K\textsubscript{ATP} channels are heteromultimers of K\textsubscript{R6.2} and a sulfonylurea receptor, SUR, an ATP binding cassette (ABC) protein with several isoforms. K\textsubscript{R6.2} forms a channel pore whose spontaneous activity and ATP sensitivity are modulated by the receptor via an unknown interaction(s). Side by side comparison of single-channel kinetics and steady-state ATP inhibition of human β-cell, SUR1/K\textsubscript{R6.2}, versus cardiac, SUR2A/K\textsubscript{R6.2} channels demonstrate that the latter have a greater mean burst duration and open probability in the absence of nucleotides and ~4-fold higher IC\textsubscript{50,ATP}. We have used matched chimeras of SUR1 and SUR2A to show that the kinetics, which determine the maximal open probability (P\textsubscript{max}), and the ATP sensitivity are functionally separable and to identify the two segments of SUR responsible for these isoform differences. A region within the first five transmembrane domains specifies the interburst kinetics, whereas a C-terminal segment determines the sensitivity to inhibitory ATP. The separable effects of SUR on ATP inhibition and channel kinetics implies that the cytoplasmic C terminus of SUR either directly modulates the affinity of a weak ATP binding site on the inward rectifier or affects linkage between the binding site and the gate. This is the first identification of parts of an ABC protein that interact with an ion channel subunit to modulate the spontaneous activity and ATP sensitivity of the heteromeric channel.

K\textsubscript{ATP} channels are heteromultimers of a sulfonylurea receptor, SUR, a member of the ATP binding cassette (ABC) superfamily, and a potassium inward rectifier, K\textsubscript{R6.2} (1–4). Both SUR channels demonstrate that the latter have a greater mean burst duration and open probability in the absence of nucleotides and ~4-fold higher IC\textsubscript{50,ATP}. We have used matched chimeras of SUR1 and SUR2A to show that the kinetics, which determine the maximal open probability (P\textsubscript{max}), and the ATP sensitivity are functionally separable and to identify the two segments of SUR responsible for these isoform differences. A region within the first five transmembrane domains specifies the interburst kinetics, whereas a C-terminal segment determines the sensitivity to inhibitory ATP. The separable effects of SUR on ATP inhibition and channel kinetics implies that the cytoplasmic C terminus of SUR either directly modulates the affinity of a weak ATP binding site on the inward rectifier or affects linkage between the binding site and the gate. This is the first identification of parts of an ABC protein that interact with an ion channel subunit to modulate the spontaneous activity and ATP sensitivity of the heteromeric channel.

**EXPERIMENTAL PROCEDURES**

**Molecular Biology**—The human SUR1 and SUR2A cDNAs and their genes have been described previously (3). Human K\textsubscript{R6.2} was obtained from genomic DNA by PCR and was sequenced on both strands. The matched SUR chimeras were engineered using naturally occurring endonuclease restriction sites in the SUR1 and SUR2A cDNAs where possible. When necessary, matching restriction sites were engineered into the complementary cDNA using overlapping PCR primers. These were combined with appropriate flanking forward and reverse primers including unique restriction enzyme sites. Two amplifications were carried out; the first set of reactions matched the forward and reverse flanking primers with the appropriate overlapping primer. The resulting PCR products were purified by agarose gel electrophoresis, mixed, and used as the template for the second PCR reaction with the flanking primers. The product of the second reaction was cut with an appropriate pair of restriction enzymes and used to replace the corresponding wild-type fragment. The desired SUR fragments were then swapped using standard subcloning methods. All the PCR products and restriction sites were sequenced to verify the constructions. The restriction sites used, the amino acids, and their positions in the SUR1 and SUR2A genes have been described previously (3). Human K\textsubscript{R6.2} was obtained from genomic DNA by PCR and was sequenced on both strands. The matched SUR chimeras were engineered using naturally occurring endonuclease restriction sites in the SUR1 and SUR2A cDNAs where possible. When necessary, matching restriction sites were engineered into the complementary cDNA using overlapping PCR primers. These were combined with appropriate flanking forward and reverse primers including unique restriction enzyme sites. 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COSm6 cells were used in these studies because previous work has shown they have no endogenous SUR1 detectable with radio-labeled glibenclamide binding assays (11), and expression of either SUR1 or KIR6.2 alone produces no novel K channels detectable by 86Rb efflux assays or single-channel recording (9, 12).

Patch-clamp Recording and Single-channel Current Analysis—The properties of the reconstituted channels were analyzed in inside-out configurations using the patch-clamp technique as described previously (9). All experiments were done at 23–24 °C. Pipettes were filled with the quasi-physiological external solution containing 140 mM NaCl, 5 mM KCl, 1 mM MgCl2, 1 mM CaCl2, 10 mM HEPES, pH 7.4 (NaOH) or the K+-rich external solution containing: 145 mM KCl, 1 mM MgCl2, 1 mM CaCl2, 10 mM HEPES, pH 7.4 (KOH) and had a resistance of 4–8 megaohms. Smaller pipettes were used in some experiments to record single-channel currents. An internal solution, nominally Mg²⁺-free, containing 140 mM KCl, 5 mM EDTA, 5 mM HEPES, 10 mM KOH, pH 7.2 (KOH) was used as the control bath solution. The disodium salt of ATP and other compounds were from Sigma. Bathing solutions were applied using a programmable rapid solution changer (RSC-200, Biologic Inc, Claix, France). Quasi-steady-state sensitivity to inhibitory ATP was estimated using experimental conditions and a protocol designed to minimize variability in the apparent IC₅₀(ATP) as described under “Experimental Procedures.” The mean IC₅₀(ATP), ± error values and a slope factor (h) obtained from the best fit to a conventional pseudo-Hill equation using Origin 5.0 Professional (Microcal Software, Inc., Northampton, MA) are shown. The IC₅₀(ATP) values for the two channels are significantly different and markedly lower than determined for human K₉.6.2ΔC35 channels shown for comparison.

Fig. 1. A comparison of single-channel kinetics and ATP inhibition of SUR1/K₉.6.2 versus SUR2A/K₉.6.2 channels. A, a representative example of currents through SUR1/K₉.6.2 (left) and SUR2A/K₉.6.2 (right) channels recorded at −40 mV with the K⁺-rich external pipette and internal control solutions. The dwell time distributions are shown below the traces. The mean burst duration at a burst criterion of 2 ms, Tb, was <30 ms for all 4 patches containing a single SUR1/K₉.6.2 channel but was >298 ms for all 5 patches containing single SUR2A/K₉.6.2 channels. B, the steady-state ATP inhibition of the SUR1/K₉.6.2 (upper trace) and SUR2A/K₉.6.2 (lower trace) channel was examined as described under “Experimental Procedures.” The mean IC₅₀(ATP), ± error values and a slope factor (h) obtained from the best fit to a conventional pseudo-Hill equation using Origin 5.0 Professional (Microcal Software, Inc., Northampton, MA) are shown. The IC₅₀(ATP) values for the two channels are significantly different and markedly lower than determined for human K₉.6.2ΔC35 channels shown for comparison.
wardly directed currents, only patch current records with no superimposed openings were used in which the cumulative Po increased linearly for more than a half-minute during continuous recording immediately after patch isolation. For heteromeric SUR/KIR6.2 channels displaying Po > 0.5, these criteria were sufficient to be confident that only single channels without significant run-down were being analyzed. The kinetic analysis including the burst analysis at an optimal burst criteria was performed using pClamp7 and BioQuest software as described previously (9). Averaged data are expressed as means ±S.E. for n ≥ 5 with error bars equal to the S.E. unless otherwise noted. Significance was evaluated using the Student’s t test; differences with values of p < 0.05 were considered to be significant.

RESULTS

SUR Isoforms Specify the Kinetics and ATP Inhibition of K_ATP Channels—A quantitative side by side comparison of the kinetics and steady-state ATP-inhibition of human SUR1/KIR6.2 and human SUR2A/KIR6.2 channels was carried out to define the differences in gating and ATP sensitivity of β-cell and cardiac K_ATP channels. As shown in Fig. 1, the SUR2A/KIR6.2 channels display longer bursts and spend less time in interburst gaps than the SUR1/KIR6.2 channels, whereas the intraburst kinetics of the two channels are indistinguishable. As a consequence, a hallmark of the SUR2A/KIR6.2 channels is a higher Pm_{max} (0.91 ± 0.01 versus 0.64 ± 0.03 at −40 mV, respectively). The SUR1/KIR6.2 channels, on the other hand, are ~4-fold more sensitive to ATP than the SUR2A/KIR6.2 channels (IC_{50,ATP} = 5.9 ± 0.5 versus 23.4 ± 2.6 μM, see also Ref. 9). Both channel isoforms are significantly more sensitive to inhibitory ATP and both have severalfold higher Pm_{max} values than homomeric KIR6.2 channels, 0.09 ± 0.01 and 221.1 ± 12.1 μM for the human KIR6.2A/C35 channels, which were analyzed in parallel (Ref. 13, see also Refs. 9, 14–16).

Two Distinct Region of SUR Specify Spontaneous Bursting and ATP Inhibition of K_ATP Channel Isoforms—Matched pairs of human SUR1 and SUR2A chimeras were co-expressed with KIR6.2 to delineate the regions of SUR that affect the interburst kinetics and ATP inhibition of K_ATP channels. The chimeras were generated by “swapping” progressively longer segments, chosen to include the major structural features of SUR, as presented in Table I and shown diagrammatically in Fig. 3. Fig. 2 illustrates the analytical strategy using one pair of chimeras in which the N-terminal segment of SUR1 (or SUR2A) that included the first 29 amino acids and the first two sets of transmembrane domains, TMDI-N and TMDI-C, were swapped onto the reciprocal C-terminal segment of SUR2A (or SUR1) including NBF1, TMDI, NBF2, and the C terminus. The SUR1−SUR2A/KIR6.2 channels displayed all the kinetic properties of the β-cell channel but required ~4-fold more ATP for half-maximal inhibition. The matched partner, SUR2A−SUR1/KIR6.2, displayed the closed time distribution, high Pm_{max} and bursting pattern of a cardiac K_ATP channel but was inhibited by ATP with an IC_{50,ATP} value appropriate for the β-cell K_ATP channel. The intraburst kinetics of the two chimeric channels illustrated in Fig. 2 are the same as they are for the channels assembled from the other matched pairs. Analysis of the other pairs of chimeras (Fig. 3) demonstrated that the TMD1-N segment determines the higher Pm_{max} of the cardiac channel, whereas the C-terminal region specifies the lower IC_{50,ATP} of the β-cell channel. Swapping a 52-amino acid segment, C52, the last 42 of which actually differ, was sufficient to completely specify the IC_{50,ATP} value associated with the donor SUR isoform. Smaller swaps, 27- and 12-amino acids, respectively, C27 and C12, produced a graded effect, and, therefore, we have not attempted to localize the molecular determinant(s) to a specific residue; rather, the results suggest the last 42 amino acids are important and may form a contact surface with KIR6.2.

The results show there are two structural elements of SUR critical for determining the isoform-dependent components of gating and its modulation by inhibitory ATP and that these elements are distinct from the two nucleotide binding folds. This is consistent with observations that mutations in the nucleotide binding folds (17–19), including those which eliminate ATP labeling of SUR1 (20), do not affect inhibition by ATP. In our hands, for example, the human SUR1K_{719H}/K_{R6.2}, SUR1K_{719H}/K_{R1385F}/K_{R6.2}, and SUR1K_{1506A}/K_{R6.2} channels are all inhibited by ATP with IC_{50,ATP} values of 5–7 μM (data not shown).

DISCUSSION

This is the first identification of two segments of the sulfonylurea receptor that specify the intrinsic activity and ATP sensitivity of the inward rectifier subunit of K_ATP channels. The results show that the modulation of channel kinetics and apparent sensitivity to inhibitory ATP are separable. Recent reports have focused on the behavior of the homomeric KIR6.2 channels that can be studied either by removing an endoplasmic reticulum retention signal(s) (as shown by B. Schwappach, N. Zerangue, Y. N. Jan, and L. Y. Jan (21)) from the C terminus of KIR6.2 (14, 15) or by strong overexpression (16). Although the homomeric channels display the wild type unitary conductance and are weakly inhibited by ATP, they lack the hallmark properties of native, heteromeric K_ATP channels including a high open probability, correct bursting, high sensitivity to ATP, and responsiveness to sulfonylureas, potassium channel openers, and MgADP (14, 15). The properties of the homomeric channels...
channels are unlike any known native K\textsuperscript{+} channels, indicating their physiological relevance is questionable. Co-expression of K\textsubscript{IR6.2} with a SUR, on the other hand, generates a K\textsuperscript{+} conductance that accurately reflects those found in a variety of different tissues, with the isoform or tissue-specific properties being specified by the SUR involved. This report provides evidence for allosteric interactions between K\textsubscript{IR6.2} and two regions of SUR that are involved in two defining properties of K\textsubscript{ATP} channels, their spontaneous bursting behavior and ATP sensitivity. TMDI-N contains a critical determinant of bursting behavior, whereas the last 42 amino acids of SUR specify the effect of ATP on gating. These results do not contradict previous reports that mutations in the nucleotide binding folds of SUR have negligible effects on the ATP inhibition of K\textsubscript{ATP} channels; rather, together the two results indicate that whereas ATP binding and possible hydrolysis on SUR is not required for ATP inhibition, interaction of K\textsubscript{IR6.2} with other regions of the receptor are critical for normal ATP inhibitory gating.

FIG. 2. A comparative analysis of one pair of matched chimeric SUR/K\textsubscript{IR6.2} channels. A, a schematic representation of one of the matched pairs of chimeric SURs, which were co-expressed with wild type K\textsubscript{IR6.2}. The swapped segments from SUR1 and SUR2A are shown in gray and black, respectively. The chimeras are named, as illustrated, according to which isoform contributed the N terminus. B, an analysis of the closed time distributions for the two chimeric channels used to evaluate differences in their kinetics, done as described in Fig. 1A. Note the greater T\textsubscript{b,ms} and significantly smaller slow component of the closed time probability density function for the SUR2A\textemdash SUR1/K\textsubscript{IR6.2} versus SUR1\textemdash SUR2A/K\textsubscript{IR6.2} channel that determines its higher F\textsubscript{max} (0.91 ± 0.01 versus 0.64 ± 0.03; n = 4 for both channels). C, determination of the ATP inhibition of SUR2A\textemdash SUR1/K\textsubscript{IR6.2} (upper trace and open squares) and SUR1\textemdash SUR2A/K\textsubscript{IR6.2} (lower trace and open circles) channels demonstrates that this pair of chimeric channels has mixed properties. The SUR2A\textemdash SUR1/K\textsubscript{IR6.2} channels show the kinetics of cardiac channels but are inhibited with the IC\textsubscript{50,ATP} of β-cell K\textsubscript{ATP} channels, whereas the SUR1\textemdash SUR2A/K\textsubscript{IR6.2} channels have β-cell kinetics and the IC\textsubscript{50,ATP} of cardiac channels. ATP inhibition was analyzed as in Fig. 1B.
The chimeric channel experiments provide additional insight into the mechanism of inhibition of KATP channels by ATP and imply SUR either modulates the affinity of Kir6.2 for ATP or alters a link between the nucleotide binding site and the gate. We develop the following argument using a minimal linear kinetic scheme for KATP channel gating based on a simple model introduced originally by Gillis et al. (22).

As the intraburst transitions between C1 and O, specified by \( \tau_{O} \) and \( \tau_{C} \), are the same for the two channels (Fig. 1), the higher \( P_{\text{max}} \) of the SUR2A/Kir6.2 channels implies the cardiac channel will spend less time in interburst closed states that bind ATP. Therefore, the reduced apparent sensitivity of the SUR2A/Kir6.2 channels versus the SUR1/Kir6.2 channels to ATP could be attributed solely to a reduction in the fraction of time the former channel spends in interburst closed states. If this were correct, the SUR isoforms could modulate the apparent ATP sensitivity of the chimeric channels without substantially altering their bursting. This argument is consistent with the observation that homomeric Kir6.2AC channels have a reduced sensitivity to ATP (14, 15), yet spend a greatly increased fraction of time in interburst closed states proposed to bind ATP. Thus the differences in ATP sensitivity of the KATP channel isoforms that are attributable to SUR and the enhancement of ATP sensitivity seen when Kir6.2 is co-expressed with a SUR cannot be due solely to changes in channel kinetics that affect the occupancy of interburst closed states that bind ATP, as has been suggested from studies on several Kir6.2 mutations (15, 24–26) and N-terminal truncations of Kir6.2 (23) that produce parallel increases in \( P_{\text{max}} \) and IC\(_{50}\)(ATP). These results imply that SUR must either directly modulate the affinity of Kir6.2 for ATP in closed state or affect the linkage between the binding site(s) and the gate. We hypothesize that modulation of the affinity of Kir6.2 for ATP by SUR could result either from allosteric interactions or by formation of a shared ATP binding site. Further studies including direct measurement of ATP binding are needed to resolve these possibilities.

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