoforms (Hambrock et al., 1998; Schwanstecher et al., 1998; Dörzscher et al., 1999; Hambrock et al., 1999; Russ et al., 1999).

It was the aim of this study to investigate the interaction of HMR 1883 and glibenclamide with the recombinant K<sub>ATP</sub> channels and SURs in electrophysiological and radioligand binding studies; particular emphasis was given to the interaction with SUR2A and SUR2B. The results show that HMR 1883 exhibits a unique selectivity profile at the different SURs and that binding of HMR 1883 to SUR2 is more sensitive to inhibition by nucleotides than that of glibenclamide.

Materials and Methods

Cell Culture, Transfection, and Membrane Preparation. Human embryonic kidney 293 cells were cultured as described previously (Hambrock et al., 1998) in minimum essential medium containing glutamine and supplemented with 10% fetal bovine serum and 20 μg/ml gentamycin. Cells were transfected with the pcDNA 3.1 vector (Invitrogen, Karlsruhe, Germany) containing the coding sequence of rat SUR1, murine SUR2A, or murine SUR2B (GenBank accession numbers L40624 (Aguilar-Bryan et al., 1995), D86037, and D86038, respectively (Isomoto et al., 1996)). Cells stably transfected with these proteins were isolated in the presence of the antibiotic geneticin and expressed the different SUR subtypes at levels of 1.0 ± 0.2 pmol/mg of protein. For patch clamp experiments, cells were transiently transfected with SUR1+Kir6.2 (murine Kir6.2, D50581 (Yamada et al., 1997); murine Kir6.2, D50581 (Inagaki et al., 1995)) at a molar plasmid ratio of 1:1 using lipofectAMINE and OPTIMEM (Invitrogen) according to the manufacturer’s instructions. pEGFP-C1 vector (CLONTECH, Palo Alto, CA), encoding for green fluorescent protein, was added for easy identification of transfected cells (Russ et al., 1999). Cells were allowed to express transfected DNA for 48 h and were then used for electrophysiological experiments.

Membranes for binding studies were prepared from cells stably expressing SUR as described (Hambrock et al., 1998). Five days prior to membrane preparation, the antibiotic was withdrawn. Cells were harvested, centrifuged for 6 min at 500g and 37°C, and lysed by addition of ice-cold hypotonic buffer (5 ml per culture dish) containing 10 mM HEPES and 1 mM EGTA, pH 7.4. The lysate was centrifuged at 105,000g and 4°C for 60 min. The resulting membrane pellet was resuspended in a buffer (5 mM HEPES, 5 mM KCl, and 139 mM NaCl, pH 7.4, at 4°C) at a protein concentration of ~1.5 to 3 mg of protein/ml and frozen at −80°C. Protein concentration was determined according to Lowry et al. (1951) using bovine serum albumin as the standard.

Radioligand Binding Experiments. Membranes (final protein concentration 0.1–0.5 mg of protein/ml) were added to the incubation buffer (139 mM NaCl, 5 mM KCl, 5 mM HEPES, and 2.2 mM MgCl<sub>2</sub>) supplemented with 1 mM Na<sub>a</sub>ATP, the radioligand ([<sup>3</sup>H]glibenclamide = 1.0–1.6 nM or [<sup>3</sup>H]P1075 = 1.5–3 nM), and the inhibitor of interest at 37°C. In case that an ATP-regenerating system was coupled, creatine kinase (5 U/ml) and creatine phosphate (3 mM) were added in the presence of 10 mM Mg<sup>2+</sup> as described in Hambrock et al. (1999). For low MgATP (3 μM), the incubation medium contained 3 μM ATP and 1 mM Mg<sup>2+</sup>. At equilibrium ([SUR1 + [<sup>3</sup>H]glibenclamide, 15 min; SUR2A + [<sup>3</sup>H]P1075, 13 min; and SUR2B + [<sup>3</sup>H]P1075, 30 min), incubation was stopped by diluting 0.3 ml aliquots in triplicate into 8 ml of quench solution (50 mM tris-(hydroxymethyl)-aminoethane and 154 mM NaCl, pH 7.4) at 0°C. The solution was filtered over Whatman GF/B filters and filters were washed twice with 8 ml of quench solution. Nonspecific binding was determined in the presence of 1 μM (unlabeled) glibenclamide (SUR1) or 10 μM P1075 (SUR2) and did not exceed 20% of total binding with the exception of the [<sup>3</sup>H]P1075 binding experiments in the presence of low ATP (3 μM) where it reached up to 40% of total binding.

Patch-Clamp Experiments. The patch-clamp technique was used in the whole-cell and inside-out configuration. Transfected human embryonic kidney 293 cells showing green fluorescent protein fluorescence were chosen. Patch pipettes were drawn from borosilicate glass capillaries (GC 150, Harvard Apparatus, Edenbridge, UK).

Fig. 1. Structures of glibenclamide and HMR 1883. In HMR 1883, the benzamido moiety of glibenclamide is retained; however, the sulfonylhthiourea part of HMR 1883 continues in the meta position of the central benzene ring. The pK<sub>a</sub> values of the nitrogen next to the sulfonyl group are 4.4 (HMR 1883) and 6.2 (glibenclamide), respectively (Gögelein et al., 1999).

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**Structure Images**

1. **glibenclamide**: The structure shows a benzamide moiety retained, with a sulfonylurea part continuing in the meta position of the central benzene ring. The pK<sub>a</sub> values for nitrogen next to the sulfonyl group are 4.4 (HMR 1883) and 6.2 (glibenclamide), respectively.
2. **HMR 1883**: The structure displays a unique selectivity profile at different SURs, with increased sensitivity to inhibition by nucleotides compared to glibenclamide.**
and heat polished using a horizontal microelectrode puller (Zeitz, Augsburg, Germany). For inside-out patches, bath and pipette were filled with a high K'-Ringer solution containing (in mM): KCl, 142; NaCl, 2.8; MgCl₂, 1; CaCl₂, 1; D(+)-glucose, 11; and HEPES, 10, titrated to pH 7.4 with NaOH at 22°C. After filling with buffer, pipettes had a resistance of 1 to 1.5 MΩ. After excision of the patch, the pipette was moved in front of a pipe with a high K'/H₂O²⁻ pipettes had a resistance of 1 to 1.5 MΩ. After excision of the patch, the pipette was moved in front of a pipe with a high K'-Ringer solution containing (in mM): KCl, 143; MgCl₂, 0.85; CaCl₂, 1; ethylene glycol-bis-(2-aminoethyl ether)-N,N,N',N'-tetraacetic acid, 5; D(+)-glucose, 11; and HEPES, 10, titrated to pH 7.2 with NaOH at 22°C. HMR 1883, glibenclamide, and ATP (free Mg²⁺ was kept constant) were dissolved as described below and added to the patch solution. Patches were clamped to −50 mV. All responses were normalized to the effect of 1 μM glibenclamide (100% block).

Experiments with the whole-cell configuration were performed as described by Russ et al. (1999). The bath solution was (in mM): NaCl, 142; KCl, 2.8; MgCl₂, 1; CaCl₂, 1; D(+)-glucose, 11; and HEPES, 10, titrated to pH 7.4 with NaOH at 37°C. Patch pipettes were filled with (in mM): K-glutamate, 132; NaCl, 10; MgCl₂, 2; HEPES, 10; ethylene glycol-bis-(2-aminoethyl ether)-N,N,N',N'-tetraacetic acid, 1; Li₂GDP, 1; and Na₂ATP, 0.3, titrated to pH 7.2 with NaOH and had a resistance of 3 to 5 MΩ. Cells were clamped at −60 mV.

Data were recorded with an EPC 9 amplifier (HEKA, Lambrecht, Germany) using the “Pulse” software (HEKA). Signals were filtered at 200 Hz using the four-pole Bessel filter of the EPC9 amplifier and sampled with 1 kHz.

Data Analysis. Concentration dependencies were analyzed by fitting the logistic form of the Hill equation,

\[
y = \frac{100}{(1 + 10^{x_{50}} \cdot pK - x_{50})}
\]

to the data. \( n = n_H \) is the Hill coefficient, \( x \) the concentration of the compound under study, and \( K \) is the midpoint of the curve with \( px = -\log x \) and \( pK = -\log K \). The dependence of the midpoint of an inhibition curve (IC₅₀ value) on the concentration of the radioligand, \( L \), was calculated according to the equation (Cheng and Prusoff, 1973),

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

where \( K_i \) is the inhibition constant and \( K_D \) the equilibrium dissociation constant of the radioligand.

Fits of the equations to the data were performed according to the method of least-squares using the programs FigP (Biosoft, Cambridge, UK) or SigmaPlot 4.01 (Statistical Product and Service Solutions Inc., IL, USA). Individual binding competition experiments were analyzed according to eq. 1. Amplitudes and \( pK \) values are normally distributed and were compared by the two-tailed unpaired Student’s \( t \) test. In the text, \( pK ± S.E.M. \) or \( K \) values with the 95% confidence interval in parentheses are given. Propagation of errors was taken into account.

Chemicals. [³H]P1075 (specific activity 4.5 TBq (117 Ci) mmol⁻¹) was purchased from Amersham Pharmacal Biotech (Freiburg, Germany) and [³H]glibenclamide (specific activity 1.85 TBq (50 Ci) mmol⁻¹) from DuPont NEN (Bad Homburg, Germany). The reagents and media used for cell culture and transfection were from Invitrogen (Eggenstein, Germany). Na₂ATP and Li₂GDP were from Roche Molecular Biochemicals (Mannheim, Germany); creatine kinase, creatine phosphate, glibenclamide, and noradrenaline from Sigma (Deisenhofen, Germany). HMR 1883 was the kind gift of Aventis (Frankfurt, Germany) and P1075 of Leo Pharmaceuticals (Ballerup, Denmark). KₐTP channel inhibitors were dissolved in dimethyl sulfoxide/ethanol (1:1) and further diluted with the same solvent or with incubation buffer. In binding studies, the final solvent concentration in the assays was always below 0.3%, in electrophysiological studies ≤ 0.1%.

Results

Binding of HMR 1883 and Glibenclamide to Recombinant SURs. The interaction of HMR 1883 and glibenclamide with SUR1 was studied in [³H]glibenclamide competition assays. In the presence of MgATP (1 mM), HMR 1883 inhibited specific [³H]glibenclamide binding completely and in a monophasic manner (\( n_H = 1 \)) with an inhibition constant (\( K_i \) value) of 63 μM (Table 1). The \( K_i \) value (\( = K_p \)) for glibenclamide was 1.5 nM. In the absence of MgATP, both inhibition curves were shifted to the left by a factor of about 7 (Table 1).

The SUR2 subtypes have a lower affinity for sulfonylureas than SUR1, rendering [³H]glibenclamide competition assays in membranes difficult (Dörsschner et al., 1999; Russ et al., 1999; Hambrock et al., 2001). Therefore, the interaction of the compounds with SUR2 was studied using the [³H]P1075 (Bray and Quast, 1992). The inhibition curves are presented in Fig. 2, and the parameters from the curve fits are summarized in Table 1. In the presence of 1 mM MgATP, HMR 1883 inhibited opening blocker to SUR2A with a \( K_i \) value of 14 μM; for glibenclamide, \( K_i \) was 0.5 μM. We had observed earlier that in a typical incubation solution containing nominally 1 mM MgATP/0 ADP, the ADP content had increased to ~100 μM at the end of the incubation period (Hambrock et al., 1999). Therefore, experiments were performed also in the presence of an ATP-regenerating system that reduces the ADP content by about 90% (Hambrock et al.,

TABLE 1

<table>
<thead>
<tr>
<th>SUR</th>
<th>Radioligand</th>
<th>Conditions</th>
<th>MgATP²</th>
<th>HMR 1883 ( K_i )</th>
<th>GBC ( K_i )</th>
<th>Selectivity² GBC/HMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUR1</td>
<td>[³H]GBC</td>
<td></td>
<td>1 mM</td>
<td>63 (59, 69)</td>
<td>0.0015 (0.0013, 0.0018)</td>
<td>4 × 10⁴</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>9.5 (7.9, 11.5)</td>
<td>0.00222 (0.00013, 0.00038)</td>
<td>4 × 10⁴</td>
</tr>
<tr>
<td>SUR2A</td>
<td>[³H]P1075</td>
<td></td>
<td>1 + ARS</td>
<td>4.4 (3.8, 5.0)</td>
<td>0.23 (0.22, 0.24)</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.003</td>
<td>1.8 (1.3, 2.5)</td>
<td>0.12 (0.10, 0.15)</td>
<td>15</td>
</tr>
<tr>
<td>SUR2B</td>
<td>[³H]P1075</td>
<td></td>
<td>1 + ARS</td>
<td>44 (36, 52)²</td>
<td>0.91 (0.89, 1.20)</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.003</td>
<td>12 (8.6, 18)</td>
<td>0.59 (0.47, 0.74)</td>
<td>7</td>
</tr>
</tbody>
</table>

² [³H]Glibenclamide concentration was = 1 nM; [³H]P1075, = 3 nM (SUR2A) or 2 nM (SUR2B). The \( K_i \) values of [³H]P1075 binding used for the Cheng-Prusoff correction were, respectively, for SUR2A (1 mM MgATP/1 mM MgATP + ATP-regenerating system)/5 μM MgATP: 15.1/12.9/17.4 μM and for SUR2H: 4.4/2.1/7.8 μM.

² ARS, ATP-regenerating system. For composition of solutions, see Fig. 2.

² Selectivity ratio = \( K_i (\text{HMR})/K_i (\text{GBC}) \).

² Hill coefficients were: \( n_H = 1.24 ± 0.01/1.35 ± 0.05 \) for 1 mM ATP/1 mM ATP + ARS, respectively; these values are significantly \( P < 0.01 \) different from 1.0.

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In this study we have compared the interactions of HMR 1883 and glibenclamide with different SURs and recombinant cardiovascular K\textsubscript{ATP} channels in smooth muscle is Kir6.2/SUR2B (Isomoto et al., 1996; Yamada et al., 1997). In inside-out patches, 1 \textmu M glibenclamide inhibited the ATP-sensitive current through the Kir6.2/SUR2B channel by 67\% (Fig. 3, lower panel), the glibenclamide-sensitive fraction of the current (mean, 54\% \pm 3\%; n = 49) was set to 100\% in each patch. HMR 1883 and glibenclamide inhibited the Kir6.2/SUR2A channel with IC\textsubscript{50} values of 0.38 and 0.026 \textmu M, respectively and Hill coefficients close to 1 (Table 2).

The recombinant form of the K\textsubscript{ATP} channel in nonvascular smooth muscle is Kir6.2/SUR2B (Isomoto et al., 1996; Yamada et al., 1997). In inside-out patches, 1 \textmu M glibenclamide inhibited the ATP-sensitive current through the Kir6.2/SUR2B channel by 67\% \pm 2\% (n = 66) and was renormalized as above. Figure 3 (bottom) shows that HMR 1883 and glibenclamide inhibited this current with IC\textsubscript{50} values of 1.2 and 0.027 \textmu M; surprisingly, the Hill coefficient of the HMR 1883 inhibition curve was significantly lower than 1 (0.6 \pm 0.1) (Fig. 3; Table 2). In contrast, glibenclamide did not discriminate between these channels and gave inhibition curves with Hill coefficients close to 1.

Attempts to measure the recombinant vascular K\textsubscript{ATP} channel, Kir6.1/SUR2B, in inside-out patches were unsuccessful. When the patches were drawn into solutions containing 3 \textmu M MgATP, i.e., conditions in which others observed channel activity (Satoh et al., 1998), we did not obtain measurable currents. Therefore, whole cell currents were measured. Upon dialysis of the cell with GDP (1 \textmu M) and ATP (0.3 \textmu M) in the presence of Mg\textsuperscript{2+}, an outward current developed that was blocked by HMR 1883 in a concentration-dependent manner (Fig. 4). Analysis of the concentration dependence gave an IC\textsubscript{50} value of 5.3 \textmu M and a Hill coefficient of 1.1 (Russ et al., 1999). The inhibition by either compound depended little on voltage.

**Discussion**

**Interaction with SUR1.** In this study we have compared the interactions of HMR 1883 and glibenclamide with different SURs and recombinant cardiovascular K\textsubscript{ATP} channels in the presence of 1 mM MgATP, HMR 1883 inhibited [\textsuperscript{3}H]P1075 binding with midpoint at 44 \textmu M and was about 15 times less potent than glibenclamide in this respect (Fig. 2; Table 1). Reduction of ADP by coupling of an ATP-regenerating system shifted the inhibition curve of HMR 1883 to the left by a factor of 2.2 and reduction of MgATP to 3 \textmu M by 10-fold; for glibenclamide, the maximum shift (reduction of MgATP) was \~5-fold. Interestingly, the Hill coefficients of the HMR 1883 inhibition curves in the presence of 1 mM MgATP were slightly but significantly higher than 1 (1.24 \pm 0.01 in the absence and 1.35 \pm 0.05 in the presence of the ATP-regenerating system, respectively; Table 1). In contrast, \textit{n}_H was close to 1 for HMR 1883 at 3 \textmu M MgATP and for all glibenclamide inhibition curves.

**Interaction with Recombinant K\textsubscript{ATP} Channels.** The inhibition of the recombinant cardiac K\textsubscript{ATP} channel Kir6.2/SUR2A by HMR 1883 and glibenclamide is shown in Fig. 3. The upper panel illustrates the current after patch excision into nucleotide-free solution, run down of the current, inhibition by MgATP, and refreshment upon washout of the nucleotide. HMR 1883 (10 \textmu M) inhibited the current by 57\%, and glibenclamide, at the saturating concentration of 1 \textmu M, by 67\%. For evaluation of the concentration-inhibition curves (Fig. 3, lower panel), the glibenclamide-sensitive fraction of the current (mean, 54\% \pm 3\%; n = 49) was set to 100\% in each patch. HMR 1883 and glibenclamide inhibited the Kir6.2/SUR2A channel with IC\textsubscript{50} values of 0.38 and 0.026 \textmu M, respectively and Hill coefficients close to 1 (Table 2).

In addition, experiments were performed at 3 \textmu M MgATP, the EC\textsubscript{50} value for activation of opener binding to SUR2 (Hambrock et al., 1998, 1999; Schwanstecher et al., 1998). Figure 2 and Table 1 show that under these conditions, the inhibition curves of HMR 1883 and glibenclamide were shifted increasingly to the left and that this shift was larger for HMR 1883. Decreasing the MgATP concentration from 1 mM to 3 \textmu M shifted the HMR 1883 inhibition curve leftward by a factor of 7.9 \pm 0.8 and that of glibenclamide by 4.2 \pm 0.4, and this difference is highly significant (P < 0.01, two-tailed \textit{t} test on \textit{p}_K values).

The interaction of the two compounds with SUR2B was investigated in the same manner as that with SUR2A. In the presence of 1 mM MgATP, HMR 1883 inhibited [\textsuperscript{3}H]P1075 binding with midpoint at 44 \textmu M and was about 15 times less potent than glibenclamide in this respect (Fig. 2; Table 1). Reduction of ADP by coupling of an ATP-regenerating system shifted the inhibition curve of HMR 1883 to the left by a factor of 2.2 and reduction of MgATP to 3 \textmu M by 10-fold; for glibenclamide, the maximum shift (reduction of MgATP) was \~5-fold. Interestingly, the Hill coefficients of the HMR 1883 inhibition curves in the presence of 1 mM MgATP were slightly but significantly higher than 1 (1.24 \pm 0.01 in the absence and 1.35 \pm 0.05 in the presence of the ATP-regenerating system, respectively; Table 1). In contrast, \textit{n}_H was close to 1 for HMR 1883 at 3 \textmu M MgATP and for all glibenclamide inhibition curves.

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Interaction of HMR 1883 with SURs and K<sub>ATP</sub> Channels

Fig. 3. Inhibition by HMR 1883 and glibenclamide of Kir6.2/SUR2A and Kir6.2/SUR2B currents in inside-out patches at −50 mV. Upper panel, original recording from a patch containing Kir6.2/SUR2A channels in symmetrical high K<sup>+</sup> buffer. After excision of the patch into nucleotide-free solution, the current was subject to rundown. Exposure to MgATP (1 mM) led to total inhibition (8-pA leak current indicated by the dotted line), and after washout, some refreshment was observed. Application of 10 μM HMR 1883 inhibited the current by 57% as indicated by the comparison of the lower line with the extrapolated current in the absence of inhibitor (upper line). After washout and application of MgATP (1 mM), 1 μM glibenclamide inhibited the current by 67%. Lower panel, concentration-inhibition curves of Kir6.2/SUR2A and 2B currents by glibenclamide, ntration-inhibition curves of Kir6.2/SUR2A and 2B currents by glibenclamide experiments, HMR 1883 was applied (lower line), and after washout, some refreshment was observed. Application of 1 mM MgATP (1 mM) led to total inhibition (8-pA leak current indicated by the dotted line), and after washout, some refreshment was observed. Application of 10 μM HMR 1883 inhibited the current by 57% as indicated by the comparison of the lower line with the extrapolated current in the absence of inhibitor (upper line). After washout and application of MgATP (1 mM), 1 μM glibenclamide inhibited the current by 67%. Lower panel, concentration-inhibition curves of Kir6.2/SUR2A and 2B currents by glibenclamide (n = 4–5) and HMR 1883 (n = 3–12). The glibenclamide (1 μM)-sensitive fraction of the ATP-inhibited current, I,<sub>ATP</sub>, was renormalized to 100%. Asterisks indicate significant difference between the block of Kir6.2/SUR2A and Kir6.2/SUR2B channels, respectively, by given concentrations of HMR 1883. For parameters of the Hill fit to the data, see Table 2.

The Hill coefficients of the HMR 1883 inhibition curves at SUR2B in the presence of high MgATP were significantly lower than the true binding affinity (K<sub>i</sub> lower than 10<sup>−8</sup>-fold; Russ et al., 1999), and this may be similar for SUR2A (Hambrock et al., 1999; Russ et al., 1999). Therefore, the following information can be extracted from the data in Table 1. The K<sub>i</sub> values of HMR 1883 in SUR2A- and SUR2B-containing membranes are 7- to 28-fold higher than those of glibenclamide, depending on the nucleotides present. First, this shows that glibenclamide binds to the two SUR2 subtypes with higher affinity than HMR 1883. Second, the interaction of the two compounds with SUR2 is differentially regulated by nucleotides. At both SUR2 subtypes, reduction of MgATP shifted the K<sub>i</sub> value of HMR 1883 significantly more toward the left (8–10-fold) than that of glibenclamide (~4-fold). Assuming that HMR 1883 binds to the sulfonylurea site of SUR, this result suggests that the negative allosteric coupling between the sulfonylurea site and the nucleotide binding sites of SUR2 is stronger when the sulfonylurea site is occupied by HMR 1883 than by glibenclamide. We have indeed observed that the MgATP shift of ligands to the sulfonylurea site on SUR2B is variable and can even change sign from a rightward shift (HMR 1883 > glibenclamide > phloxein B) to leftward (4,4-diisothiocyanatostilbene-2,2′-disulfonic acid) (Russ et al., 2000). In addition, there is a subtle difference between the SUR2 subtypes when the effect of MgADP reduction (presence of ATP-regenerating system) on the K<sub>i</sub> values of HMR 1883 is compared with that of ATP reduction: For SUR2A, MgADP is the more prominent negative allosteric regulator of HMR 1883 binding, whereas at SUR2B, MgATP is more important. For glibenclamide, such differences are not observed; both maneuvers affect the K<sub>i</sub> values of glibenclamide in a uniform manner. Obviously, these predictions need confirmation in electrophysiological experiments and such studies are underway.

The Hill coefficients of the HMR 1883 inhibition curves at SUR2B in the presence of high MgATP were slightly but significantly higher than 1 (Table 1). In principle, this could be due to the combination of the negative and positive allosteric interactions between the nucleotide-, opener-, and sulfonylurea-sites at SUR in the following way. Occupation of the sulfonylurea-site by HMR 1883 reduces [H]P1075 binding by the negative allosteric interaction between these two sites. In addition, it decreases ATP binding (Ueda et al., 1999), which, in turn, weakens opener binding since this depends on occupation of the nucleotide binding site (Ham-
HMR 1883 [data for glibenclamide from Russ et al. (1999); Schwanstecher et al., 1998). Since at
Kir6.2/SUR2A; however this was not the case. Therefore, additional
allosteric interactions between opener and binding sites on different subunits of

**Electrophysiological Experiments.** In inside-out patches and in the absence of nucleotides, HMR 1883 inhibited the recombiant cardiac (Kir6.2/SUR2A) and the nonvascular smooth muscle KATP channel (Kir6.2/SUR2B) with IC$_{50}$ values of 0.38 and 1.2 µM, respectively. In contrast, glibenclamide was more potent and did not discriminate between these channels (IC$_{50} = 26$–27 nM). Quantitatively, HMR 1883 was 15-fold weaker than glibenclamide at the SUR2A-containing channel and 44-fold weaker at the SUR2B-containing channel; in addition, the Kir6.2/SUR2B inhibition curve was surprisingly flat ($n_H = 0.6$). When the two compounds were compared at the recombinant vascular KATP channel, Kir6.1/SUR2B, in the whole cell configuration and in the presence of nucleotides, HMR 1883 was ~100-fold less potent than glibenclamide and the HMR 1883 inhibition curve was steep ($n_H = 1.7$).

The paradoxical observation that inhibition of the Kir6.2/SUR2B channel by HMR 1883 occurred with a Hill coefficient of 0.6 ± 0.2 as compared with a value of 1.7 ± 0.2 for the Kir6.1/SUR2B channel deserves comment. Part of the explanation for this difference may lie in the different experimental conditions (inside-out patch, nominally nucleotide-free, and 22°C in case of Kir6.2/SUR2B versus whole cell recording, high intracellular GDP/ATP and 37°C for Kir6.1/SUR2B); in particular the different nucleotide concentrations may again play an important role. Hill coefficients >1 for HMR 1883 but not for glibenclamide have also been observed by Göggelein et al. (1998) in various whole cell preparations or in intact tissues, this may reflect the stronger negative allosteric coupling between HMR 1883 and nucleotide binding at SUR2 (see above).

Although the results of the electrophysiological studies cannot be quantitatively compared with the binding studies (see above), the two approaches showed that under all conditions tested, HMR 1883 was markedly (7- to 100-fold) weaker than glibenclamide in its interaction with SUR2A or SUR2B.

**Selectivity of HMR 1883 for the Cardiac over the Vascular KATP Channel.** An important point for the intended therapeutic application of HMR 1883 is the selectivity of the compound for the cardiac over the vascular K$_{ATP}$ channel. The present data give only qualitative indications. From the $[^3]$H/P1075 inhibition experiments at high MgATP one obtains potency differences of 4.5 and 3.1 in the presence

**Table 2**

<table>
<thead>
<tr>
<th>Channel</th>
<th>Configuration</th>
<th>IC$_{50}$ (µM)</th>
<th>$n_H$</th>
<th>IC$_{50}$ (µM)</th>
<th>$n_H$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kir6.2/SUR2A</td>
<td>inside-out</td>
<td>0.38 (0.28, 0.51)</td>
<td>1.2 ± 0.2</td>
<td>0.026 (0.023, 0.029)</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Kir6.2/SUR2B</td>
<td>inside-out</td>
<td>1.2 (0.9, 1.6)</td>
<td>0.6 ± 0.1</td>
<td>0.027 (0.022, 0.032)</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Kir6.1/SUR2B</td>
<td>whole cell</td>
<td>5.3 (4.7, 6.0)</td>
<td>1.7 ± 0.2</td>
<td>0.043 (0.033, 0.055)</td>
<td>1.1 ± 0.2</td>
</tr>
</tbody>
</table>

*Experiments performed at 22°C.*
and absence of the ATP-regenerating system. In inside-out patches and in the absence of nucleotides, the potency difference of HMR 1883 inhibiting the Kir2.2/SUR2A (cardiac) and the Kir6.2/SUR2B (nonvascular smooth muscle) channel was 3-fold; however, due to the flat inhibition curve of the SUR2B-containing channel, up to 10 times higher concentrations of HMR 1883 are required to produce substantial (~80%) block. Since coexpression of SUR2B with Kir.6.x affects glibenclamide potency in a manner depending on the Kir6.x subtype (Hambrock et al., 2001), the situation may again be different when potency of the two blockers at Kir6.2/SUR2A channel is compared with the recombiant vascular KATP channel, Kir6.1/SUR2B. In any event, experiments in Langendorff-perfused rabbit hearts (Gögelein et al., 1998) and in conscious dogs with a healed myocardial infarction which were subjected to acute coronary artery occlusion (Billman et al., 1998) have shown that HMR 1883, at concentrations sufficient to close the cardiocyte channel, did not affect the channel in coronary myocytes. This indicates a sufficient selectivity of the compound in these more “realistic” models where the channel is in its native environment and in contact with physiological (or pathophysiological) levels of nucleotides.

In conclusion, we have shown here that the interaction of the novel sulfonylthiourea HMR 1883 with several KATP channels and SURs is very different from that of glibenclamide. The stronger inhibitory effect of nucleotides on HMR 1883 binding to the SUR2 subtypes as compared with glibenclamide might reflect a stronger negative allosteric coupling between the nucleotide binding and the sulfonylurea sites for HMR 1883, making this sulfonylthiourea an interesting tool for further studies.

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