ATP-sensitive potassium channels (KATP) are implicated in a diverse array of physiological functions. Previous work has shown that alternative usage of exons 14, 39, and 40 of the muscle-specific KATP channel regulatory subunit, sur2, occurs in tissue-specific patterns. Here, we show that exon 17 of the first nucleotide binding fold of sur2 is also alternatively spliced. RNase protection demonstrates that SUR2(Δ17) predominates in skeletal muscle and gut and is also expressed in bladder, fat, heart, lung, liver, and kidney. Polymerase chain reaction and restriction digest analysis of sur2 cDNA demonstrate the existence of at least five sur2 splice variants as follows: SUR2(39), SUR2(40), SUR2(Δ17/39), SUR2(Δ17/40), and SUR2(Δ14/Δ39). Electrophysiological recordings of excised, inside-out patches from COS cells cotransfected with Kir6.2 and the sur2 variants demonstrated that exon 17 splicing alters KATP sensitivity to ATP block by 2-fold from ~40 to ~90 μM for exon 17 and Δ17, respectively. Single channel kinetic analysis of SUR2(39) and SUR2(Δ17/39) demonstrated that both exhibited characteristic KATP kinetics but that SUR2(Δ17/39) exhibited longer mean burst durations and shorter mean interburst dwell times. In sum, alternative splicing of sur2 enhances the observed diversity of KATP and may contribute to tissue-specific modulation of ATP sensitivity.

ATP-sensitive potassium channels (KATP), first described in cardiac myocytes (1, 2), are weak inwardly rectifying K+ channels, which are inhibited by intracellular ATP and activated by Mg-ADP (3, 4). KATP are found in a diversity of tissues, including cardiac, skeletal, and smooth muscles, brain, and the β-cell of the pancreas (5, 6). In these cells, KATP couple metabolic stresses to membrane excitability (5) and therefore play a key role in regulating physiological events such as muscle contraction, control of vascular tone, and insulin secretion (7–9). KATP are also hetero-octameric complexes (17, 18) consisting of at least two types of subunits as follows: a member of the small inward rectifier potassium channel family, Kir6.1 (ubiquitous KATP) or Kir6.2 (β-cell inward rectifier), and a sulfonylurea receptor regulatory subunit, sur1 or sur2, which are members of the ATP binding cassette superfamily (ABC proteins) (14–16, 19–21). The coexpression of both types of subunits yielded K+ currents inhibitable by cytoplasmic ATP and sulfonylurea compounds (16, 20). When the same pore protein, Kir6.2, is expressed either with sur1 or sur2, the resulting channels exhibit identical conductive properties but differ in their ATP block profile and drug responses. Channels from sur1 were inhibited half-maximally with 10 μM ATP and were responsive to the potassium channel opener, diazoxide (20), whereas channels from sur2 demonstrated a half-maximal block at 100 μM ATP and were insensitive to diazoxide (16). To date, five species of sur mRNA transcribed from two different sur genes have been isolated and shown to yield functionally distinct KATP, when expressed with Kir6.2. sur1 (14) confers typical β-cell KATP properties. sur2 identified by Inagaki et al. (16) resembles the cardiac type KATP. A variant of the sur2 carboxy-terminal domain (CTD) arises from the excision of the final 129 coding bp of sur2 (along with 48 bp of the sur2 3' untranslated sequence) and results in the expression of a unique CTD from the use of 129 bp of the 3'-untranslated sequence as coding sequence. This splice variant is described as SUR2B. SUR2B yields recombinant KATP channels with restored diazoxide sensitivity (22) and resembles a smooth muscle type KATP (16, 20, 22). The expression pattern of these sur species corresponds to their tissue-predictive functional properties. A third sur2 variant lacking exon 14, named SUR2A (by coincidence, as Isomoto et al. (22) denoted the original sur2 as SUR2A), has been identified only in heart (15). Each of the identified sur2 variants results from a single exon alternative splicing event. Examination of the mouse sur2 genomic structure reveals that the sur2 intron/exon boundaries are comparable to the published genomic structure of human SUR1, with 40 exons available for the mRNA coding region. Here, we adopt a system of sur2 nomenclature based on the exons in.
volved in alternative splicing to clarify the identified sur2 variants, as well as future yet to be described variants resulting from alternative splicing. The alternate carboxyl terminus usage of exon 40 replacing exon 39, SUR2B, is denoted as SUR2(40). Likewise, we identify the age of exon 40 replacing exon 39, SUR2B, is denoted as from alternative splicing. The alternate carboxyl terminus us-

Exon 17 encodes the 13 amino acids next to the Walker A motif containing exon 16 of the first nucleotide binding fold (NBF). The Walker A motif is highly conserved in many ATP-binding proteins (21, 24) and is required for the functional integrity of the NBF. In sur1, mutations of the conserved lysine residue (Lys-719 in sur1) in the Walker A motif of NBF1 produce a decrease in ATP binding to the sur (25). This variant lysine residue has been associated with facilitation of ATP hydrolysis (26–28). Electrophysiological experiments also im-

Inagaki et al. (16, 19, 20) that uses exon 39 as SUR2(39), and our previously identified cardiac-restricted variant, SUR2A, is thus designated SUR2(Δ14/39).

Probe Generation (S1 Nuclease)—A full-length sur2 cdNA with exon 39 CTD was subcloned into a pcDNA3 vector. The construct was digested with XbaI and primed with an antisense oligonucleotide directed to exon 39, 5′-TGGACGAGTTTGGACGATAGA-3′. Run-off primer extension was performed in the presence of 3000 Ci/mmol α-32PdCTP (Amersham Pharmacia Biotech) with Klenow polymerase (Ambion). The labeled probes were purified from the template by denaturing polyacrylamide electrophoresis, and the band containing the probe was excised and eluted from the gel overnight in TE at 37 °C. A β-actin loading control probe was generated in a similar fashion using Tri-actin mouse 125 as a template (Ambion).

Solution Hybridization—40 μg of total RNA, isolated by TRIzol extraction, was coprecipitated with 5 × 108 cpm labeled probe, resus- pended in hybridization solution (Ambion), and allowed to hybridize for 18 h at 44 °C. The hybridized RNA were digested with RNase A and T1 for 30 min (RNase protection assay) or with S1 nuclease at 37 °C for 30 min, and the protected products were resolved on a standard denaturing sequencing gel. The gel was autoradiographically exposed to Bi- max XR film (Kodak) overnight at −80 °C or was exposed to a phos- phorimaging cassette (Molecular Dynamics) for 3 h at room temperature for quantitation.

Cell Culture and Transfection

Full-length sur2 cDNA coding regions (bp 1–4684) were subcloned into a mammalian expression vector (pcDNA3, Invitrogen) using stand-

ARD molecular biological techniques (30). COS-1 and COS-7 cells (ATCC), grown in Dulbecco’s modified Eagle’s medium/high glucose + 10% fetal calf serum (Life Technologies, Inc.), were plated at a density of 1 × 105 cells onto scored glass coverslips in 60-mm plates, 12–18 h prior to transfection. Each plate was transfected with 5 μg of total plasmid DNA, with a molar ratio of 4:1 SUR2-pcDNA3/mβ-cell inward rectifier-pCMV6b-GFP-greenlantern (Life Technologies, Inc.), using the Superfect transfection system (Qiagen). The cells were used for recording 72 h post-transfection.

Electrophysiology—Cells were selected for recording based on their green fluorescence at 525 nm when excited at 480 nm due to the coexpression of green fluorescent protein. Pipettes (1–3 meégohms) were filled with “pipette solution” containing (mM) 140 KCl, 0.22 MgCl2, 5 HEPES-KOH or HEPES-NaOH at pH 7.4. In some experiments, 130 NaCl + 10 KCl was iso-osmotically substituted for 140 NaCl as indicated in the text. Transfected cells were placed in a chamber bath perfused with “internal solution” containing (mM) 140 KCl, 0.2 Mgl2, 2 EGTA, 5 HEPES-KOH, pH 7.3; ATP, tolbutamide, and gly-

bureide were added to the internal solutions as indicated in the text. The solutions superfusing the patch membrane were switched within 1 s using a fast solution switching system, DAD12 (ALA Instruments). Inside-out patches were recorded with an EPC-7 amplifier (List Electronics) for single channel and Axxo200B (Axon Instruments) for multiple channel analy-

sis. Single or multiple channel current recordings were filtered at 0.5–2 kHz, sampled at 5 kHz, and then stored digitally direct via the PCLAMP (Axon Instruments) and “Aquire” (Bruxton Corp.) software packages for subsequent offline data analysis.

In patches containing a single active channel, channel open activity was assessed by an open probability (P_o) as follows. A 50% threshold criterion was used to detect an event, and all events were visually confirmed. The dwell time at each level was determined using FETCHAN software (Axon Instruments). From this, the P_o was calculated using the equation derived by Spruce et al. (34). Patches containing multiple channels (<5 channels) were calculated in a similar fashion. In macro-patch recordings (>5 channels), an apparent open probability (N P_o) was employed. The N P_o was calculated as the average current in a 10-s time window divided by the known single channel current amplitude under the same recording conditions.

EXPERIMENTAL PROCEDURES

RT-PCR Analysis of Exon 17

Total RNA was isolated from whole organ mouse tissues by TRIzol extraction (Life Technologies, Inc.). The RNA was primed with the sur2 gene-specific primer (5′-CGTACCCCGACTCCATTGCGCC-3′), and with oligo(dT), and reverse-transcribed with Superscript II (Life Technologies, Inc.) following the manufacturer’s instruction. Single-stranded cDNA (1 μl) was used as template for polymerase chain reaction amplification using Klenataq polymerase (CLONTECH) under the following conditions: 35 cycles of 96 °C × 30 s melting, 58 °C × 30 s annealing, 72 °C × 1 min extension. The primers are as follows: forward (in exon 16) 5′-CA TCTTCTCTTTCTGCTTGGCC-3′; reverse (in exon 21) 5′-CCTCTTCAATTTCAGTTGTCTCC-3′.

Long RT-PCR for Detection of Migrating Region Splicing and Alternative CTD Usage

—Total RNA was isolated by TRIzol extraction (Life Technologies, Inc.) from the indicated whole organ mouse tissues. The RNA was primed with oligo(dT) and reverse-transcribed with Superscript II (Life Technologies, Inc.). Exon 14/Exon 39–40 Relationship—Primers (forward, 5′-GAA GCT-GGGCGAGCTCAAGAG-3′; reverse, 5′-GATCGGCGCCATTTCTGAG TGTGGACAGA TCCTGA-3) were designed to reside 500 bp 5′ to exon 14 and 3′ of exon 40. These primers were used to amplify cDNA using Klenataq polymerase under the following conditions: 36 cycles of 96 °C × 30 s melting, 68 °C × 3 min annealing/extension. The products were purified by Qiabyte PCR purification (Qiagen) and digested with HaelII (New England Biolabs). The digested products were resolved on 0.8% SeaKem agarose gels (FMC), and the corresponding sur2 frag-

ments with and without exon 14 were gel-purified using Qiagen II gel purification (Qiagen). The purified DNA fragments were digested with XbaI (New England Biolabs) and then resolved on 2.0% SeaKem agarose gels.

Exon 17/Exon 39–40 Relationship—Primers were generated to re-

side entirely within exon 17 and exon 39 or designed to exclude the two exons leaving 3–4 bp overhanging the splice junction at the 3′ end of the primer (3′ overhangs are underlined). The primers are as follows: exon 17, 5′-GTTAAGGA ATCTGAGCCCTTTTGGAGAAGCCCGAAGAAG-3′;-strand 17, 5′-CCCTGAGAAAG AAATTTCTTTGAGAGCCACAC-3′; exon 39, 5′-TAGGCGAAGCGGTGCTAATAGA-3′; strand 39, 5′-CTCGAGCACGTCAGAATGGTGTGAACCCGATGAG-3′. PCR conditions and agarose gel electrophoresis are as stated above.

RNase Protection Assay and S1 Nuclease Assay

Probe Generation (RNase)—A 375-bp cDNA probe corresponding to the sur2 coding region bp 1879–2254 was generated by PCR with Pfu polymerase (Stratagene) and subcloned into Bluescript KS+ (Strat-

agene). Sequence integrity and orientation was confirmed by dye ter-

For single channel kinetic analysis, idealized current reconstructions for each recording were generated using "TAC/TACfit" program (Bruxton Corp.) based on a half-maximal amplitude threshold criterion. Open and closed time histogram analysis was performed on recordings demonstrating only a single level of channel activity. Burst durations were resolved at a critical time cutoff of 3.5 ms to distinguish a gap between two bursts from a closure within a burst (31, 32) (Equation 1).

\[
a_{1}[\exp(-t_{\text{cw}}/\tau_{1})] - a_{2}[\exp(-t_{\text{cw}}/\tau_{2}) - \exp(-t_{\text{cw}}/\tau_{3})]
\]

(Eq. 1)

where \(\tau_{1}\) and \(\tau_{2}\) are the time constants for closed intervals within a burst and between bursts; \(a_{1}\) and \(a_{2}\) are the areas of exponential fits corresponding to \(\tau_{1}\) and \(\tau_{2}\), and \(t_{\text{cw}}\), the "dead time" of underestimated events, corresponds to double the sampling rate (400 \(\mu\)s). Distributions of open and closed time events were plotted against a logarithmic time scale with event durations log-binned at 50-\(\mu\)s resolution. Exponential fits to the histograms were performed by a maximum likelihood fitting strategy. Values for kinetic rate constants were determined based on the relationships described by Sakmann and Trube (33), Gillis et al. (31), and Alekseev et al. (32).

**RESULTS**

**sur2 Variants and Function**

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**RESULTS**

**sur2 Exon 17 Exhibits Tissue-restricted Alternative Splicing**—Our initial characterization of sur2 included the description of an alternatively spliced coding region variant, \(\text{SUR2}(\Delta 14/39)\), restricted to heart expression (15). Further examination of the PCR products spanning this alternatively spliced region suggested a novel coding region variant. Primers designed to amplify from the coding region 2126–2415 bp (exons 16–21) resolved a novel product from the full-length sur2 PCR product (Fig. 1A). This smaller variant demonstrated wide tissue expression distribution in mouse and was detected in every tissue examined. It was prominent in bladder, gut, kidney, and skeletal muscle and was weakly detected in all other tissues. The sequence of this product indicated it lacked 39 bp (13 amino acids), from bp 2190 to 2229 of the sur2 coding region, corresponding precisely to a single exon, exon 17, based on the human \(\text{SUR1}\) genomic structure. Subsequent mapping of the mouse sur2 genomic intron/exon boundaries demonstrated that the splice junction for this species indeed corresponded to exon 17, with appropriate flanking splice donor/acceptor sequences (data not shown). Previous work indicated that the beginning of exon 17 "wobbled" by \(\pm 1\) amino acid, which was attributed to an alternate recognition of the splice acceptor site at the intron/exon junction. This is a distinct event from the alternative splicing of exon 17 we present here.

Solution hybridization and RNA protection with a labeled antisense RNA probe spanning exons 13–18 (bp 1879–2259 of the coding region) was employed both to confirm the RT-PCR splicing data and to quantitate the relative mRNA tissue expression level for each of the three identified splice variants in...
Corresponding 10 ng of the indicated cDNA template. 9th to 14th lanes species amplified according to the diagram. The presence of a 2–2.2-kilobase pair amplified product indicates the existence of the particular and exon 40 species resolved as 760- and 581-bp fragments, respectively.

Mouse heart cDNA. The products were digested and resolved by agarose gel electrophoresis in two steps as indicated in the schematic. Exon 39 and exon 40 species resolved as 760- and 581-bp fragments, respectively. In heart, full-length SUR2 was expressed in highest levels in heart, skeletal muscle, and bladder but at much lower levels than the corresponding exon 39 compared with exon 40 (Fig. 1C). Weak expression of exon 39 was also noted in adipose tissue and in bladder but at much lower levels than the corresponding expression of the exon 40 variants. The exon 40 variant was present in all tissues.

Exon 14 Splicing Only Occurs with Carboxyl Tail Exon 39—Demonstrating three mid-coding region sur2 splice variants (full-length, Δ14, and Δ17) and two carboxyl-terminal tails (exons 39 and 40) suggests that six possible sur2 variations could be generated if the mid-coding region splice events occur independently of alternate carboxyl-tail usage. To determine if exon 14 splice variants occurred with both exon 39 and exon 40, long PCR was employed spanning exons 9–40 (Fig. 2A). Exon 14 and exon Δ14 amplified products could be identified and separated by a unique HaeII restriction site present in exon 14, 500 bp 3’ to the sense primer. From these amplified products, HaeII digestion yielded PCR products differing by 500 bp. The digested products were separated by gel electrophoresis, and the exon 14 and exon Δ14 fragments were excised from the agarose gel. The presence or absence of exon 17 (≥ 35 bp), present only within exon 14+ variants, resolved as a nearly indiscernible doublet indicated as Δ14+ in Fig. 2A. These two exon 17 species, as both possessed exon 14, were copurified for the subsequent digestion step. The gel-purified fragments were digested a second time with a unique XbaI site 5’ to exon 39. Exon 39 and 40 variants resolved as 760- and 581-bp fragments, respectively. The results demonstrated that exon 14 containing sur2 variants were present with both exon 39 and 40 CTD tails, but the SUR2(Δ14) variant, which was expressed

**Fig. 2. Relationship between midcoding region exon usage and carboxyl-terminal usage.** A, PCR restriction digestion to evaluate exon 14 and CTD usage. Primers defined PCR products that spanned bp 1639–5240 of the full-length sur2 transcript, amplified from oligo(dT)-primed mouse heart cDNA. The products were digested and resolved by agarose gel electrophoresis in two steps as indicated in the schematic. Exon 39 and exon 40 species resolved as 760- and 581-bp fragments, respectively. B, exon 17 and carboxyl-terminal usage. Primer combinations and their corresponding sur2 targets are depicted in the schematic. Each lane of the resolved PCR products indicates the primer set employed and the sur2 variant; the lack of a product indicates its absence. The 9th to 14th lanes represent a relevant positive and negative control for each primer using 10 ng of the indicated cDNA template.
in heart alone, only existed with the exon 39 CTD.

Exon 17 Splicing Occurs Independently with Respect to Splicing of the CTD—A similar PCR/restriction digestion approach was not available to determine the relationship between exon 17 splicing and the alternate carboxyl terminus, as no unique restriction sites existed in exon 17. Instead, primers were designed to reside entirely within exons 17 and 39 or to straddle these exons and thus exclude them (Fig. 2B). The straddling primers annealed to the splice junction asymmetrically; a majority of the 5’ end of each primer annealed to one exon leaving 3–4 bp of the primer’s 3’ end to anneal to the exon following the splice junction. By this design, these primers could only anneal to those sur2 variants lacking either exon 17 or 39 to provide a productively primed template for polymerase extension (scheme, Fig. 2B). The four designed primers were used in combination to test each of the four possible splice forms for exons 17/17 and 39/40, resulting in a 2–2-kilobase pair amplified product (depending on the primer pair) if the variant was expressed in the tissue or no amplified product if the tissue lacked the variant. Heart and skeletal muscle were tested, as most of the other tissues were known to contain only the exon 40 CTD (Fig. 1 and Ref. 22). As such, these tissues must contain either 17/40 or Δ17/40 as determined by the earlier RT-PCR and the S1 nuclease (Fig. 1). Fat and bladder, which also express minor levels of exon 39, were not examined at this time. As seen in Fig. 2B, both skeletal muscle and heart expressed all four of the possible exon 17 variants.

In summary, five sur2 isoforms are generated by the alternative splicing of the coding region: SUR2(39), SUR2(Δ14/39), SUR2(Δ17/39), SUR2(40), and SUR2(Δ17/40). Their tissue expression pattern is summarized in Fig. 3.

Single Channel Currents Resulting from the Coexpression of sur2 Splice Variants with mKir6.2—Coexpression of four of the five sur2 variants and mKir6.2 in COS-1 or COS-7 elicited currents with identical single channel conductance (Fig. 4, A and B), and kinetic properties similar to cardiac and skeletal muscle K<sub>ATP</sub> (7, 32, 34). One variant, SUR2(Δ14/39), failed to generate current. The current-voltage relationship for each variant demonstrated weak inward rectification and an estimated reversal potential of approximately −58 mV (Fig. 4B), a value close to the K<sup>+</sup> equilibrium potential of −65 mV. The single channel conductance was approximately 30 pS for all variants at a membrane potential of 0 mV. In addition, each coexpressed variant exhibited characteristic K<sub>ATP</sub> channel inhibition at 1 mM cytoplasmic ATP. Application of 100 μM tolbutamide or 1 μM glyburide to exon SUR2(39) or SUR2(Δ17/39) expressing variants showed roughly equal channel block (data not shown). These results demonstrate that the four sur2 variants possessing exon 14 yield typical K<sub>ATP</sub>.

Exon 17 Splicing Alters ATP Sensitivity—The four exon 14-containing sur2 variants were inhibited by ATP in a dose-dependent manner, with a 2-fold difference in ATP sensitivity based on the presence or absence of exon 17. ATP in the presence of 0.2 mM Mg<sub>Cl</sub>2 was rapidly applied to the cytoplasmic side of the patch membrane at concentrations varying from 1 to 1000 μM. To avoid bias from any channel run-down, the concentration of ATP was stepped in both increasing and decreasing concentrations. Fig. 5A shows representative current records for the four functional sur2 variants. The open probability, or the averaged current at each ATP concentration, was normalized to that in the absence of ATP. The normalized current values, or relative current, represent the ATP block and are summarized in Fig. 5B. The concentration-dependent block of ATP was quantified by fitting the mean data points with a sigmoidal Hill equation (Fig. 5B). Half-maximal inhibitory concentrations, K<sub>1/2</sub>, and the Hill coefficients, H, for ATP block of channel activity were determined and are listed in Table 1. The data show significant differences between variants with exon 17 and variants without exon 17. Deletion of exon 17 increased the K<sub>1/2</sub> for ATP block approximately 2-fold, regardless of CTD usage. The Hill coefficients for all variants are approximately 1 and the differences among them are insignificant.

Next, we explored whether the differences in single channel gating could account for the reduced ATP sensitivity. Given the observation that CTD usage did not affect ATP sensitivity, we restricted the study of single channel kinetics to variants with exon 39. The single channel behavior of SUR2(39) was compared with SUR2(Δ17/39) at low (1 μM) and high (50 μM) internal ATP, in symmetrical K<sup>+</sup> (140 mM) at a membrane potential of −60 mV. Both variants exhibited kinetics characteristic of striated muscle K<sub>ATP</sub>, resolving into long bursts of channel openings that contained brief intraburst closures separated by interburst gaps (Fig. 6A) (7, 34, 35). Open and closed time constants were obtained using a maximal likelihood fit to the dwell time histograms. The distribution of open and closed times within a burst for each variant were accurately described with a single exponential function. The averaged open time constants, τ<sub>O</sub> and τ<sub>C</sub>, for SUR2(39)·τ<sub>O</sub> = 3.19 ± 0.09 ms and τ<sub>C</sub> =
**Fig. 4. Electrophysiological recordings or COS-7 cells expressing sur2 splice variants cotransfected with Kir6.2.** A, current traces of single channels recorded from KATP generated by coexpressing mouse Kir6.2 and the indicated mouse sur2 variants. C and O represent closed and open channel levels, respectively. The holding potential was 0 mV for all traces. [K’]/[K], = 140/10 ms/mA. Outward current is plotted as an upward deflection. B, single channel current I–V relationships for recombinant KATP. The current amplitudes were measured from all points amplitude histograms. Each data point represents 1–6 patches.

0.37 ± 0.02 ms (n = 7) and SUR2(Δ17/39)-τ_{C1} = 3.30 ± 0.10 ms and τ_{C1} = 0.36 ± 0.04 ms (n = 14) were not significantly different from each other nor did they differ from the corresponding averaged τ_{O} and τ_{C1} values measured in the presence of 50 μM internal ATP, which were SUR2(39)-τ_{O} = 3.12 ± 0.15 ms and τ_{C1} = 0.35 ± 0.01 ms (n = 8) and SUR2(Δ17/39)-τ_{O} = 3.04 ± 0.19 ms and τ_{C1} = 0.37 ± 0.01 ms (n = 8).

The distributions of gaps between bursts for both variants required at least two exponents to fit (Fig. 6B), in close correspondence to previously reported data from cardiac and skeletal K_{ATP}, which has been described by a four-state linear sequential model (32, 35). The suitability of such a model, consistent with the model we derived from our duration histogram analysis (Fig. 7A), was tested independently by subjecting our raw single channel recordings to a maximum likelihood computational method for the modeling of an aggregated Markov process, as described by Qin et al. (36). The resulting four-state linear sequential model generated by this method was identical to the model based on our histogram analysis and to the previously described models (32, 35). The rate constants for this kinetic scheme, summarized in Fig. 7A, were calculated from the parameters determined by the maximum likelihood fits to the dwell time histograms (Fig. 6B). At 1 μM ATP, SUR2(39) differed significantly from SUR2(Δ17/39) for rate constants directed away from the open state: k_{C2} = 5.8 s^{-1} versus 3.4 s^{-1} (p < 0.05) and k_{C3} = 6.9 s^{-1} versus 5.4 s^{-1} (p < 0.05). At 50 μM ATP, both variants exhibited significant increases in the rate constants directed away from the open state (k_{C2} and k_{C3}), but the magnitude of the change was similar: k_{C2} for SUR2(39) increased 2.9-fold versus a 2.3-fold increase in k_{C2} for SUR2(Δ17/39), and k_{C3} for SUR2(39) increased 4.6-fold versus a 3.8-fold increase in k_{C3} for SUR2(Δ17/39). An appreciable difference between two variants was reflected in the rate constant directed toward the open state, k_{O2}, at 50 μM ATP. Whereas k_{O2} at 1 μM ATP showed no difference between the variants, a 2-fold decrease in k_{O2} was observed for SUR2(39) at 50 μM ATP. The k_{O2} for SUR2(Δ17/39) remained unchanged with increases in the ATP concentration. The difference in k_{O2} was reflected both in the calculated mean burst duration and in the mean interburst gap duration for each variant (Fig. 7B). The mean burst duration of SUR2(Δ17/39) in 1 μM ATP, 1.5-fold longer than that of SUR2(39) (220 ± 14 ms versus 166 ± 13 ms), did not differ from its duration at 50 μM ATP. In contrast, the mean burst duration for SUR2(39) decreased 2-fold between 1 and 50 μM ATP. The mean interburst gap durations did not differ significantly between the two variants at 1 μM ATP. The mean interburst gap duration for SUR2(39) increased 7.2-fold at 50 μM ATP (from 45 ± 15 to 325 ± 58 ms), whereas SUR2(Δ17/39) increased only 1.9-fold (32 ± 5 to 63 ± 26 ms).

**DISCUSSION**

**Contribution of Alternative Splicing of sur2 to K_{ATP} Channel Diversity—K_{ATP} channel properties vary widely in different cell types, particularly with regard to nucleotide block and drug sensitivity (3, 4, 37, 38). This diversity may meet the various physiological and pathophysiological roles of K_{ATP} in the wide range of tissues. Many factors such as pH, cell membrane composition, and other nucleotides affect ATP and pharmacological sensitivity of K_{ATP} and contribute to the observed diversity in tissue response (3, 4, 9, 39–41). In addition, channel subunit isoform composition may also intrinsically regulate K_{ATP} response. The cloning of cDNAs for K_{ATP} subunits, sur1 (14), sur2 (15, 16), Kir6.1 (19) and Kir6.2 (20), and the demonstration of distinct nucleotide and pharmacological sensitivities between recombinant K_{ATP} (16, 20) indicated that a degree of the observed K_{ATP} diversity may result from the assembly of different channel subunit isoforms. Specifically, the sur2 subunit has been found to confer characteristic features for K_{ATP} including drug sensitivity and ADP activation. The distinct tissue expression patterns reported for sur1 and sur2 isoforms likely contribute to observed tissue-specific K_{ATP} channel diversity. Furthermore, Isomoto et al. (22) demonstrated that the alternative CTD usage of SUR2(40) results in recombinant K_{ATP} with altered response to the antihypertensive drug diazoxide. They argued that the properties of the reconstructed channel correspond to the native smooth muscle K_{ATP}. Here, we identify alternative splicing of exon 17, which alters nucleotide sensitivity by 2-fold. The alternate usage of exon 17 in sur2 follows a consistent pattern with previously observed tissue-specific differences in ATP sensitivities for K_{ATP} and as such may be a contributing factor to the overall tissue differences in ATP sensitivities. For example, cardiac myocytes, which express sur1, full-length sur2, and SUR2(Δ17), ATP block over the range 20–100 μM has been reported (4, 42); vascular smooth muscle, likely only expressing full-length sur2, ~30 μM (43); kidney, expressing full-length sur2 and SUR2(Δ17), ~100 μM (44); skeletal muscle, which predominantly expresses SUR2(Δ17), ~130 μM (7); and the β-cell, which expresses sur1, ~10–20 μM (3). It is important to note, however, that Kir6.2 is expressed primarily in the pancreas and in heart (20), suggesting that sur2 must associate with another Kir, possibly Kir6.1, in other tissues and thus may possess significantly different ATP inhibition properties. Indeed, when SUR2(40) has been coexpressed with Kir6.1 the resulting sulfonylurea-sensitive channels were unlike tradi-
tional $K_{\text{ATP}}$ in that they did not spontaneously open when excised into an ATP/free bath solution (45).

$K_{\text{ATP}}$ molecular heterogeneity is further extended with the identification of five different sur2 variants (Fig. 2). The exon selection and relative transcriptional levels occur in a tissue-specific pattern with $D_{14}$ usage restricted to heart and $D_{17}$ predominant in skeletal muscle and gut and high in bladder. Interestingly, as exon 39/40 usage has already proven to discriminate diazoxide sensitivity, it is conceivable that exon 17 and $D_{17}$ may prove to be targets for unique drug interactions, thus providing $K_{\text{ATP}}$ drug/tissue specificity for some tissues, such as bladder or gut.

Previously, we described the identification of an sur2 variant lacking exon 14, SUR2(D14/39) (15). In this study we tested the functional expression of this variant with Kir6.2 and found that it yielded undetectable currents. This information is important in the following two respects: first, it reveals that the peptide segment for exon 14 may be critical to form functional chan-

**FIG. 5.** Dose-dependent responses of ATP for sur2 splice variants expressed in COS-6 cells. A, representative current recordings of ATP block for reconstituted $K_{\text{ATP}}$ from the coexpression of Kir6.2 and the indicated sur2 variants. Currents were filtered at 200 Hz. The holding potential was set at 0 mV. $[K^+]_i = 140/10$ mM/mM. Outward current is plotted as an upward deflection. $i = 0$ indicates the level where all channels are closed, and individual channel levels are marked to indicate the number of channels per patch. ATP was applied to the internal face of the membrane at the micromolar concentrations indicated. The fast downward deflections in the data traces correspond to solution change artifacts. 1 mM ATP was included in the internal solutions whenever the ATP concentration is not provided. B, dose-response relationships of ATP block for each sur2 variant coexpressed with Kir6.2. The curves represent fits of the experimental data to the Hill equation: $I_{rel} = 1/(1 + ([\text{ATP}]/K_{1/2(\text{ATP})})^H)$, where $I_{rel}$ is the current amplitude relative to the peak current at 1 mM ATP, $K_{1/2(\text{ATP})}$ is the concentration for half-maximal ATP block, and $H$ is the Hill coefficient. The data points are the mean ± S.D. from the number of experiments listed in Table I. * indicates a significant difference of $p < 0.05$ between the relative current levels for the exon 17+ variants versus the exon 17 variants, as determined by the paired Student's $t$ test.

| ATP block of $K_{\text{ATP}}$ expressed from SUR2 splice variants and Kir6.2 |
|-----------------|-----------------|-----------------|-----------------|
|                   | SUR2(39)        | SUR2(Δ17/39)    | SUR2(40)        | SUR2(Δ17/40)    |
| Number of tests  | 15              | 9               | 4               | 4               |
| [ATP] block, $K_{1/2}$ | 44.0 ± 7.4*    | 90.0 ± 22.3*    | 35.8 ± 6.1**   | 88.6 ± 27**    |
| Hill Coefficient, $H$ | 0.90 ± 0.10    | 0.90 ± 0.18     | 1.13 ± 0.14     | 0.78 ± 0.16     |

Levels of significance were determined using the paired Student's $t$ test. Both half-maximal inhibition ($K_{1/2}$) and the Hill coefficients were determined from the best fits of the data to the Hill equation as demonstrated in Fig. 5B. For comparison * versus * and ** versus **. Both are $p < 0.01$.  

Table I  
ATP block of $K_{\text{ATP}}$ expressed from SUR2 splice variants and Kir6.2.
nels; second, this variant has the potential to serve as a negative suppressor of the sur2 and so K<sub>ATP</sub> expression. However, we did not find an obvious suppressing effect in the test of the coexpression of this variant with SUR2(39) using transient transfection. Direct methods to assay the levels and subcellular expression patterns of SUR2(D14/39) will be needed to confirm this result.

The approach of chimeric SUR/Kir fusion proteins has provided strong evidence that the K<sub>ATP</sub> complex consists of a hetero-octamer, with the sur and Kir subunits in equal stoichiometry (17, 18). Of potential consequence, sur1 and sur2 demonstrate overlapping expression in heart and possibly skeletal muscle (16, 46), and sur2 and SUR2(Δ17) variants show overlapping expression in most tissues (Fig. 1, A and B). If the subunit isoforms and variants are coexpressed in the same cell, it is possible that individual K<sub>ATP</sub> subunits could coassemble into heterogeneous K<sub>ATP</sub> octameric complexes. This would significantly expand the K<sub>ATP</sub> subunit combinations possible. Considering that our tissue distribution data were collected from the whole organs that contained different types of cells, it still remains to be determined whether the variants are indeed coexpressed in a single cell.

As both full-length and SUR2(Δ17) mRNA species are present in most tissues where sur2 is expressed, the alternative splicing of exon 17 may provide an intriguing mechanism to regulate K<sub>ATP</sub> sensitivity with respect to the cellular metabolic environment. Recently, Akao et al. (47) demonstrated that the Kir6.2 subunit of the cardiac K<sub>ATP</sub> is regulated at the transcriptional level in response to induced cardiac ischemia, and we have observed altered sur2 transcript levels in cardiac and skeletal muscle tissues of rats subjected to fasting. Additionally, Xie and McCobb (48) observed an example of environmentally controlled splice regulation by demonstrating that stress hormones could regulate the alternative splicing of a K<sub>1</sub> channel transcript. This suggests transcriptional or post-transcriptional regulation (including exon usage) may have a role for altering, or resetting, overall ATP sensitivity of K<sub>ATP</sub> current in cells experiencing periods of metabolic stress. Thus, by expressing different sur2 variants in different cells within a tissue, or within a single cell to form chimeric channels which may also involve a dynamic regulation of subunit expression levels, the alternative splicing of sur2 reported here is a significant mechanism employed in vivo to broaden the repertoire of K<sub>ATP</sub>.

ATP Block and the Underlying Kinetic Mechanism of Reconstructed K<sub>ATP</sub>—A novel finding in theses studies is the alternation in ATP block in response to exon 17 usage. We found that the K<sub>15</sub> for ATP block were around 40 μM for full-length sur2 and 90 μM for SUR2(Δ17) variants. Interestingly, the CTD

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4 W. A. Chutkow and Z. Fan, unpublished observations.
usage did not significantly affect ATP sensitivity. The $K_{17}$ for exon 17 containing sur2 are similar to those previously reported (16, 22, 46). The Hill coefficient, which is determined by the nature of the ATP binding process, reportedly varies between 1 and 2 in both native striated muscle KATP and recombinant KATP (5, 17, 22). Here, under uniform conditions, we found that the Hill coefficient for all four variants was approximtely 1, suggesting that the overall ATP binding to block the channels follows an apparent single site binding process.

The ATP block of striated muscle KATP has been described with a four-state linear sequential model (32, 35), similar to the model presented in Fig. 7. In this model the transition O ↔ C1 is ATP-independent, whereas the transitions C3 ↔ C2 ↔ O are ATP-dependent processes. In agreement with the observation in native cardiac KATP (35), our data indicated that the four rate constants, $k_{22}, k_{23}, k_{2O}, k_{O2}$, all varied with internal ATP concentration, with $k_{2O}$ the least variable. The ATP dependence of the rate constants leading to the open state, $k_{22}$ and $k_{2O}$ for the SUR2(39) variant, indicated possible cooperativity of several ATP-binding sites or the regulation of ATP block by a fourth channel has been described, an engineered truncation of Kir6.2 lacking its carboxyl-terminal 26 residues (Kir6.2Δc26), which expressed as an ATP-sensitive channel in the absence of the sur subunit (51). From a casual inspection of the four different recombinant KATP channel burst lengths, the burst duration sequence is SUR2(Δ17/39) > SUR2(39) > sur1 > Kir6.2Δc26, in the absence of ATP (Fig. 7 (20, 51)). This sequence indicates the importance of sur in the determination of the burst kinetics of this channel.

Structural Basis of Exon 17 Affecting KATP Function—The peptide segment encoded by exon 17 is a part of the NBF1 structure and is in close proximity to the Walker A motif. Its location suggests an intimate association between this segment and the putative ATP binding pocket of NBF1. The highly conserved Walker A motif is believed to be a critical part of a site for ATP binding and hydrolysis (52, 53). A consensus structure model has been proposed for the nucleotide binding pocket of NBF-containing proteins which consists of a series of six α-coil/β-strands (54). According to this model, the segment of exon 17 begins with the end of β-strand 2 and encompasses a critical residue predicted to participate in nucleotide hydrolysis. Alternatively, sequence homology between sur2 and other ABC proteins suggests that the exon 17 segment may comprise a portion of the putative switch I region identified in G-binding proteins which is predicted to affect nucleotide binding and/or signal transduction upon nucleotide hydrolysis (23, 27). The NBF of some ABC proteins has been shown to possess ATPase activity (26, 28). Although Ueda et al. (25) have demonstrated ATP binding to sur1, intrinsic ATPase activity by sur has yet to be directly demonstrated.

Our finding of the reduction in ATP sensitivity with the removal of exon 17 suggests that the exon 17 segment contributes to nucleotide responsiveness of the Kir6.2-sur2 complex. However, our electrophysiological data are insufficient to distinguish between the direct involvement of exon 17 structure in ATP binding causing channel block or an indirect effect on the mechanism of ATP block. Shyng et al. (29) proposed a model for sur as an ATP-supersensitizing switch. In this model, the sur subunit enhances the intrinsic ATP block of Kir. ATP hydrolysis at one or both of sur’s NBFs uncouples the ATP hypersensitivity sur imposes on Kir. Our finding suggests that the capability of SUR2(Δ17) of enhancing ATP sensitivity is significantly diminished or lost. In the context of the supersensitizing model, Δ17 could result in reduced ATP affinity at NBF1 thus preventing the key substrate for the hypersensitizing switch from binding, or Δ17 enhances the rate of ATP hydrolysis and facilitates an uncoupling to the desensitized state, or perhaps both. Alternatively, should exon 17 play a role in the signal transduction through which a hydrolytic signal induces an sur conformational change to the desensitized state, SUR2(Δ17) might then resemble an sur locked into a desensitized conformation. To test the validity of these interpretations, it will be important to determine ATP binding and hydrolysis at NBF1 for the two splice forms.

In conclusion, we have described five distinct sur2 transcripts resulting from the alternative splicing of the single sur2 gene, and we demonstrated that the alternative splicing of exon 17 affects the ATP block sensitivity of recombinant KATP. Together with the tissue-specific expression, alternative splicing of sur2 may contribute a meaningful role in determining KATP physiological functions.

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