Toward Understanding the Assembly and Structure of K\textsubscript{ATP} Channels

LYDIA AGUILAR-BRYAN, JOHN P. CLEMENT IV, GABRIELA GONZALEZ, KUMUD KUNJILWAR, ANDREY BABENKO, AND JOSEPH BRYAN

Departments of Cell Biology and Medicine, Baylor College of Medicine, Houston, Texas

I. INTRODUCTION

Adenosine 5'-triphosphate-sensitive potassium (K\textsubscript{ATP}) channels couple metabolic events to membrane electrical activity in a variety of cell types. The cloning and reconstitution of the subunits of these channels demonstrate they are heteromultimers of inwardly rectifying potassium channel subunits (K\textsubscript{IR}6.x) and sulfonylurea receptors (SUR), members of the ATP-binding cassette (ABC) superfamily. Recent studies indicate that SUR and K\textsubscript{IR}6.x associate with 1:1 stoichiometry to assemble a large tetrameric channel, (SUR/K\textsubscript{IR}6.x)\textsubscript{4}. The K\textsubscript{IR}6.x subunits form the channel pore, whereas SUR is required for activation and regulation. Two K\textsubscript{IR}6.x genes and two SUR genes have been identified, and combinations of subunits give rise to K\textsubscript{ATP} channel subtypes found in pancreatic \( b \)-cells, neurons, and cardiac, skeletal, and smooth muscle. Mutations in both the SUR1 and K\textsubscript{IR}6.2 genes have been shown to cause familial hyperinsulinism, indicating the importance of the pancreatic \( b \)-cell channel in the regulation of insulin secretion. The availability of cloned K\textsubscript{ATP} channel genes opens the way for characterization of this family of ion channels and identification of additional genetic defects.
opening of these channels sets the resting potential, and their closure allows the activity of other channels to depolarize the plasma membrane. β-Cells are relatively small cells with a high input impedance, and opening of a small number of K<sub>ATP</sub> channels will set their resting membrane potential. Mutations in the channel subunits have been shown to cause familial hyperinsulinism or persistent hyperinsulinemic hypoglycemia of infancy (PHHI). The phenotype of this disorder of newborns and infants, an inappropriately high rate of insulin secretion despite severe hypoglycemia, is the result of a loss of K<sub>ATP</sub> channel activity. This loss “resets” the resting membrane potential to a higher value that is within the “voltage window” for steady-state L-type Ca<sup>2+</sup> channel currents. Loss of K<sub>ATP</sub> channels thus causes a persistent Ca<sup>2+</sup> influx that maintains an increased intracellular Ca<sup>2+</sup> concentration that drives insulin secretion.

In cardiac and skeletal myocytes, and in vascular smooth muscle and neurons, the question of whether K<sub>ATP</sub> channels are active under normal physiological conditions remains controversial. However, it is accepted that K<sub>ATP</sub> channels can be opened, even in isolated, quiescent myocytes, by hypoxic or ischemic conditions, and their outward current is sufficient to reduce the duration of the action potential (13, 17, 39). This reduction slows the influx of Ca<sup>2+</sup>, decreasing the contractile force, which reduces the consumption of ATP, thus protecting the cell during periods of metabolic impairment. In smooth muscle cells, which like β-cells have a high input resistance, a small number of K<sub>ATP</sub> channels can influence vascular tone. In neurons, opening of K<sub>ATP</sub> channels reduces the frequency of action potentials, again serving a protective function.

Our cloning and reconstitution studies demonstrate K<sub>ATP</sub> channels are heteromultimers of two types of subunits (Fig. 1): a large subunit that binds sulfonylureas and ATP and belongs to the ATP-binding cassette (ABC) superfamily and a small subunit, which belongs to the inwardly rectifying potassium channel (K<sub>IR</sub>) superfamily. Beauty is said to lie in the eye of the beholder; this seems equally true for the subunits of K<sub>ATP</sub> channels. Some workers consider the K<sub>IR</sub> subunits to be of paramount importance, whereas others find the sulfonylurea receptors (SURs) more interesting and important. We stress that both subunits are required to form an active K<sub>ATP</sub> channel and that full understanding of this family of channels must include consideration of the important features of both subunits and requires integration of ideas from the ABC protein superfamily field, in addition to those from the potassium channel field. Various aspects of the physiology and electrophysiology of K<sub>ATP</sub> channel function have been reviewed extensively (1, 8, 15, 28, 36, 37, 69, 77, 80, 90, 92, 97, 103, 109). This review focuses on the assembly and structure of K<sub>ATP</sub> channels. This area, made possible by the cloning and reconstitution of channel subunits, is critical for understanding the molecular physiology of these channels. The story is incomplete, but we hope this review may serve as a pointer for further studies.

II. ADENOSINE 5’-TRIPHOSPHATE-SENSITIVE POTASSIUM CHANNEL GENES

A. Sulfonylurea Receptor Genes

Two SUR genes have been identified in rodents and humans. The more completely characterized human genes are discussed here (Figs. 2 and 3): SUR1 (OMIM 600509) (4) which specifies the high-affinity SUR (1581 or 1582
amino acids) found in pancreatic α-, β-, and δ-cells, brain, and other neuroendocrine cells, and SUR2 (OMIM 601439) (60, 63) which encodes two low-affinity receptors (1549 amino acids) that are thought to form K<sub>ATP</sub> channels in cardiac, skeletal, and vascular and nonvascular smooth muscle.

The human SUR1 gene, 39 exons spanning >100 kb of DNA, was localized to the short arm of chromosome 11, at 11p15.1 (107). This was informative, since the susceptibility gene for familial hyperinsulinism, or PHHI (OMIM 256450), had been localized to this region (50, 89, 106). Persistent hyperinsulinenemic hypoglycemia of infancy is characterized by an excessive release of insulin despite severe hypoglycemia. Analysis of a small set of PHHI families demonstrated that two mutations in SUR1 segregated with the disorder (105). Further studies have confirmed this finding (see the section on PHHI on the OMIM home page at the National Center for Biotechnology Information at http://www3.ncbi.nlm.nih.gov/Omim/ for the most recent progress in this area). Dunne et al. (32) have established that islet cells, taken from a patient with a known mutation in SUR1, lack active K<sub>ATP</sub> channels.

The human SUR2 gene, 38 exons spanning >100 kb of DNA, localizes to the short arm of chromosome 12 at 12p11.12 (18). To date, no disease(s) has been associated with this gene. The SUR2 gene specifies at least two main types of low-affinity receptors, designated SUR2A and SUR2B, which result from differential usage of two COOH-terminal exons as illustrated in Figures 2 and 3. In the human SUR2 gene, the two terminal exons are each 45 amino acids, and the exon order is A, B (Fig. 2). The two receptors are identical except for the terminal 45 residues (Fig. 3). The initial reports show that coexpression of SUR2A and SUR2B with mouse K<sub>IR6.2</sub> generates K<sub>ATP</sub> channels with differing pharmacological properties (60, 63). On the basis of these distinctions, SUR2A/K<sub>IR6.2</sub> is presumed to be the cardiac type channel, whereas the SUR2B/K<sub>IR6.2</sub> channel is presumed to be the vascular smooth muscle type.

Chutkow et al. (18) reported cloning two SUR2 cDNAs from rat and human libraries. The longer cDNA encodes a receptor (unfortunately designated “SUR2B”) that is nearly identical to the rat SUR2A sequence reported by Inagaki et al. (60). In keeping with the chronological order of expression and with the exon ordering in the genome, we equate this with SUR2A. The second cDNA described by these authors has the SUR2A COOH terminus, but has a deletion of exon 14 (marked as Δ14 in Fig. 2). This cDNA, which appears to be restricted to cardiac tissue, has not been expressed, and it is unknown whether it will form a functional channel. We propose to rename this splice variant SUR2AΔ14. Using reverse transcriptase-polymerase chain reaction (PCR), we have identified two additional minor RNAs in heart and skeletal muscle with deletions of exon 17 and exons 17 + 18 (marked as SUR2Δ17 and SUR2Δ17,18, respectively, in Fig. 2). It is not clear if the proteins produced from these
FIG. 3. Comparison of human SUR1 and SUR2 protein sequences. Positions of intron-exon boundaries are marked with solid bars. Eighteenth exon of SUR1, deleted in SUR2, is lightly shaded. Dashed positions are inserted gaps.
RNAs form functional channels or are physiologically significant. Similarly, full-length cDNAs have not been cloned, and it is not clear if the deletions are in SUR2A, SUR2B, or both RNAs. The combinatorial possibilities are intriguing and would increase the diversity of $K_{ATP}$ channel types, possibly providing tissue-specific channel subtypes of physiological and therapeutic importance.

Comparison of the intron-exon boundaries of the human SUR1 and SUR2 genes points to their evolutionary relationship (Fig. 3). The exon sizes are nearly identical. The apparent one exon difference, 39 for the SUR1 gene versus 38 for the SUR2 gene, results from the deletion of a small 36-bp exon, exon 18 in SUR1, from the SUR2 gene (Fig. 3). The terminal 45 amino acids of SUR2B more closely resemble the COOH terminus of SUR1. Interestingly, both the SUR1/KIR6.2 and SUR2B/KIR6.2 channels are activated by diazoxide, whereas the SUR2A/KIR6.2 channel is not.

B. KIR6.2 Genes

Two KIR genes have been identified that encode subunits that form $K_{ATP}$ channels when coexpressed with SURs. KIR6.1, also termed uKATP-1 since it was found to be widely (ubiquitously) distributed, was reported originally to be a $K_{ATP}$ channel in its own right (62), but this observation does not appear to be reproducible. Further work has shown that KIR6.1 can be activated by SUR1 (7, 19) and SUR2B, but not SUR2A (Bryan, Clement, and Aguilar-Bryan, unpublished data). Interestingly, the SUR1/ KIR6.1 channel is detectable by whole cell recording or $^{86}$Rb efflux methods but appears to “rundown” too rapidly in inside-out patches to be characterized at the single-channel level (53). The native partner for KIR6.1 has not been conclusively identified, although Yamada et al. (115) have suggested the SUR2B/KIR6.1 channel may be the ADP-sensitive conductance described originally (14, 64, 77, 118) in vascular smooth muscle. The ubiquitous distribution of this subunit suggests possible localization in blood vessels, or the more speculative possibility that KIR6.1 is part of the mitochondrial $K_{ATP}$ channel described by Garlid and colleagues (46–48, 88).

The KIR6.1 cDNAs were cloned originally by homology screening using a fragment of KIR3.4 [GIRK1/(66)]. The human gene encoding KIR6.1, designated KCNJ8, is ~9.7 kb in length with three exons. The encoded protein, 424 amino acids, is 98% identical to rat KIR6.1 (uKATP-1). KCNJ8 was mapped to chromosome 12p11.23 using fluorescence in situ hybridization (61). This is close to the SUR2 gene at 12p11.12, but the distance is too large for successful PCR as was done for SUR1 and KIR6.2.

The KIR6.2 cDNAs (OMIM 600937) were cloned by homology screening with KIR6.1 (59). The 390-amino acid protein, also referred to as BIR (for $\beta$-cell inward rectifier) has been shown to reconstitute $K_{ATP}$ channels when coexpressed with SUR1 (59) and with SUR2A (60) and SUR2B (63). The SUR1/KIR6.2 pairing has been confirmed (93). The intronless human KIR6.2 gene was mapped to the same region on chromosome 11 where SUR1 mapped. Additional PCR experiments showed that the KIR6.2 gene was ~4,500 bp 3′ of the SUR1 gene (1, 59). Recently, an 86-kb fragment of human genomic DNA from chromosome 11p14.3 has been put into the Genbank (Evans et al., accession no. U90583), which contains the entire sequence of human KIR6.2 and the 3′-end of SUR1. Mutations in KIR6.2 have also been shown to segregate with PHHI (78, 105). Coexpression of one mutation in the M2 segment of KIR6.2, L147P, described by Thomas et al. (105) with wild-type SUR1 failed to reconstitute active channels (Bryan et al., unpublished data). The result establishes that KIR6.2 is a component of the $\beta$-cell $K_{ATP}$ channel and confirms the reconstitution experiments, described in section IV, where SUR1 and KIR6.2 associate to form $\beta$-cell type $K_{ATP}$ channels. Both SUR2A and SUR2B have been reconstituted with KIR6.2; the pharmacological differences between the reconstituted channels suggest that the SUR2A/KIR6.2 pair forms the cardiac-like $K_{ATP}$ channel (60), whereas the SUR2B/KIR6.2 pair is a vascular smooth muscle-like channel (63). A major caveat to these conclusions is that it has not been established that KIR6.2 is the second subunit in these tissues.

These first members, SUR and KIR6.x, of the $K_{ATP}$ channel family have been available for only a short time. Intensive efforts are underway to screen for other possible members of the family, both receptors and inward-rectifier subunits. The proximity of the two pairs of SUR/ KIR genes strongly suggests the duplication of a gene pair that then diverged during evolution. This has led us to speculate on the existence of a primitive fused SUR-KIR gene that split to form the original gene pair. As described in section X, artificially fused recombinant proteins can form active channels. It will be interesting to discover whether there are additional SUR/KIR gene pairs and whether there is a modern day fused SUR-KIR gene.

III. HALLMARKS OF THE ADENOSINE 5′-TRIPHOSPHATE BINDING CASSETTE PROTEIN FAMILY

The SURs belong to a large family of proteins referred to as the ATP binding cassette or ABC transporter superfamily, which has a large number of member sequences in the DNA database specifying ABC proteins from diverse organisms, bacteria through human. The yeast genome project has identified 29 ABC proteins in this organism alone (23, 67, 74). Mutations in various members of the superfamily have been associated with human genetic disorders including cystic fibrosis, adrenoleukodystrophy,
and familial hyperinsulinism to name only a few (22, 23). The SURs are most closely related, by amino acid sequence similarity, to the largest members of the superfamily. Although they share sequence similarity with cystic fibrosis transconductance regulators (CFTRs) and multidrug resistance proteins (MDRs), SURs have the greatest similarity to members of the multidrug resistance-associated protein (MRP) branch of the ABC superfamily (20). In addition to MRPs and SURs, this subfamily includes other important transporters, including the yeast sodium transporter (101), the liver canicular multispecific organic anion transporter (16, 104), and the epithelial basolateral chloride conductance regulator (112). Several authors have used multisequence alignments to illustrate the similarities within the subfamily (74, 111).

The greatest similarities between the SURs and other ABC proteins are found within the nucleotide binding folds (NBFs; Fig. 1). The receptor NBFs have all of the hallmarks of the ABC family, including the Walker A (-GlyXXGlyXGlyLysSer/Thr, where X is any amino acid) and B (-YYYYAsp-, where Y is a hydrophobic amino acid) consensus motifs (113) and a conserved -LeuSerGlyGlyGlyGln- sequence. Manavalan et al. (73) have pointed out the sequence similarities between CFTR and G proteins. X-ray crystal structures for the three major classes of GTPases, the small p21ras-like proteins, the heterotrimeric G proteins, and elongation factor-Tu, indicate that the residues of the Walker A motif interact with magnesium ion, and with the oxygen atoms of the α- and β-phosphates (see Ref. 56 for a review). The Gln in the -LeuSerGlyGlyGlyn- sequence has been proposed to act as a general base during nucleotide hydrolysis, but this has been difficult to prove (55). A major question is whether SURs will hydrolyze nucleotides and how this hydrolysis might be coupled to the regulation of channel activity.

The exact topology of the SURs has not been determined. Both MDR (52) and CFTR (91) are characterized by a core or module consisting of a set of six transmembrane spanning domains preceding a NBF. Two cores are connected by a linker region to form the MDR prototype. The linker region appears to be quite variable. In CFTR, for example, the linker forms a regulatory “R” domain between the two cores (91). Phosphorylation of the R domain by adenosine 3’,5’-cyclic monophosphate-dependent protein kinase regulates the chloride channel activity of this protein. Simple hydrophobicity analysis (38) suggested that SUR1 had a similar general topology with added hydrophobic segments. The 13 transmembrane spanning domain topology shown in Figure 1 was proposed based on such analysis with two constraints: 1) that the glycosylated NH2 terminus was extracellular and 2) that the two NBFs were intracellular (4). The predicted topology was similar (9 + 4 vs. 8 + 4) to that proposed initially for MRP (20), which had a highly hydrophobic NH2-terminal extension that added additional transmembrane spanning domains to the canonical MDR topology. The SUR1 linker region has a highly acidic domain containing a stretch of glutamic acid residues. The topology of the MRP/SUR subfamily has not been tested experimentally. More extensive predictions based on multisequence alignments of new members of the MRP subfamily have suggested an alternative model that unifies the MDR, CFTR, and MRP/SUR topologies (111). On the basis of the glycosylation pattern of SUR1 and MRP, the NH2 terminus of the MRP/SUR subfamily is predicted to be extracellular. Comparison of the hydrophobicity profiles of the NH2-terminal extension predicts five additional transmembrane spanning domains for this subfamily (Fig. 1). We present both topologies in Figure 1 but have retained our original model in subsequent figures with the understanding that the actual topology remains to be determined experimentally.

Adenosine 5’-triphosphatase activity is a hallmark of the superfamily (see Refs. 6, 21, 26, 43, 45, 67, 91, 94, 98 for reviews). Hydrolysis is usually coupled to the transport of a substrate or, in the case of CFTR, to gating of the chloride channel and thus regulation of ion flux. Early studies on KATP channels using nonhydrolyzable ATP analogs showed inhibition of activity and led to the idea that nucleotide hydrolysis did not play a role in regulation of channel activity (for example, Refs. 9, 12, 29, 34, 70, 85). These observations were based on starting with active channels in freshly excised inside-out patches in ATP-free solutions, then observing that nonhydrolyzable analogs effectively blocked channel activity. Several pieces of information that could be interpreted as effects on ATP hydrolysis, including stimulation of KATP channel activity by vanadate and aluminum fluoride (30, 87) as well as “refreshment” (31) or “reactivation” (35, 42) of rundown channels, have been interpreted as evidence for involvement of G proteins (87), a requirement for phosphorylation (68, 82; but see Refs. 24, 44, 58 for another view), for depolymerization of the actin cytoskeleton (44), or for hydrolysis of phosphatidylinositol 4,5-bisphosphate (40). It remains unresolved which of these mechanisms is of physiological importance. Recent work with mutations in the NBFs of SUR1 suggests ATP hydrolysis will be involved in understanding, for example, the activation of an ATP inhibited channel by ADP (54, 81). A direct demonstration of ATP hydrolysis by an SUR will be a critical first step in understanding how this channel is regulated.

IV. HALLMARKS OF THE INWARDLY RECTIFYING POTASSIUM CHANNEL FAMILY

Sequence similarities suggested KIR6.1 and KIR6.2 were weakly rectifying members of the KIR superfamily. Sequence analysis predicted two transmembrane spanning domains, M1 and M2, flanking a “P” or pore loop
with a -GlyPheGly- sequence in place of the more common
-GlyTyrGly- motif that contributes to the potassium selectivity of other potassium channels (27) (Fig. 1).

The Kir channels, as their name implies, are inwardly rectifying channels that pass potassium better in the inward than outward direction. A major component of the inward rectification mechanism is due to blockade of the permeation path by polyamines and/or magnesium at voltages above the reversal potential, as illustrated schematically in Figure 4 (41, 71, 102; see Ref. 27 for a review).

The degree of rectification is dependent on the amino acids in the pore. Mutation of one residue in the M2 domain converts the weakly rectifying ROMK1 channel, for example, to a strong rectifier (25, 72, 102). Similarly, IRK1, a strong rectifier, can be changed to a weak one by making the D172N mutation in M2 (100, 102, 116). The equivalent residue in Kir6.2 is N160, and the N160D mutant exhibits strong rectification (Fig. 4), suggesting that M2 forms part of the permeation path of Kir6.2. A comparison of the human Kir6.1 and Kir6.2 sequences is shown in Figure 5.

V. SULFONYLUREA RECEPTORS 1 AND 2 HAVE DIFFERING AFFINITIES FOR SULFONYLUREAS

The β-cell SUR is identified by its ability to bind hypoglycemic agents like glibenclamide (nM range) and tolbutamide (μM range) with high affinity. This area has been reviewed extensively (1, 10, 11, 86). There is consensus that membranes isolated from pancreatic β-cells, various β-cell lines, other neuroendocrine cell lines, and brain contain high-affinity receptors with dissociation constant \( K_D \) values in the low nanomolar range (0.5–10 nM) and that the binding data are well fit by a single-site model (Hill coefficient of 1). Earlier measurements of binding constants and the half-maximal concentrations required to block \( K_{ATP} \) channel activity and stimulate insulin release from β-cells were in reasonable agreement for different sulfonyleureas, indicating the receptor was a physiologically important component of \( K_{ATP} \) channels (2, 84, 120). This conclusion was confirmed with the discovery that mutations in \( K_{ATP} \) channel subunits cause PHHI, as discussed above (1, 32, 33, 89, 105, 107, 108).

![Figure 4. Polyamines and inward rectification of ATP-sensitive potassium (\( K_{ATP} \)) channels. A: a schematic representation of mechanism of inward rectification based on “long-pore plugging” model of Lopatin et al. (71). Polyamines, like spermine, enter and bind in the pore, thus blocking flow of potassium ions. Affinity of polyamine binding depends on residues within pore. In Kir6.2, changing asparagine-160 to aspartic acid increases affinity and alters rectification properties as indicated in B. Following Lopatin et al. (71), 2 molecules of spermine are shown in the pore. B: current (I)-voltage (V) relationships for \( K_{ATP} \) channels reconstituted using hamster SUR1 and either wild-type Kir6.2 or Kir6.2 with N160D mutation. I-V data were obtained from excised patches by ramping holding potential from 0 to +180 mV, holding for 400 ms to allow spermine to enter pore, then rapidly ramping, 0.68 mV/ms, to 100 mV. Current was sampled every 0.2 ms during ramp; 10 recordings were then averaged. Comparison of I-V curves for SUR1/Kir6.2 versus SUR1/Kir6.2 N160D shows N160D mutation greatly reduces outward current if spermine is present (solid line, no spermine; dotted line, 10 μM spermine; dashed line, 100 μM spermine). In addition, a “bump” is obvious in SUR1/Kir6.2 N160D curves in the -50 to 0 mV range, which reflects longer time required for spermine to exit N160D channel. \( E_K \), potassium equilibrium potential.](http://physrev.physiology.org/)
Expression of rodent SUR1 cDNAs in COSm6 cells generated high-affinity binding with \( K_D \) values of 2 and 10 nM for the rat and hamster receptors, respectively (4) (see Fig. 6). The binding data for the recombinant receptors are well fit by a single binding site model (Hill coefficient of 1), and there is no evidence at present that endogenous COS cell factors are required for reconstitution of high-affinity binding. The reasons for stressing that single binding site models appear to account adequately for the interactions of SUR1 with sulfonylureas will become apparent below where the structure of KATP channels is proposed to be a tetramer of SUR/KIR. Figure 6 shows a comparison of the binding activity for \([^{125}I]iodomiglubinamide\) in membranes isolated from either COSm6 cells expressing the hamster SUR1 receptor or HIT T15 cells (3, 75). The \( K_D \) values, 9 versus 7 nM, are indistinguishable. In this example, the levels of expression differ by approximately a factor of 100, with a maximum binding value for the COS cells of 140 pmol receptor/mg membrane protein (4). \([^{[3]}H]glibenclamide\) (65), \([^{[125]}I]iodomiglubinamide\) (2), and azido-[\(^{[125]}I]\]iodoglibenclamide (95, 96) will photolabel the SUR1 receptor(s) when used at nanomolar concentrations. This property has proven to be a useful means to identify the high-affinity receptor (Fig. 6, inset).

Expression of rat SUR2A cDNA in COSm6 cells generated a low-affinity SUR with an estimated \( K_D \) of 10 \( \mu M \) (59). Figure 6 gives a comparison of iodo[glibenclamide binding activity for the SUR1 and SUR2A receptors. These results are in agreement with the general observation that higher concentrations of sulfonylureas are required to inhibit, for example, \( K_{ATP} \) channels in cardiac cells. Similarly, photolabeling of SUR2 is minimal using nanomolar concentrations of the iodo- or azido-iodo derivatives of glibenclamide (Fig. 6, inset).

VI. COEXPRESSION OF SULFONYLUREA RECEPTOR 1 AND INWARDLY RECTIFYING POTASSIUM CHANNEL 6.2 GENERATES \( \beta \)-CELL-TYPE ADENOSINE 5'-TRIPHOSPHATE-SENSITIVE POTASSIUM CHANNELS

Cloning and expression of the \( \beta \)-cell SUR in COSm6 cells demonstrated that SUR1 was sufficient to reconstitute high-affinity sulfonylurea binding, but not channel activity (4). The coexpression of SUR1 with \( K_{IR} \) generated potassium-selective channels that could be activated by metabolic poisoning or the potassium channel opener diazoxide, whereas expression of \( K_{IR} \) alone generated no channel activity (59). Whole cell channel activity for the hamster SUR1 and mouse \( K_{IR} \) channel was inhibited half-maximally by sulfonylureas like glibenclamide (1.875). The \( K_D \) values, 9 versus 7 nM, are indistinguishable. In this example, the levels of expression differ by approximately a factor of 100, with a maximum binding value for the COS cells of 140 pmol receptor/mg membrane protein (4). \([^{[3]}H]glibenclamide\) (65), \([^{[125]}I]iodomiglubinamide\) (2), and azido-[\(^{[125]}I]\]iodoglibenclamide (95, 96) will photolabel the SUR1 receptor(s) when used at nanomolar concentrations. This property has proven to be a useful means to identify the high-affinity receptor (Fig. 6, inset).
through the SUR2A/KIR6.2 channel by glibenclamide was 350 nM versus <10 nM for the β-cell channel. Similarly, the response to potassium channel openers was different. The SUR1/KIR6.2 and SUR2B/KIR6.2 channels were activated by diazoxide, whereas the SUR2A/KIR6.2 channels were not, up to concentrations near the solubility limit for the drug. The SUR2/KIR6.2 channels, on the other hand, were activated by pinacidil and cromakalim, whereas the β-cell channels were poorly responsive to these compounds. The nucleotide sensitivities of the reconstituted channels also differed. The reported IC50 values for inhibition by ATP were 100 μM (MgATP) for the rat SUR2A/mouse KIR6.2 channel (60) and 300 μM (MgATP) and 68 μM (ATP4+) for the mouse SUR2B/mouse KIR6.2 channel (63) versus ~10 μM (MgATP) for the hamster SUR1/mouse KIR6.2 channel (59). The significance of these differences with respect to receptor subtype is not completely clear because we measured an IC50 of ~50 μM for the human SUR1/mouse KIR6.2 channel (Fig. 7). Similarly, our unpublished estimate of

![Graph](https://example.com/graph1.png)

FIG. 6. Comparison of sulfonylurea binding to SUR1, SUR2, and hamster insulinoma tumor (HIT) cell receptors. Displacement of [125I]iodoglibenclamide by unlabeled iodoglibenclamide was compared for native receptors from HIT cells (dotted line), recombinant hamster SUR1 (circles), and recombinant rat SUR2A (squares) expressed in COSm6 cells. Inset compares photolabeling of receptors in rat insulinoma (RIN) cells and COSm6 cells transfected with either hamster SUR1 or rat SUR2A cDNA. HIT cell receptor binding data are an average from a large number of experiments (see Refs. 2, 3, and 75 for details). Error bars are ±SD; dissociation constant (Kd) values are given to nearest integer value. [SUR1 data are from Aguilar-Bryan et al. (4); rat SUR2A data are from Inagaki et al. (60).]

sured in quasi-symmetric high potassium (140 mM), with an average of ~65 pS. Taken together, the pharmacology, channel characteristics, and correlation of subunit mutations with PHHI indicate that SUR1 and KIR6.2 form the β-cell-type ATP-sensitive potassium channel.

VII. COEXPRESSION OF SULFONYLUREA RECEPTOR 2 AND INWARDLY RECTIFYING POTASSIUM CHANNEL 6.2 GENERATES CARDIAC- AND SMOOTH MUSCLE-TYPE ADENOSINE 5’-TRIPHOSPHATE-SENSITIVE POTASSIUM CHANNELS

The SUR2 receptors were cloned by homology with SUR1, and their expression with KIR6.2 also generated potassium-selective channels that were activated by metabolic inhibition. Two reports (60, 63) indicate that the pharmacology of the SUR2/KIR6.2 channels differs from that of the β-cell KATP channels. Consistent with the drug binding studies (see Fig. 6), the SUR2/KIR6.2 channels were less sensitive to sulfonylureas. The half-maximal inhibitory concentration (IC50) for 86Rb+ efflux

![Graph](https://example.com/graph2.png)

FIG. 7. Partial characterization of human SUR1/mouse KIR6.2 KATP channels. Recordings are from excised membrane patches of COSm6 cells transfected with human SUR1 and mouse KIR6.2. ATP inhibition curve is the average of 3 experiments, and bars are ±SD. Activity in 1 μM ATP was taken as 100%. Half-maximal inhibition concentration (IC50) value is ~5 times that measured for hamster SUR1/mouse KIR6.2 channel under the same conditions. I-V relationship shows inward rectification affected by magnesium and a single-channel conductance of 74.4 pA.
the steady-state IC$_{50}$ for the rat SUR2A/K$_{ir}$6.2 channel is 30 μM (MgATP) and ~100 μM for magnesium 5′-adenyllylimidodiphosphate. The general conclusion has been that the receptor subtype plays a dominant role in controlling both the pharmacology and nucleotide sensitivity of reconstituted K$_{ATP}$ channels, although the spectrum of drugs and nucleotides analyzed at this time is small. We also point out that only the three major receptor subtypes have been used in these preliminary studies. It is unclear, for example, whether the SUR2 deletions (?14, ?17, and ?17, ?18) will alter channel pharmacology in useful ways. The ?17 and ?17, ?18 deletions are in NBF1 and would be expected to alter the nucleotide binding properties of these receptors.

A prominent feature of the activity of K$_{ATP}$ channels is their bursting behavior. Work on native K$_{ATP}$ channels indicated lower rate constants for interburst transitions for cardiac (119) versus β-cell (49) K$_{ATP}$ channels. The reconstituted channels display a similar behavior, with the transition frequency between a burst and an interburst interval being dependent on the receptor subtype used (Fig. 8A). Inspection of the records on a shorter time scale suggests that the fast, flickering transitions within a burst are quite similar. In line with these qualitative observations, the quantitative analysis shown in Figure 8D demonstrates identical single exponential open-time distributions with $\tau_o$ equal to the mean open time as expected for a model with a single open state ($\tau_o = 3.14 \pm 0.17$ vs. $3.30 \pm 0.08$ ms for SUR1/K$_{ir}$6.2 vs. SUR2A/K$_{ir}$6.2, respectively; values are means ± SD, $n = 3$ for all). (Note that these are the average values from 3 experiments, whereas the values in Fig. 8 are for a single experiment.) Two exponentials were needed to describe the closed time distributions. The fast closed components within a burst were statistically equivalent ($\tau_c$ fast = 0.32 ± 0.03 vs. 0.30 ± 0.08 ms for SUR1/K$_{ir}$6.2 vs. SUR2A/K$_{ir}$6.2, respectively). The interburst intervals were significantly different for the two channel types ($\tau_c$ slow = 11.51 ± 1.30 vs. 62.70 ± 15.95 ms for SUR1/ K$_{ir}$6.2 vs. SUR2A/K$_{ir}$6.2, respectively). Therefore, the β-cell and SUR2A/K$_{ir}$6.2 cardiac K$_{ATP}$ channels can reach the same open probability, mean open probability, with quite different temporal patterns of channel activity, the β-cell channel through short frequent bursts and the cardiac-like channel by longer, less frequent bursts. The results suggest that K$_{ir}$6.2, the common subunit, determines the rapid intraburst kinetics, whereas the receptor regulates the transition from a silent to a bursting state.

VIII. ARE SULFONYLUREA RECEPTORS PROMISCUOUS?

The question of the range of inward rectifiers that sulfonylureas might partner with has been raised by Ammala et al. (7), who suggested that SUR1 conferred sensitivity to sulfonylureas on K$_{ir}$1.1 (ROMK1) (57) and other channels of unknown type in HEK 293 cells. Although this idea is interesting in that it may expand the diversity of metabolically sensitive potassium channels, it has not been possible to repeat the observations with K$_{ir}$1.1 in oocytes (53) or in COS cells (19). In addition, neither K$_{ir}$2.1 (53) nor K$_{ir}$4.3 (19) is activated or made sensitive to sulfonylureas by coexpression with SUR1. Possible interactions with strong inward rectifiers in coronary arterial myocytes have been looked for by Wellman et al. (114) but have not been observed. We conclude that, for the moment, only K$_{ir}$6.x are able to associate with SURs to generate K$_{ATP}$ channels. This raises the obvious possibility that there are other members of the K$_{ir}$6.x subfamily that remain to be discovered.

IX. COEXPRESSION OF SULFONYLUREA RECEPTOR 1 AND INWARDLY RECTIFYING POTASSIUM CHANNEL 6.2 ALTERS THE GLYCOSYLATION STATE OF THE RECEPTOR

The reconstitution experiments clearly showed that formation of K$_{ATP}$ channels required coexpression of both subunits. The simplest interpretation of these results implied association of the two subunits into a complex with fixed stoichiometry. Other interpretations have been suggested based on reports that CFTR may transport ATP, which in turn could serve as a diffusible regulator of other channels via action through purinergic receptors (5). We have examined the simpler interpretation, which better explains the available data (19).

Glibenclamide and several derivatives mentioned above (Fig. 9) have been key reagents in the cloning of the high-affinity SUR and the subsequent characterization of the interactions between SUR1 and K$_{ir}$6.2. Kramer et al. (65) were the first to show that [3H]glibenclamide photolabeled the 140-kDa receptor in rat β-cell tumor membranes. We showed that an iodinated derivative of glibenclamide, iodoglibenclamide, had the same property (2). Schwanstecher et al. (95) synthesized a 4-azido-5-iodosalicyloyl analog of glibenclamide, azido-iodoglibenclamide, and demonstrated that in addition to identifying SUR1, it specifically labeled another protein at 38–40 kDa. It is now clear that the 38- to 40-kDa protein is K$_{ir}$6.2 (19) (see Fig. 11, for example).

Several lines of evidence indicate that at least three differentially glycosylated forms of SUR1 are identified by photolabeling (Fig. 10) and can be separated using lectin chromatography (76). Site-directed mutagenesis work has shown that there are two glycosylation sites at Asn-10 and Asn-1050 (Clement and Bryan, unpublished data). Fractionation studies indicate that the complex glycosylated,
A

**Human SUR1** plus mouse K<sub>6.2</sub>

**Rat SUR2** plus mouse K<sub>6.2</sub>

150- to 170-kDa species is localized to the plasma and granule membranes, whereas the core glycosylated 140-kDa species is found only on internal membranes (83). The complex glycosylated receptor is thus expected to be in functional channels.

Expression of SUR1 alone produced only the 140-kDa species (Figs. 10 and 11 and Ref. 4), whereas coexpression with K<sub>IR6.2</sub> restored the normal glycosylation pattern (Fig. 11). After coexpression, both subunits could be cophotolabeled with <sup>125</sup>I-azidoglibenclamide, implying they are in close proximity. The <sup>125</sup>I-azidoglibenclamide is expected to be a potential cross-linker, and an additional band was observed in these photolabeling experiments at the position expected from cross-linking of SUR1 with K<sub>IR6.2</sub> (designated as “linked” in Fig. 11). The colabeling correlates with channel formation; K<sub>IR6.1</sub>, but not K<sub>IR1.1</sub> (ROMK1) or K<sub>IR3.4</sub> (CIR, rcKATP-1), was labeled (19). The results are consistent with the idea that transit of SUR1 to the cell surface requires association with K<sub>IR6.1</sub>, K<sub>IR6.2</sub>, or other undiscovered inward rectifier subunits in the K<sub>IR6.x</sub> subfamily.

**X. SULFONYLUREA RECEPTOR 1 AND INWARDLY RECTIFYING POTASSIUM CHANNEL 6.2 SUBUNITS ARE PHYSICALLY ASSOCIATED**

The association of SUR1 and K<sub>IR6.2</sub> has now been confirmed in multiple ways (19). In cells coexpressing SUR1 and K<sub>IR6.2</sub>, most of the K<sub>IR6.2</sub> appears to be associated with the complex glycosylated form of receptor (Fig. 12). The multimer formed by the associated subunits is presumed to be in the plasma membrane, but this has not
such fusions, illustrated in Figures 13 and 14, have been informative (19).

The SUR1-KIR6.2 fusion (Fig. 13) was engineered to determine if a 1:1 stoichiometry was sufficient for formation of active KATP channels. The NH2 terminus of KIR6.2 was fused to the COOH terminus of SUR1 through a linker of 6 glycine residues. Expression of SUR1-KIR6.2 in COSm6 cells produced a glibenclamide-inhibitable potassium channel that was activated by metabolic inhibition and diazoxide, indicating that the presumed 1:1 stoichiometry was sufficient to form active channels (Fig. 13). The current-voltage relationship of the SUR1-KIR6.2 channels is the same as that seen for unfused channels, indicating that constraining the respective NH2 and COOH termini does not alter the conductance properties of the pore (Fig. 15). These fusion channels show a decreased sensitivity to both glibenclamide and ATP. The decrease in sensitivity to ATP is small, ~50 versus ~10 \(\mu\)M for wild type, which is similar to the difference between hamster and human SUR1/mouse KIR6.2 channels (see Fig. 15). The reason(s) for the decreased sensitivities remains to be explored, but constraining the respective NH2 and COOH termini presumably interferes with the transduction of nucleotide-induced conformational changes in SUR1 to KIR6.2.

The SUR1-(KIR6.2)2 fusion (Fig. 14) was engineered to determine if a 1:1 stoichiometry was required for the formation of active KATP channels. If a 1:1 stoichiometry was required, active channels should not form from SUR1-(KIR6.2)2 alone. Efflux of \(^{86}\)Rb\(^+\) and excised patch experiments showed only background levels of flux and

been demonstrated directly. An estimate of the mass of the multimer formed by the 150- to 170-kDa receptor and KIR6.2, using sucrose density gradient centrifugation, gives a value of 950 kDa, which is compatible with a tetramer of SUR1/KIR6.2 (4 \(\times\) 176,000 + 4 \(\times\) 45,000 = 880,000), with additional mass attributed to glycosylation.

XI. FUSIONS OF INWARDLY RECTIFYING POTASSIUM CHANNEL 6.2 AND SULFONYLUREA RECEPTOR 1 FORM FUNCTIONAL CHANNELS

Fusions of K\(_{\text{ATP}}\) channel subunits offered the possibility of studying novel channels whose stoichiometries were defined at the subunit level. The head-to-tail organization of the SUR1 and KIR6.2 genes on chromosome 11 suggested that the two subunits might have been fused at an earlier time in evolution (1). The gene organization, plus the predicted topology of the receptor, with the NH2 terminus of SUR1 on the extracellular side of the plasma membrane, dictated the orientation of fusion constructs. Two

FIG. 9. Chemical structures of glibenclamide derivatives used to clone and characterize K\(_{\text{ATP}}\) channels.
Functional monomers of SUR1 are required to rescue SUR1-(KIR6.2)2. Coexpression of SUR1-(KIR6.2)2 with either a truncated receptor missing 184 amino acids from the COOH-terminal end, G1398P(23X) (32), or ΔF1388 (79) failed to generate active channels that could be identified by either 86Rb+ efflux or single-channel records (Fig. 14). Both of these mutant receptors are expressed and have been shown to retain high-affinity glibenclamide binding activity. The results show that two functional receptors are not sufficient to activate channel activity and imply that four functional receptors are required. The experiments leave open the question no KATP channels when SUR1-(KIR6.2)2 was expressed alone (Fig. 14). The critical issue was whether a correct stoichiometry could be restored if monomeric SUR1 was added. Coexpression of SUR1-(KIR6.2)2 + SUR1 produced potassium channels that had the general properties of KATP channels. Efflux of 86Rb+ and excised patch experiments showed these triple fusion channels were activated by metabolic inhibition and were inhibited by glibenclamide and ATP (Fig. 15). The results demonstrated that monomeric SUR1 could associate with SUR1-(KIR6.2)2, rescuing channel activity. These results implied that a 1:1 stoichiometry is required for channel activity.

FIG. 11. Coexpression of SUR1 and KIR6.2 affects receptor glycosylation and labeling of inward rectifier subunit. An autoradiogram of [125I]iodoglibenclamide labeled proteins identified when SUR1 and KIR6.2 cDNAs were transfected alone or together into COSm6 cells. Untransfected COS cells and COS cells transfected with β-galactosidase (β-gal) serve as controls. Albumin in the growth medium binds drug with low affinity and is labeled. Three higher-molecular-mass bands can be seen in cells cotransfected with both cDNAs, core and complex glycosylated receptors, and a species with molecular mass expected from cross-linked SUR1-KIR6.2. Inward rectifier subunit cophotolabels only when expressed with receptor. Labeling of both SUR1 and KIR6.2 with [125I]iodoglibenclamide can be competed by nanomolar concentrations of unlabeled drug (96).

FIG. 12. SUR1 and KIR6.2 are associated. To demonstrate physical association of SUR1 and KIR6.2, membranes were isolated from COS cells coexpressing SUR1 and KIR6.2. After labeling with [125I]iodoglibenclamide, KATP channel subunits were solubilized with detergent and fractionated on wheat germ agglutinin, a lectin which binds selectively to sialic acid residues found in complex glycosylated proteins (76). Core glycosylated receptor is not absorbed to the lectin and flows through the column. Complex glycosylated receptor and KIR6.2 are retained and can be selectively eluted with N-acetylglucosamine. KIR6.2 is not glycosylated and is not retained on the column in the absence of SUR1. Estimated molecular mass of eluted SUR1/KIR6.2 multimers is 850 kDa (19).
mer. Tucker et al. (110) have shown that expression of KIR6.2 subunits truncated at the COOH terminus (≤41 amino acids removed) produces potassium channels, which retain some sensitivity to ATP, but are insensitive to sulfonylureas, potassium channel openers, and activation by Mg-ADP. The results indicate that KIR6.2 is sufficient to form a potassium-selective pore with the conductance of a KATP channel, but without the regulatory properties.

As pointed out in section II B, there is evidence that the M2 segments of KIR subunits form part of the channel pore. The change of Asn-160 to Asp (N160D) in the putative M2 segment of KIR6.2 changes KATP channels from weak to a strong rectifiers, consistent with their forming part of the pore (see Fig. 4). The rectification requires that polyamines and/or magnesium are present at the intracellular mouth of the channel (see Fig. 4). The formation of heterologous channels by coexpression of mixtures of weak and strongly rectifying subunits has been used to estimate the stoichiometry of the pore of other KIR channels (51). Shyng et al. (99) used a modified approach with KATP channels composed of unfused subunits of whether incorporation of a single mutant receptor (or KIR subunit) into the multimer would destroy channel activity. Overall, the experiments with fusions of KATP channel subunits show that a 1:1 SUR1~KIR6.2 stoichiometry is both sufficient and necessary for the formation of an active channel.

XII. INWARDLY RECTIFYING POTASSIUM CHANNEL 6.2 FORMS THE β-CELL ADENOSINE 5'-TRIPHOSPHATE-SENSITIVE POTASSIUM CHANNEL PORE

Several lines of evidence indicate that KIR6.2 is the pore-forming subunit and that it assembles as a tetra-
FIG. 15. Comparison of current-voltage relationships and ATP inhibition of fused and unfused K<sub>ATP</sub> channels. Current-voltage relationships for fused and unfused channels are indistinguishable in presence of magnesium as shown here. A least-squares fit to data below the potassium equilibrium potential gave a value of 69 ± 1 pS (straight line). Fusion channels are less sensitive to ATP, with IC<sub>50</sub> values of 8.5, 35, and 114 μM. For these experiments, 100% activity is taken as that determined in presence of 1 μM ATP. [From Clement et al. (19). Copyright is held by Cell Press.]

and have shown that coexpression of SUR1 with a 1:1 mixture of wild-type K<sub>R6.2</sub> and K<sub>R6.2N160D</sub> produces five channel types distinguishable by their rectification properties. This is the result expected for a channel with a tetrameric pore.

Expression of SUR1~(K<sub>R6.2</sub>)<sub>2</sub> and SUR1~(K<sub>R6.2N160D</sub>)<sub>2</sub> with monomeric SUR1 generated weakly and strongly rectifying channels (19). Expression of a mixture of the fusion proteins with monomeric SUR1 generated three channel subtypes, the two parental species plus the heterologous channel, which could be discriminated based on their conductance-voltage relationships. The current-voltage response of the mixture differed from either parental type, and the calculated relative conductance-voltage plots could be fit with three Boltzmann functions more adequately than with either two or four. The result implies that the permeation pathway must be formed from two SUR1~(K<sub>R6.2</sub>)<sub>2</sub> proteins (Fig. 16).

FIG. 16. Triple fusion proteins can make heterologous K<sub>ATP</sub> channels. A plot of relative conductance (G<sub>REL</sub>) as a function of voltage shows that expression of triple fusion proteins carrying the N160D K<sub>R6.2</sub> mutation (heavily shaded construct) exhibit strong rectification (squares). Expression of triple fusion proteins constructed with wild-type K<sub>R6.2</sub> (lightly shaded construct), on the other hand, shows weak rectification characteristic of wild-type K<sub>ATP</sub> channels. Coexpression of a mixture of weak and strongly rectifying triple fusion proteins yields a complex conductance versus voltage curve that can be well fit with 3 Boltzmann equations. Results indicate that a heterologous channel (depicted as containing both shaded constructs) with intermediate rectification properties is formed in addition to the expected weak and strongly rectifying species. Data support the idea that K<sub>R6.2</sub> is part of the permeation pathway, whereas SUR1 is required to put the channel in an operational state. [From Clement et al. (19). Copyright is held by Cell Press.]

XIII. IS THE COMPLEX GLYCOSYLATED 950-KILODALTON COMPLEX THE ADENOSINE 5’-TRIPHOSPHATE-SENSITIVE POTASSIUM CHANNEL?

The available evidence argues that the 950-kDa multimer is the K<sub>ATP</sub> channel accessible on the plasma membrane. 1) All of the available data (19), outlined above, indicate the active channel is a tetramer of SUR1/K<sub>R6.2</sub>, i.e., (SUR1/K<sub>R6.2</sub>)<sub>4</sub>. 2) There is a strong correlation between K<sub>ATP</sub> channel activity, cophotolabeling, and the appearance of the 150- to 170-kDa glycosylated species of SUR1. Two inward rectifiers, K<sub>R1.1</sub> and K<sub>R3.4</sub>, which do not affect glycosylation or cophotolabeling, do not form K<sub>ATP</sub> channels with SUR1. 3) Complex glycosylation occurs in the medial Golgi after core glycosylation has taken place in the endoplasmic reticulum; thus the 150- to 170-kDa receptors, not the 140-kDa species, are expected to be at the plasma membrane, and fractionation studies place the 150- to 170-kDa species in the plasma membrane (83).
**XIV. A MODEL AND CONCLUSIONS**

The cloning of the high-affinity SUR and members of the KIR6.x family provided the reagents needed to begin molecular studies on K<sub>ATP</sub> channels. The data are consistent with the tetrameric model shown in Figure 17, which schematically illustrates the overall architecture and proposed stoichiometries of the K<sub>ATP</sub> channel constructs discussed in this review. The model focuses attention on the assembly pathway and raises the question of whether either SURs or KIR6.x can assemble tetramers independently.

A comparison of the tetrameric structure with the known pharmacology of K<sub>ATP</sub> channels raises additional interesting questions concerning stoichiometries of channel inhibition and activation. There are numerous reports on the inhibition of K<sub>ATP</sub> channel activity by sulfonylureas with inhibition constants and K<sub>D</sub> values for the SUR1/ KIR6.2 channel covering a range from nanomolar to micromolar depending on the drug used. Interestingly, the reported Hill coefficients are centered on a value of one. These results would appear to be at odds with a tetrameric structure unless binding of a single sulfonylurea molecule will block channel activity. Although this remains to be demonstrated, the observation that four functional receptors are required for channel activity is consistent with this idea. Similarly, the proposed model and requirement for four functional receptors suggests that mutations in SUR, or KIR6.2, should be dominant negative in character. Preliminary evidence indicates that coexpression of mutant receptors with wild-type SUR1 plus KIR6.2 results in a decreased number of active channels. Familial hyperinsulinism, on the other hand, is considered to be a recessive genetic disorder, raising the question of why dominant negative effects are not observed in the pancreatic β-cell and whether they might become apparent in other cell types.

The tetrameric structure raises the possibility that a different type of “promiscuity” may be possible. It seems reasonable to speculate that if the different receptor species are expressed in the same cell type, they may form channels that are heterologous with respect to receptor type. This possibility could lead to K<sub>ATP</sub> channels with different pharmacological properties and should be testable experimentally.

Stoichiometry questions now need to be raised concerning inhibition of channel activity by nucleotides and activation by MgADP and potassium channel openers like diazoxide and pinacilid. However, the major question of whether one or both NBFs on SURs can hydrolyze nucleotides, as implied by their family heritage, must be resolved before these questions can be framed in a meaningful way.

**REFERENCES**


