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ATP-Sensitive K^+ Channel Modulator Binding to Sulfonylurea Receptors SUR2A and SUR2B: Opposite Effects of MgADP

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ABSTRACT

K_{ATP} channels are heteromeric complexes of inwardly rectifying K^+ channel subunits and sulfonylurea receptors (SURs). SUR2A and SUR2B, which differ within the carboxyl terminal exon 38, are characteristic for the cardiac and smooth muscle type channels, respectively. Here we compare binding of the tritiated K_{ATP} channel opener, [^{3}H]P1075, to membranes from human embryonic kidney (HEK) cells transfected with murine SUR2A and 2B at 37°C. Binding to both SURs required addition of Mg^{2+} and ATP in the low micromolar range. In the presence of MgATP, micromolar concentrations of MgADP, formed by the ATPase activity of the membrane preparation, increased binding to SUR2A but inhibited binding to SUR2B. Decreasing temperatures strongly reduced [^{3}H]P1075 binding to SUR2A, whereas binding to SUR2B was increased in a bell-shaped manner. Kinetic experiments revealed a faster dissociation of the [^{3}H]P1075-SUR2A complex, whereas the association rate constants for [^{3}H]P1075 binding to SUR2A and 2B were similar. Openers inhibited [^{3}H]P1075 binding to SUR2A with potencies ≈4 times lower than to SUR2B; in contrast, glibenclamide inhibited [^{3}H]P1075 binding to SUR2A ≈8 times more potently than to SUR2B. The data suggest that SUR2A and 2B represent the opener receptors of cardiac and vascular smooth muscle K_{ATP} channels, respectively, and show that MgADP is an important modulator of opener binding to SUR. The different carboxyl termini of SUR2A and 2B lead to differences in the MgADP dependence and the thermodynamics of [^{3}H]P1075 binding, as well as in the affinities for openers and glibenclamide, underlining the importance of this part of the molecule for K_{ATP} channel modulator binding.

ATP-sensitive K^+ channels (K_{ATP} channels), first discovered in the heart (Noma, 1983; Trube and Hescheler, 1984), link the membrane potential to the metabolic state of the cell as reflected by the levels of nucleoside triphosphates and diphosphates (Ashcroft and Ashcroft, 1990; Quast, 1996; Yokoshiki et al., 1998). K_{ATP} channels have been shown to be a heteromeric complex of pore-forming subunits, which belong to the class of inwardly rectifying K^+ channels (Kir6.x), and of sulfonylurea binding subunits (SURs) (Inagaki et al., 1995; Sakura et al., 1995; reviews: Ashcroft and Gribble, 1998; Babenko et al., 1998). SURs are members of the ATP binding cassette proteins (ABC proteins) and contain binding sites for sulfonylureas and nucleotides (Aguilar-Bryan et al., 1995; Inagaki et al., 1996; Isomoto et al., 1996). Based on multisequence alignments and hydropathy analyses, the classical topology of ABC proteins (i.e., 12 transmembrane helices with two intracellular nucleotide binding folds) has been proposed for the SURs with an extension of five transmembrane helices at the N terminus (Tusnády et al., 1997). Electrophysiological studies on recombinant K_{ATP} channels have shown that the SURs confer on the channel complex the sensitivity to the sulfonylureas, the openers, and the activating nucleotides and that they account for the major pharmacological differences between the K_{ATP} channels in various tissues (review: Babenko et al., 1998). The channel of the pancreatic β-cell contains SUR1 (Inagaki et al., 1995; Sakura et al., 1995); current evidence suggests that the SUR in heart and skeletal muscle is SUR2A (Inagaki et al., 1996) and that in smooth muscle it is SUR2B (Isomoto et al., 1996; Yamada et al., 1997). In the SURs cloned to date (human, rat, and murine) there are species differences in the amino acid sequence (Isomoto et al., 1996; Aguilar-Bryan et al., 1998). However, SUR2A and 2B of the same species are splice variants differing only within the last carboxyl terminal exon (Aguilar-Bryan et al., 1998); in the case of the murine SUR2 isoforms, this involves the last 42 amino acids (Isomoto et al., 1996).

Recent studies examining the binding of the tritiated K_{ATP} channel opener [^{3}H]P1075 ([^{3}H]-*N*-cyano-*N*′-(1,1-dimethyl-

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ABBREVIATIONS: ABC proteins, ATP binding cassette proteins; HEK cells, human embryonic kidney cells; K_{ATP} channel, ATP-sensitive K^+ channel; P1075, [^{3}H]-*N*-cyano-*N*′-(1,1-dimethylpropyl)-*N*″-3-pyridylguanidine); SUR, sulfonylurea receptor.

propyl)-*N*″-3-pyridylguanidine; Bray and Quast, 1992) to recombinant SUR2A (rat SUR2A, Schwanstecher et al., 1998) and SUR2B (murine SUR2B, Hambrock et al., 1998; human SUR2B, Schwanstecher et al., 1998) have provided strong support for the contention that these SURs are indeed the receptors for openers and sulfonylureas of the native K_{ATP} channels in cardiac and vascular smooth muscle. Binding studies with [^{3}H]P1075 to rat cardiocytes in culture (Lemoine et al., 1996) and to cardiac membranes from rat (Löffler-Walz and Quast, 1998) and dog (Atwal et al., 1998), as well as to rat aortic rings (Bray and Quast, 1992; Quast et al., 1993), have provided detailed information on the ligand binding properties of native cardiac and vascular K_{ATP} channels.

In this study we investigated [^{3}H]P1075 binding to membranes from human embryonic kidney (HEK)293 cells transfected with murine SUR2A and 2B. In agreement with other studies (Hambrock et al., 1998; Schwanstecher et al., 1998) we found that opener binding to SUR requires the presence of MgATP; the EC_{50} values were 5 μM (SUR2A) and 3 μM (SUR2B). HPLC analysis, however, showed, that most of the nucleotide (3 μM ATP) added to the solution had been hydrolyzed at the end of the incubation period. In addition, we show for the first time that MgADP, which opens the channel by interacting with SUR (Nichols et al., 1996; Satoh et al., 1998; reviews: Ashcroft and Gribble, 1998; Babenko et al., 1998), also affects opener binding to SUR2A and 2B, modulating it in opposite directions. Significant differences between the two SURs were also observed in the kinetics and thermodynamics of [^{3}H]P1075 binding and the modulator binding profile.

Experimental Procedures

Cell Culture, Transfection, and Membrane Preparation. HEK 293 cells were cultured in plastic dishes with a diameter of 9.4 cm at 37°C in a humidified atmosphere with 95% air and 5% CO_2 in minimum essential medium (MEM) containing glutamine and supplemented with 10% fetal bovine serum and 20 μg ml^{-1} gentamycin. At 60 to 80% confluence (10–16 million cells per dish), cells were transfected with the pcDNA 3.1 vector (Invitrogen, San Diego, CA) containing the coding sequence of murine SUR2A or murine SUR2B (GenBank accession numbers D86037 and D86038, respectively; Isomoto et al., 1996). Transfections were performed using lipofectAMINE and OPTIMEM (Life Technologies, Eggenstein, Germany) according to the manufacturer's instructions with 4 μg DNA and 25 μl lipofectAMINE per culture dish. Cells were allowed to express transfected DNA for 48 h. Control experiments were performed by omitting either DNA or lipofectAMINE. Isolation of stably transfected cells was achieved as described previously (Hambrock et al., 1998).

Membranes from control and from stably transfected cells were prepared at a confluence of 70 to 80% (13–16 million cells per dish). Cells were suspended by rinsing with medium and centrifuged for 6 min at 500 g at 4°C. The pelleted cells were lysed by addition of ice-cold hypotonic buffer (5 ml per culture dish) containing: 10 mM HEPES and 1 mM EGTA at pH 7.4. Cell rupture was assured by microscopy and the lysate centrifuged at 10^5 g and 4°C for 60 min. The resulting membrane pellet was resuspended in a buffer containing: 5 mM HEPES, 5 mM KCl, 139 mM NaCl, and 0 or 1 mM $MgCl_2$ (see below) at pH 7.4 and 4°C at a protein concentration of ≈ 3.0 mg ml^{-1} (SUR2A) or ≈ 1.5 mg ml^{-1} (SUR2B) and frozen at −80°C. Protein concentration was determined according to Lowry et al. (1951) using BSA as the standard.

Kinetics of [^{3}H]P1075 Binding. Membranes were thawed and homogenized with a polytron homogenizer for 2 × 5 s at 10^4 rpm at 4°C. To measure the association kinetics, membranes [final protein concentration: 200 μg ml^{-1} (SUR2A) and 50 μg ml^{-1}(SUR2B)] were added to the incubation buffer containing: 139 mM NaCl, 5 mM KCl, 5 mM HEPES, 3.8 mM $MgCl_2$, and 3 mM Na_2ATP, so that the concentration of free Mg^{2+} was 1 mM (see below) and were supplemented with [^{3}H]P1075 (2–5 nM) at 37°C. Aliquots (300 μl) were withdrawn at different times for separation of bound and free ligand by dilution into 8 ml of ice-cold quench solution (50 mM Tris and 154 mM NaCl, pH 7.4) and rapid filtration under vacuum over Whatman GF/B filters. Filters were washed twice with 8 ml of ice-cold quench solution and counted for ^{3}H in the presence of 6 ml of scintillant (Ultima Gold; Packard Instruments, Meriden, CT). Nonspecific binding was determined in the presence of 10 μM unlabeled P1075 and did not change with time. Because the label concentration (L) was in large excess over the concentrations of binding sites, the data were fitted to a single exponential as function of time (t),

$$B = B_{eq} * [1 - \exp(-k_{app} * t)] \quad \text{with}$$
$$k_{app} = k_+ * L + k_- = k_-(1 + L/K_D) \tag{1}$$

where B_{eq} denotes the concentration of the receptor-label complex at equilibrium and k_{app} the apparent rate constant of association. For a simple bimolecular association reaction, k_{app} depends on the rate constants of association and dissociation (k_+, k_-) and on the concentration of L as written in eq. 1 (Tallarida, 1995). $K_D = k_-/k_+$ is the equilibrium dissociation constant.

Dissociation was initiated by addition of P1075 (10 μM) to the receptor-label complex at equilibrium after incubation of the membrane preparation with [^{3}H]P1075 (1.5–3 nM) at 37°C for 10 min (SUR2A) or 30 min (SUR2B). Aliquots were then withdrawn to follow the dissociation kinetics, which were fitted to the equation of exponential decay,

$$B = B_{eq} * \exp(-k_- * t) \tag{2}$$

with B_{eq} and k_- defined as above.

Equilibrium Competition Experiments. Membranes (SUR2A: 150 μg protein ml^{-1}; SUR2B: 60 μg protein ml^{-1}) were added to the incubation buffer described above containing [^{3}H]P1075 (1.5–3 nM) and the inhibitor of interest in a total volume of 1 ml at pH 7.4 and 37°C. After equilibrium had been reached (SUR2A: 13 min; SUR2B: 30 min), incubation was stopped by diluting 0.3-ml aliquots in triplicate into 8 ml of ice-cold quench solution and filtrating as indicated above. Concentration dependencies were analyzed by fitting the logistic form of the Hill equation,

$$y = b + (a - b)/(1 + 10^{n * (px - pK)}). \tag{3}$$

Here, b denotes the starting level of the curve, a the level at saturation, so that a-b represents the extent of the effect (amplitude); n (=n_H) is the Hill coefficient, x the concentration of the compound under study and K the midpoint of the curve with px = −logx and pK = −log$K$. The dependence of the midpoint of an inhibition curve (IC_{50} value) on the concentration of the radioligand, L, was calculated according to the Cheng-Prusoff equation (Cheng and Prusoff, 1973),

$$IC_{50} = K_i * (1 + L/K_D) \tag{4}$$

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

To measure the dependence of [^{3}H]P1075 binding to SUR2A/2B on the free Mg^{2+} or the total ATP concentration, the concentrations of $MgCl_2$, EDTA, and Na_2ATP were adjusted as indicated in *Results*. Free Mg^{2+} concentrations in the presence of varying concentrations of ATP (added as Na_2ATP) and EDTA (1 mM) were calculated using a program written by Drs. T. Suzuki (Australian National University, Canberra, Australia) and U. Russ (University of Tübingen,

Germany) using the pK values and enthalpies of the MgATP and MgEDTA complexes compiled by Smith and Martell (1989).

Determination of Nucleotides. Adenine nucleotides were analyzed with HPLC using a Grom-Sil 120 ODS-3 CP column (5 μm, 125 × 4 mm i.d.; Sykam, München, Germany) and an UV detector (UVIS 200; Sykam) for absorbance recording at 254 nm. The column was eluted at 30°C with a flow rate of 1 ml min^{-1} and a low pressure gradient. Eluent A consisted of 65 mM potassium phosphate, pH 4.6, and 5 mM tetrabutylammonium sulfate as ion-pair forming agent. Solvent B was solvent A + 40% (v/v) acetonitrile. The mobile phase was kept at 100% solvent A for 3 min after injection of the sample (50 μl). After 4 min, a linear gradient was started to increase solvent B to 23% at 10 min and to 60% at 17 min; thereafter, solvent B was kept to 60% for 3 min. To reequilibrate the system, solvent A had to be kept at 100% for 8 min before the next sample injection. The chromatogram was completed within 30 min. AMP, ADP, and ATP were identified by their retention times (AMP, 5.8 min; ADP, 10.25 min; and ATP, 13.15 min) and quantified by peak area measurement by means of online computing integrator (AXXIOM chromatographic system 747; Sykam). Samples were prepared as described for the equilibrium binding studies. Incubation was stopped by filtration using FP 030/30 filters (Schleicher & Schuell, Dassel, Germany) and the filtrate stored immediately at −80°C for HPLC analysis.

Data Analysis. Fits of the equations to the data were performed according to the method of least-squares using the FigP program (Biosoft, Cambridge, UK). Errors in the parameters derived from the fit to a single curve were estimated using the univariate approximation (Draper and Smith, 1981) and assuming that amplitudes and pK values are normally distributed. 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 according to Bevington (1969).

Materials. [^{3}H]P1075 (specific activity 121 Ci mmol^{-1}) was purchased from Amersham Buchler (Braunschweig, Germany). The reagents and media used for cell culture and transfection were purchased from Life Technologies. Na_2ATP was purchased from Boehringer Mannheim (Mannheim, Germany); EDTA and UTP were purchased from Fluka (Deisenhofen, Germany); and creatine phosphate, creatine kinase, phosphoenol pyruvate, and pyruvate kinase were purchased from Sigma (Deisenhofen, Germany). Acetonitril and tetrabutylammonium sulfate (HPLC grade) were obtained from Merck (Darmstadt, Germany). The following drugs were kind gifts of the pharmaceutical companies indicated in parentheses: aprikalim (Rhône-Poulenc Rorer, Paris, France), AZ-DF 265 (4-[[*N*-(α-phenyl-2-piperidino-benzyl) carbamoyl]methyl] benzoic acid (Thomae, Biberach, Germany), diazoxide (Essex Pharma, München, Germany), levcromakalim (SmithKline-Beecham, Harlow, UK), nicorandil (Chugai, Tokyo, Japan), P1075 (*N*-cyano-*N*′-(1,1-dimethylpropyl)-*N*″-3-pyridylguanidine; Leo Pharmaceuticals, Ballerup, Denmark). Minoxidil sulfate and the active enantiomer of pinacidil ((−)pinacidil) were synthesized by Dr. W. P. Manley (Novartis, Basel, Switzerland). Glibenclamide was from Sigma. K_{ATP} channel modulators were dissolved in ethanol and dimethyl sulfoxide (1:1) and further diluted with the same solvent or with incubation buffer; the final solvent concentration in the assays was always below 0.3%.

Results

Kinetic Experiments. The association and dissociation kinetics of [^{3}H]P1075 binding at 37°C to membranes from HEK cells transfected with SUR2A and 2B are illustrated in Fig. 1. With SUR2A, the kinetics at 1.9 nM [^{3}H]P1075 were fast and close to the resolution limit of the filtration assay (fastest sampling *rate* ≈30 s per point). The rate constant of dissociation, k_-, was determined to 0.61 ± 0.01 min^{-1}, corresponding to a half-time ($T_{1/2}$) of 1.1 min; the apparent rate constant of association, k_{app}, at 1.9 nM [^{3}H]P1075 was 0.67 ± 0.03 min^{-1}. Assuming a one-step bimolecular binding mechanism, the rate constant of association, k_+, is calculated according to eq. 1 to (3.2 ± 1.6)*10^7 M^{-1} min^{-1}, where the large error in this value follows from the laws of error propagation (Bevington, 1969). From these kinetic values, K_D is calculated to 19 ± 10 nM, a value in excellent agreement with the K_i value of 17 nM determined in equilibrium competition experiments (see Fig. 7 and Table 2. Figure 1A shows that after 10 min, [^{3}H]P1075 binding to SUR2A started to decline, and, after 30 min, tended to plateau at ≈80%. Attempts to stabilize binding by coupling of ATP-regenerating systems like creatine kinase (20 U ml^{-1}) and creatine phosphate (10 mM) or pyruvate kinase (20 U ml^{-1}) and phosphoenol pyruvate (10 mM) in the presence of 13 mM Mg^{2+} did not improve stability, whereas in the presence of other nucleotides like UTP or ATPγS (3 mM, instead of ATP), stability decreased (data not shown). For equilibrium experiments, incubation was stopped after 13 min when binding was still at its maximum; in view of the rapid kinetics ($T_{1/2}$ = 1.1 min), equilibrium was reached.

The kinetics of [^{3}H]P1075 binding to SUR2B were considerably slower and binding was more stable (Fig. 1B). k_- was determined to 0.070 ± 0.002 min^{-1}, corresponding to $T_{1/2}$ = 10 min and, at 4.8 nM radiolabel, k_{app} was 0.221 ± 0.006 min^{-1}. Applying eq. 1, k_+ was calculated to (3.1 ± 0.1)*10^7 M^{-1} min$^-$, i.e., identical with the estimate for SUR2A. From these values, a K_D value of 2.2 ± 0.2 nM was calculated in good agreement with that determined from saturation bind-

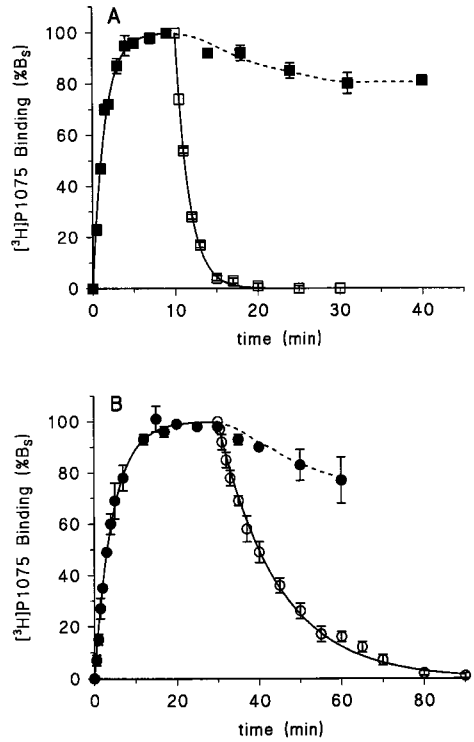

Fig. 1. Kinetics of [^{3}H]P1075 binding to membranes from HEK cells transfected with SUR2A (A) and SUR2B (B) at 37°C. A, ■, association of [^{3}H]P1075 (1.9 nM) with SUR2A and □, dissociation of the complex, induced by addition of 10 μM unlabeled P1075 after 10 min. B, ●, association of [^{3}H]P1075 (4.8 nM) with SUR2B and ○, dissociation of the complex, addition of 10 μM unlabeled P1075 after 30 min. Note different time scales in A and B. Fit of monoexponential kinetics to data (n = 3) gave for k_{app} 0.67 ± 0.03/0.221 ± 0.006 min^{-1} and for k_- 0.61 ± 0.01/0.070 ± 0.002 min^{-1} for SUR2A/2B, respectively. Data are normalized to percentage of specific binding (B_s) at maximum, which was 57 ± 4/230 ± 10 fmol mg protein^{-1} for SUR2A/2B, respectively.

ing and homologous competition experiments (3.4 nM at 1 mM $[Mg^{2+}]_{free}$; Hambrock et al., 1998). According to the slower kinetics observed with SUR2B, incubation time for equilibrium experiments was set to 30 min.

ATP Saturation Curves. Figure 2A shows the dependence of [^{3}H]P1075 binding to SUR2A on [ATP] at a total Mg^{2+} concentration of 2.2 mM. ATP supported binding with an EC_{50} value of 5 μM and Hill coefficient of 1. This value was 20 times lower than that found for the ATP dependence of [^{3}H]P1075 binding in rat cardiac membranes (EC_{50} = 100 μM; Löffler-Walz and Quast, 1998); however, the latter experiments had to be performed in the presence of an ATP-regenerating system to assure continued presence of ATP in spite of a high nucleotidase activity of the preparation (Löffler-Walz and Quast, 1998; see also Dickinson et al., 1997). Initially, we used the system also employed with cardiac membranes (Löffler-Walz and Quast, 1998), which consisted of creatine phosphate (20 mM), creatine kinase (50 U ml^{-1}), and Mg^{2+} (25 mM) in the presence of 20 mM HEPES to preserve pH (Stryer, 1995). Later experiments showed that with SUR_2 this could be reduced to creatine phosphate (3 mM), creatine kinase (5 U ml^{-1}), Mg^{2+} (10 mM) and 10 mM HEPES, and these concentrations were used routinely. Adding these components to the incubation solution, the ATP-dependence of [^{3}H]P1075 binding to SUR2A was shifted from 5 to 110 μM, a value similar to that observed in cardiac membranes. Control experiments showed that neither high Mg^{2+}, nor creatine phosphate, nor the enzyme alone had any effect and that only the three components together produced the rightward shift of the ATP activation curve (not illustrated).

Figure 2B shows similar experiments performed with SUR2B. At 2.2 mM total Mg^{2+} and in the absence of the ATP-regenerating system, ATP enabled [^{3}H]P1075 binding with an EC_{50} value of 3 μM and Hill coefficient of 1 reaching a plateau normalized to 100% (binding at 1 mM ATP). Increasing [ATP] (added as Na_2ATP) beyond 1 mM led to a further increase in binding up to 166%. When ATP was added as MgATP, the second phase was not observed (Fig. 2B). This indicated that the increase in binding at [Na_2ATP] >1 mM was due to the increasing depletion of the free Mg^{2+} concentration ($[Mg^{2+}]_{free}$) by ATP. In the presence of the ATP-regenerating system, the midpoint of the activation curve remained unchanged; however, the amplitude increased to 180% (n = 4), i.e., to a value similar to that reached before by depletion of $[Mg^{2+}]_{free}$ (Fig. 2B).

MgADP Dependence. It was thought that the effects of the ATP-regenerating system reflected the depletion of ADP. This hypothesis was tested in experiments performed at 30 μM ATP, a concentration at which [^{3}H]P1075 binding to both isoforms of SUR2 is essentially saturated under control conditions but where coupling of the ATP-regenerating system should produce a major effect (Fig. 2). Indeed, adding the ATP-regenerating system depressed binding to SUR2A to 45% but increased binding to SUR2B to 188% of control (Fig. 3). Addition of 1 mM ADP reversed the effects of the ATP-regenerating system and brought binding back to control values (Fig. 3). Analogous experiments were performed using the phosphoenolpyruvate (3 mM)/pyruvate kinase (5 U ml^{-1}) system (Mg^{2+}= 10 mM). Coupling of this system produced effects similar to those obtained with the creatine-based system; again these changes were reversed by addition of ADP (n = 3; not shown). These experiments clearly show that it is

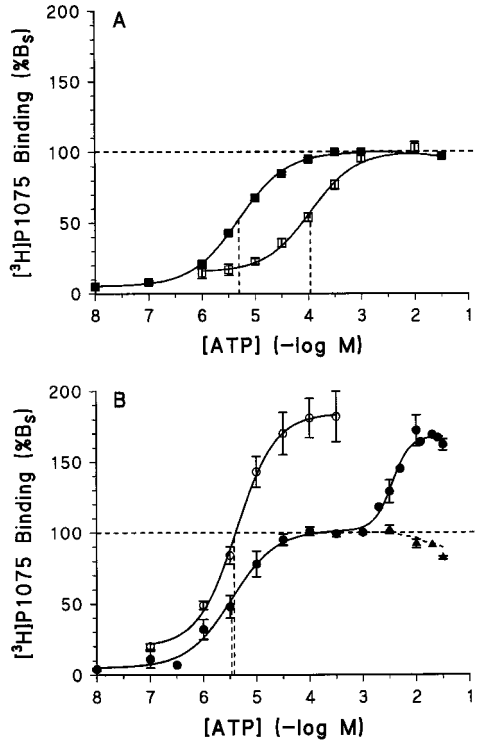

Fig. 2. Dependence of [^{3}H]P1075 binding to SUR2A and 2B on total ATP concentration, $[ATP]_o$, and effect of an ATP-regenerating system. ATP was added as Na_2ATP. A, SUR2A: in absence (■)/presence (□) of an ATP-regenerating system, ATP activated binding to SUR2A with pEC_{50} values of 5.30 ± 0.01/3.96 ± 0.05 and Hill coefficients = 0.97 ± 0.03/1.03 ± 0.11, respectively. B, SUR2B: ●, absence of ATP-regenerating system: ATP activated binding with pEC_{50} = 5.47 ± 0.08 and n_H = 1.04 ± 0.18; at ATP >1 mM, binding increased further to a final level of 166 ± 4%. ▲, Addition of ATP as MgATP. ○, Presence of ATP-regenerating system. ATP activated binding with pEC_{50} = 5.37 ± 0.04; amplitude = 183 ± 10%; n_H = 1.18 ± 0.11. Membranes (SUR2A/2B) were incubated with [^{3}H]P1075 (2.5/1.5 nM) for 13/30 min at 37°C in presence of 2.2 mM Mg^{2+}(n = 4 for each point). The ATP- regenerating system consisted of creatine phosphate (3 mM), creatine kinase (5 U ml^{-1}), Mg^{2+} (10 mM) in presence of 10 mM HEPES to buffer pH against activity of the ATP-regenerating system that consumes 1 proton/cycle (Stryer, 1995). Data were normalized with respect to (specific) binding at 1 mM ATP, which was 58 ± 3/150 ± 50 fmol mg $protein^{-1}$ for SUR2A/SUR2B, respectively.

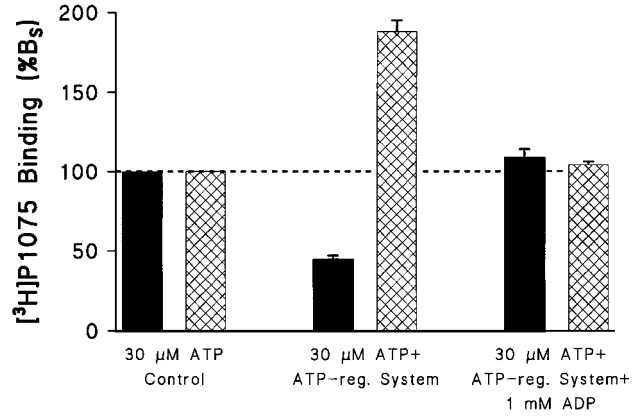

Fig. 3. Reversal of effects of ATP-regenerating system by ADP. ATP-regenerating system was: creatine phosphate (3 mM), creatine kinase (5 U ml^{-1}), and Mg^{2+} (10 mM). Binding after addition of 30 μM ATP under control conditions was 48 ± 3 fmol mg $protein^{-1}$ and 120 ± 15 fmol mg $protein^{-1}$ at 2.5 and 1.5 nM [^{3}H]P1075 for SUR2A (solid bars) and SUR2B (crosshatched bars), respectively.

depletion of ADP by the ATP-regenerating systems that produced the observed effects.

Because ADP was recognized as a modulator of [^{3}H]P1075 binding, it was of interest to determine its concentration at the end of the incubation period. HPLC analysis (Table 1) showed that stock solutions of ATP (3000, 1000, 30, and 3 μM), adjusted to pH 7.4 in the absence of membranes and incubated at 37°C for 13 or 30 min (i.e., the incubation times of SUR2A and 2B), contained <1% ADP. In the presence of membranes (SUR2A, 150 μg protein ml^{-1}; SUR2B, 60 μg protein ml^{-1}) and at 1000 and 3000 μM ATP, ADP at the end of incubation was increased by 5 to 15 times ranging from 58 to 92 μM. From these data the ATPase rate of the two membrane preparations was calculated to approximately 40 μM min^{-1} (mg protein ml^{-1})$^{-1}$; a similar ATPase rate was obtained with membranes from nontransfected HEK cells. In the presence of 1000 μM ATP, coupling of the ATP-regenerating system reduced ADP by 20 (SUR2A) and 4 times (SUR2B); addition of 1 mM ADP approximately restored the original ADP levels (Table 1).

Figure 4 shows the effect of ADP on [^{3}H]P1075 binding to SUR2A and 2B in the presence of 1 mM ATP and high $[Mg^{2+}]_{free}$ (≈1 mM). Binding to SUR2B decreased strongly with increasing ADP from 200% at the lowest ADP concentration attainable (13 μM; coupling of the ATP-regenerating system, Table 1) to 40% in the presence of mM ADP (data normalized with respect to binding without exogenous ADP). Due to the ATPase activity of the membrane preparation low ADP concentrations are difficult to control and this part of the concentration curve could not be completed. However, applying the Law of Mass Action to the data, one estimates a midpoint of 13 μM (95% confidence intervals: 7,24) and a maximum binding in the absence of MgADP of 340 ± 35% (not shown). In case of SUR2A, MgADP increased binding by 40 ± 3% with an EC_{50} value of 340 μM (95% confidence intervals: 257,446).

TABLE 1

Determination of adenine nucleotides in incubation samples

Samples were incubated for 13 min (SUR2A) or 30 min (SUR2B) min at 37°C in absence or presence of SUR2A (150 μg membrane protein ml^{-1}) or SUR2B (60 μg membrane protein ml^{-1}), the ATP-regenerating (reg.) system (creatine phosphate, 3 mM; creatine kinase, 5 U ml^{-1}; Mg^{2+}, 10 mM; HEPES, 10 mM) and ADP (1 mM). Each sample was prepared at least three times and analyzed three to five times.

ATP	SUR	ATP-reg.	1 mM ADP	ATP	ADP	AMP
μM				μM	μM	μM
3000	–			3030 ± 160	15 ± 1	N.D.
	2A			3010 ± 195	92 ± 7	N.D.
	2B			3055 ± 170	77 ± 8	N.D.
1000	–			950 ± 0	5 ± 1	N.D.
	2A			951 ± 61	86 ± 1	N.D.
		+		1140 ± 50	4.4 ± 0.3	N.D.
		+	+	2040 ± 20	89 ± 4	N.D.
	2B			1115 ± 75	58 ± 5	N.D.
		+		990 ± 20	13 ± 2	N.D.
		+	+	1790 ± 40	110 ± 4	N.D.
30	–			28 ± 3	0.22 ± 0.03	N.D.
	2A			12 ± 1	13 ± 1	7.5 ± 1.0
		+		36 ± 5	0.6 ± 0.1	N.D.
		+	+	940 ± 50	6 ± 1	N.D.
	2B			20 ± 1	10 ± 1	5.4 ± 1.1
		+		28 ± 2	0.6 ± 0.1	N.D.
		+	+	1025 ± 15	8.6 ± 0.3	N.D.
3	–			3.2 ± 0.4	N.D.	N.D.
	2A			N.D.	0.3 ± 0.1	3.0 ± 0.3
	2B			0.20 ± 0.01	0.5 ± 0.1	1.5 ± 0.2

N.D., not detectable.

The nucleotide composition after addition of 30 μM ATP was analyzed to complement the binding data shown in Fig. 3. In the presence of SUR2A and 2B, respectively, ATP was converted by 50 and 30% into ADP (13 and 10 μM) and AMP (7 and 6 μM). Coupling of the ATP-regenerating system reduced ADP by more than 10 times below 1 μM and addition of ADP (1 mM) restored the ADP concentration again approximately to the levels present in the absence of the ATP-regenerating system (Table 1). The nucleotide composition after addition of 3 μM ATP was also of interest, because this ATP concentration is close to the EC_{50} values of MgATP for activation of [^{3}H]P1075 binding (Fig. 2). Table 1 shows that at the end of equilibration time, little ATP (≤0.2 μM) and ADP (0.3 and 0.5 μM) were present and AMP was dominant.

Mg^{2+} Dependence. The dependence of [^{3}H]P1075 binding to SUR2A and 2B on $[Mg^{2+}]_{free}$ at saturating [ATP] (3 mM) is illustrated in Fig. 5. Both curves were biphasic. In either case, the first component of the curves was activatory with EC_{50} values of 0.8 μM (SUR2A) and 0.6 μM (SUR2B) and Hill coefficients (n_H) of 1. With SUR2A, the second component showed a further activation leading to more than a doubling of binding with EC_{50} ≈70 μM and n_H = 1. For SUR2B, the second component was inhibitory and binding decreased by about one-half with IC_{50} ≈170 μM and n_H = 1. Basal binding in the absence of Mg^{2+} was ≈10%, caused by contaminations of Na_2ATP with Mg^{2+} (Hambrock et al., 1998). In the case of SUR2B, the creatine-based ATP-regenerating system was coupled at saturating $[Mg^{2+}]_{free}$ (>3 mM). Under these conditions binding increased from 100 to 165 ± 2% (n = 4, data not illustrated). This showed that at saturating Mg^{2+} ADP is important and it suggested that the second component of the $[Mg^{2+}]_{free}$ dependence reflects changes in MgADP similar to those seen in Fig. 2. Similarly, one may speculate that the first component reflects changes in MgATP. Indeed, a replot of these data as function of

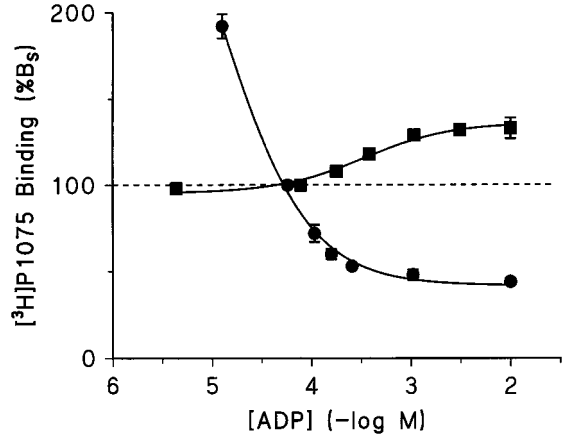

Fig. 4. Dependence of [^{3}H]P1075 binding to SUR2A (■) and 2B (●) on [ADP] in presence of 1 mM ATP and $[Mg^{2+}]_{free}$ ≈1 mM (n = 3–5). Data are normalized to 100% at standard conditions (1 mM ATP, 1 mM $[Mg^{2+}]_{free}$, no ADP added). First two ADP concentrations are from Table 1, other concentrations represent sum of endogenously formed + added ADP. Fits were performed according to eq. 3 with Hill coefficient = 1, giving for SUR2A/2B pK values of 3.47 ± 0.12/4.89 ± 0.10; starting levels (b) were 95 ± 2/340 ± 35%, and amplitudes (b-a) 41 ± 3/299 ± 34%, respectively (see text for details).

[MgATP] gave a regular concentration dependence for the first component with EC_{50} values of 42 ± 6 and 20 ± 3 μM for SUR2A and SUR2B, respectively; however, the second component of this plot was extremely steep and compressed into less than 1 order of magnitude (replot not shown).

The experiments described below were performed at 3 mM ATP and 3.8 mM Mg^{2+} ($[Mg^{2+}]_{free}$ ≈1 mM). Inspection of Figs. 2 and 4 shows that under these conditions the binding sites on SUR2A/2B for MgATP and MgADP are nearly saturated.

Temperature Dependence. Initial experiments at 0°C showed very low binding of [^{3}H]P1075 to SUR2A but good binding to SUR2B; at 37°C, however, binding to SUR2A was increased ≈10-fold and that to SUR2B was decreased by 30%. These observations prompted us to investigate the temperature dependence of binding in more detail. First, the association kinetics were measured at 24, 12, and 0°C to determine the appropriate incubation times (see legend to Fig. 6). Figure 5A shows that binding of [^{3}H]P1075 to SUR2A at equilibrium decreased continuously in the temperature range from 37 to 0°C to reach a level of 11 ± 2% at 0°C. In contrast, [^{3}H]P1075 binding to SUR2B exhibited a bell-shaped temperature dependence increasing by more than a factor of 2 at 24 and 12°C; at 0°C, binding was still 150% of that at 37°C.

Pharmacological Properties. [^{3}H]P1075 binding was inhibited by K_{ATP} channel openers and blockers with regular inhibition curves (Hill coefficient ≈1) reaching 100% with the exception of minoxidil sulfate where maximum inhibition was only 70%. Figure 7 illustrates the inhibition curves of the openers pinacidil, minoxidil sulfate, and diazoxide and of the inhibitor, glibenclamide, in SUR2A-containing membranes; the pK_i values of all compounds tested are listed in Table 2. The results for several channel modulators obtained in membranes with SUR2B have been published before (Hambrock et al., 1998); they are included in Table 2 together with additional values for pinacidil and nicorandil determined in this study. Also listed are the pK_i values of these compounds obtained against [^{3}H]P1075 in rat cardiac membranes (Löffler-Walz and Quast, 1998) and in rat aortic strips (Quast et al., 1993). The results of the correlation analysis are presented in Table 3. Excellent correlations were obtained comparing the potencies at SUR2A and SUR2B with those in heart membranes and rat aortic strips, respectively; in addition, slopes were near unity and the correlation lines were close to the line of identity. The comparison of opener potencies toward SUR2A with those toward SUR2B gave similar results concerning correlation coefficient and slope but showed that openers were on average 3.5 times more potent at SUR2B.

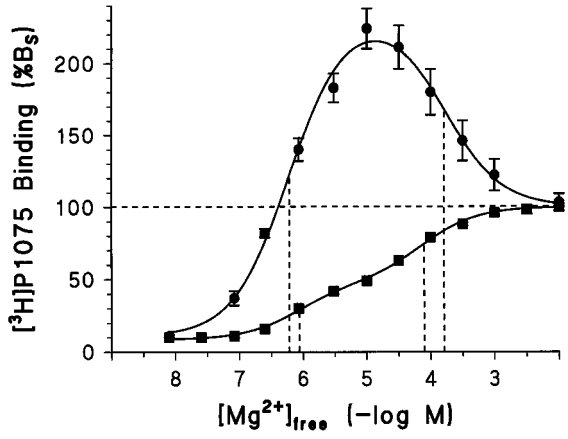

Fig. 5. Dependence of [^{3}H]P1075 binding to SUR2A (■) and 2B (●) on $[Mg^{2+}]_{free}$, in presence of 3 mM ATP and 1 mM EDTA (n = 4). Data are normalized to 100% at standard conditions (1 mM ATP, 1 mM $[Mg^{2+}]_{free}$) with absolute values given in Fig. 2. Logistic form of Hill equation with two components was fitted to data, giving for first component pEC_{50} values for SUR2A/2B of 6.08 ± 0.10/6.21 ± 0.06 and amplitudes (%) of 39 ± 3/224 ± 8; for second component, pEC_{50} values were 4.14 ± 0.06/3.77 ± 0.08 and amplitudes of 52 ± 3/-135 ± 7%.

Discussion

MgATP and [^{3}H]P1075 Binding to SUR2A and SUR2B. This study showed that addition of ATP in the

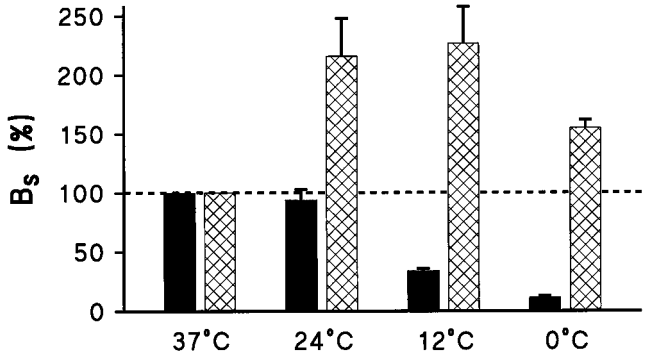

Fig. 6. Temperature dependence of [^{3}H]P1075 binding to SUR2A (solid bars) and SUR2B (cross-hatched bars). Specific binding (B_s) measured in presence of 2.8 nM [^{3}H]P1075 (SUR2A) or 4.5 nM [^{3}H]P1075 (SUR2B). Measurement of association kinetics indicated that following incubation times (minutes) were sufficient to reach equilibrium (SUR2A/2B): 37°C: 13/30; 24°C: 45/90; 12°C: 60/180; 0°C: 240 min for SUR2B. At 0°C, binding to SUR2A was too small to perform kinetic and homologous competition experiments and samples were incubated for 300 min. B_S is normalized with respect to values at 37°C, which were 55/243 fmol mg protein^{-1} for SUR2A/2B, respectively. Data are from three to four experiments.

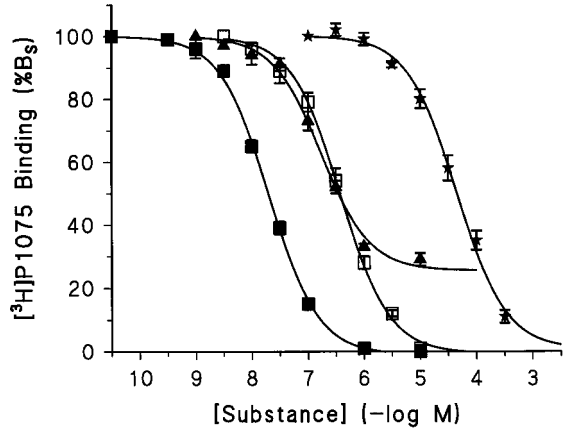

Fig. 7. Competition between [^{3}H]P1075 and selected K_{ATP} channel modulators binding to SUR2A. ■, P1075, ▲, minoxidil sulfate, □, glibenclamide, and ⋆, diazoxide. Data are means ± S.E.M. from four experiments. From fit of logistic equation to pooled data IC_{50} values of 19 nM (P1075), 166 nM (minoxidil sulfate), 370 nM (glibenclamide), and 46 μM (nicorandil) were calculated; Hill coefficients were close to 1. Note that maximum inhibition by minoxidil sulfate was only 73 ± 2%. mean pK_i values determined from fits to individual inhibition curves and corrected for presence of radiolabel are listed in Table 2. Inhibition of [^{3}H]P1075 binding was studied in presence of [^{3}H]P1075 (3 nM) and inhibitor of interest at 3 mM ATP and a free Mg^{2+} concentration of 1 mM; 100% (specific) binding corresponds to 63 ± 3 fmol mg protein^{-1} and nonspecific binding amounted to 29 ± 2% of total binding.

presence of mM Mg^{2+} enabled binding of the opener [^{3}H]P1075 to SUR2A and SUR2B with EC_{50} values of 5 and 3 μM, respectively (see also Hambrock et al., 1998; Schwanstecher et al., 1998). In addition, Schwanstecher et al. (1998) showed that nonhydrolyzable ATP-analogs do not support opener binding to SUR2B. The HPLC measurements performed in this study showed, however, that at the end of the incubation period, >90% of the 3 μM ATP originally present had been hydrolyzed and that this was due mostly to the ATPase activity of proteins other than SUR. Hence, the EC_{50} values for ATP determined here and elsewhere cannot be taken at face value. On the other hand, the ATPase rate of SUR is unknown and the nucleotides bound to SUR need not to be at equilibrium with the changing nucleotide composition of the incubation solution, in particular if the hydrolytic activity of SUR is low.

Effect of MgADP. A major new result of this study is the observation that, in the obligatory presence of MgATP, MgADP is an important modulator of [^{3}H]P1075 binding. At SUR2A, MgADP (<100 μM) shifted the MgATP dependence of binding toward the left by 20 times; higher concentrations increased binding. At SUR2B, MgADP inhibited opener binding by three- fourths. Several points deserve comment: First, the opposite direction of the MgADP effect at the two SUR2 isoforms (which only differ in their carboxyl terminus) suggests that the carboxyl terminus affects the MgADP binding site. Second, the inhibitory effect of MgADP on opener binding to SUR2B is intriguing because MgADP opens the vascular K_{ATP} channel (=nucleoside diphosphate-dependent K^+ channel, K_{NDP}; Beech et al., 1993) and the SUR2B/Kir6.1 construct (Satoh et al., 1998). Third, SUR2B, in the presence of 1 mM ATP, senses changes in the ADP concentration from 10 to 100 μM, suggesting that the nucleotide binding site that mediates the MgADP effects has a >10-fold selectivity for MgADP over MgATP. In this context it is of interest that on the multidrug resistance-associated protein, another ABC protein, a binding site with high selectivity for nucleoside diphosphates has been described that is distinct from the catalytic (nucleoside triphosphate) site (Chang et al., 1998). Occupation of this site stimulates the ATPase activity of the protein severalfold.

That Mg^{2+} is required for ADP to be effective is shown in Fig. 5, which illustrates the dependence of [^{3}H]P1075 binding to SUR2 on $[Mg^{2+}]_{free}$ at saturating [ATP]. The curves were biphasic and the experimental evidence suggests that the second component, starting at $[Mg^{2+}]_{free} \approx 10$ μM, represents the effect of MgADP. Indeed, at $[Mg^{2+}]_{free} > 10$ μM, one calculates [MgATP] >350 μM, which is saturating for [^{3}H]P1075 binding and sufficient to fuel the ATPase activity in the preparation, leading to appreciable amounts of ADP. The stability of the MgADP complex is 5 to 10 times weaker than that of MgATP (Smith and Martell, 1989), hence, it is essentially $[Mg^{2+}]_{free}$, which is limiting for formation of MgADP under the experimental conditions of Fig. 5. This gives rise to the second component of these curves with an apparent $EC_{50} \approx 100$ μM. In the cell, $[Mg^{2+}]_{free}$ has been determined to 0.5 to 1 mM; hence, Mg^{2+} is close to saturation under physiological conditions. As for the ADP levels, these are estimated to ≈100 μM in smooth muscle at rest (Butler and Davies, 1980; Taggart and Wray, 1998) and the MgADP site of SUR2B is saturated under physiological conditions. The free ADP concentration in cardiocytes at rest is ≈15 μM and reaches >100 μM in early hypoxia (Venkatesh et al., 1991); this is approximately the range over which MgADP exerts its effect on SUR2A.

Comparison of the binding data with the nucleotide measurements after addition of 30 μM ATP showed that at the end of the incubation period similar concentrations of MgADP and MgATP had developed and that MgADP strongly affected opener binding. Reduction of [MgADP] to ≈2% of [MgATP] by the ATP-regenerating system reversed the effect, supporting the estimate of a >10-fold selectivity of the MgADP site over MgATP. When the experiments were performed in the additional presence of 1 mM ADP, i.e., ATP (30 μM) + ADP (1 mM) + ATP- regenerating system, the nucleotide composition at the end of the incubation period was similar to that at 1 mM ATP in the presence of the ATP-regenerating system (ADP ≈10 μM, see Table 1). The binding result with SUR2B in Fig. 3 (last column) was, however, that of an MgADP-inhibited state which, at 1 mM ATP, requires the presence of 60 to 90 μM ADP. This showed again that the conformational state of the SUR lags behind the change in nucleotide composition of the solution (see above).

The effects of MgADP described here extend the earlier report of an inhibitory effect of $[Mg^{2+}]_{free} > 10$ μM on [^{3}H]P1075 binding to murine SUR2B where, however, additional factors like the ADP formed by the ATPase activity of SUR2B were not excluded (Hambrock et al., 1998). They are

TABLE 2

Inhibition of [^{3}H]P1075 binding by K_{ATP} channel modulators

Binding experiments with SUR2A were performed as shown in Fig. 7. Logistic equation (see *Experimental Procedures*) was fitted to individual competition curves (n = 4) yielding pIC_{50}, amplitudes (≈100% with exception of minoxidil sulphate, see below) and Hill coefficients n_H (≈1). pIC_{50} values were corrected for presence of the radiolabel according to equation of Cheng-Prusoff which, on logarithmic scale, corresponded to addition of 0.07. Data for SUR2B are from Hambrock et al. (1998) with exception of values for nicorandil and (−) pinacidil, which were determined in this study. pK_i values in rat heart microsomes are from Löffler-Walz and Quast (1998) with a Cheng-Prusoff correction of 0.06, and, those for rat aortic strips from Quast et al., 1993. Temperature was 37°C in all cases. In all four preparations, minoxidil sulfate inhibited [^{3}H]P1075 binding only by 68 to 75%.

K_{ATP} Channel Modulator	SUR2A	SUR2B	Rat Cardiac Membranes	Rat Aortic Strips
	pK_i	pK_i	pK_i	pK_i
P1075	7.78 ± 0.02	8.48 ± 0.07	8.14 ± 0.04	8.54 ± 0.03
(−)Pinacidil	7.11 ± 0.04	7.66 ± 0.02	7.03 ± 0.05	7.66 ± 0.06
Minoxidil sulfate	6.85 ± 0.05	7.28 ± 0.02	7.19 ± 0.08	7.45 ± 0.10
Levcromakalim	6.37 ± 0.04	6.95 ± 0.03	6.62 ± 0.03	7.33 ± 0.06
Aprikalim	5.92 ± 0.01	6.49 ± 0.08	5.92 ± 0.05	7.05 ± 0.05
Diazoxide	4.47 ± 0.07	5.11 ± 0.06	4.72 ± 0.05	4.66 ± 0.03
Nicorandil	4.73 ± 0.04	5.08 ± 0.06	5.03 ± 0.04	5.31 ± 0.06
Glibenclamide	6.50 ± 0.04	5.62 ± 0.05	7.12 ± 0.09	6.36 ± 0.04
AZ-DF 265	5.51 ± 0.04	5.20 ± 0.05	5.95 ± 0.11	5.95 ± 0.03

not necessarily in contradiction with the lack of effect of MgADP and MgGDP on P1075 binding to SUR2B reported by Schwanstecher et al. (1998). In their study, performed at room temperature and with human SUR2B, the MgADP site on SUR2B may have been saturated before the addition of nucleoside diphosphates.

Temperature Dependence. A surprising result of this study is the opposite temperature dependence of [^{3}H]P1075 binding to SUR2A and 2B; falling temperatures decreased binding to SUR2A monotonously but induced a bell-shaped increase in binding to SUR2B. Necessarily, these changes reflect the thermodynamics of the interaction of the opener and of the nucleotides with SUR and the temperature dependence of ADP formation. Lowering temperature will lead to a decrease in the amount of ADP formed and this may contribute to the observed decrease in binding to SUR2A and to the increase with SUR2B. A detailed interpretation of the data requires the direct measurement of nucleotide binding to SUR which, in turn, requires very high expression of the proteins.

Pharmacological Properties. Openers representative of the different chemical families of this class of drugs as well as glibenclamide inhibited [^{3}H]P1075 binding to murine SUR2A with Hill coefficient of 1 and to completion; the exception was minoxidil sulfate with only 73% inhibition. Similar observations had been made for minoxidil sulfate in membranes from HEK cells transfected with murine SUR2B (Hambrock et al., 1998), in cardiac membranes (Löffler-Walz and Quast, 1998), in A10 cells (a cell line derived from embryonic rat aorta; Russ et al., 1997), and in calf coronary myocytes (Lemoine et al., 1996). For human SUR2B expressed in COS cells, Schwanstecher et al. (1998) reported a biphasic inhibition curve with about equal amplitudes for the low- and the high-affinity component. These results have mostly been interpreted as reflecting heterogeneity of the otherwise homogeneous opener sites, although allosteric mechanisms were not ruled out (Hambrock et al., 1998; Löffler-Walz and Quast, 1998; Schwanstecher et al., 1998). Alternatively, minoxidil sulfate could transfer its sulfate group to a neighboring amino acid, thereby inhibiting further binding of the drug to the receptor site (W. P. Manley, personal communication); protein sulfation by minoxidil sulfate has been reported to occur easily (Meisheri et al., 1993).

The potencies (pK_i values) of the K_{ATP} channel modulators for binding to SUR2A are similar to those obtained in membranes from rat heart (Table 3). This suggests that SUR2A is the functionally relevant receptor for the openers in heart; however, the correlation of the binding data with electrophysiological studies in cardiocytes is not so clear (see also Löffler-Walz and Quast, 1998). Diazoxide has been accepted as the diagnostic compound to differentiate between vascular and cardiac K_{ATP} channels because it activates the native channel in the vasculature (Quast and Cook, 1989) and the recombinant channel SUR2B/Kir6.2 (Isomoto et al., 1996), but not the channel in the heart nor the recombinant channel SUR2A/Kir6.2 (Inagaki et al., 1996; Okuyama et al., 1998). In the binding experiments, however, diazoxide exhibits a regular competition pattern at SUR2B and 2A being only four times weaker at SUR2A (Table 1, see also Schwanstecher et al., 1998). Similarly, nicorandil is a rather selective opener of the vascular channel, with a 100-fold higher potency at the recombinant vascular channel, SUR2B/Kir6.2 than at SUR2A/Kir6.2 (Shindo et al., 1998) but only a 2-fold difference in binding. Taken together, these results raise questions concerning the relationship between binding of openers and activation of the cardiac channel.

In case of SUR2B, there is little doubt that this SUR is indeed the drug receptor for the openers in vascular smooth muscle. First, the pK_i values for binding to SUR2B are very similar to those obtained in rat aortic strips (Tables 2 and 3). Second, opener binding in rat aorta correlates very well with opener-induced vasorelaxation and channel opening (measured by $^{86}Rb^+$ efflux; Quast et al., 1993). Third, mutations in SUR2B affected opener binding and activation of mutant SUR2B/wild-type Kir6.2 channels in a similar way (Schwanstecher et al., 1998).

When opener binding to murine SUR2A is compared with that to murine SUR2B, an excellent correlation is obtained again (Table 3). In the mean, the openers bind 3.5 times weaker to SUR2A; glibenclamide, however, is about 7.6 times more potent than at SUR2B. Hence, it appears that the pharmacological profile of SUR2A is slightly shifted in the direction of SUR1, where the openers are very weak and glibenclamide is very potent (see e.g., Schwanstecher et al., 1998). This is paradoxical because the carboxyl terminus of SUR2B shows much higher homology to SUR1 than that of SUR2A (Isomoto et al., 1996; Inagaki et al., 1996; review: Aguilar-Bryan et al., 1998). These observations suggest that the carboxyl terminus is important for ligand binding to SUR, but that the remainder of the molecule plays a decisive part.

Conclusion. This study has shown that MgADP stimulates opener binding to SUR2A but inhibits binding to SUR2B. One may speculate that the carboxyl termini of these SURs fold back to affect the interaction of MgADP with its binding site. Finally, the results suggest that the carboxyl terminus may form part of the binding pockets for openers and glibenclamide; alternatively, it may affect these binding pockets allosterically. Further work, including mutational analyses of the SURs is required to decide between these possibilities.

TABLE 3

Comparison of K_{ATP} channel modulators in different preparations

Linear correlation analysis of the pK_i values listed in Table 2 gave correlation coefficients (r), slopes (s), and mean distance (d) from line of identity as listed below. s = 1 means that K_i values on the two axes are proportional to one another and 10^d gives factor by which, in mean, K_i values on ordinate differ from those on abscissa.

Abscissa	Ordinate	r	Slope	d
Rat cardiac membranes	SUR2A	0.98	0.98 ± 0.08	−0.28 ± 0.07
Rat aortic strips	SUR2B	0.95	0.96 ± 0.12	−0.27 ± 0.13
SUR2B	SUR2A	1.00	0.96 ± 0.04	−0.55 ± 0.04[a]

[a] Correlation analysis taking into account openers only.

Acknowledgments

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