A Novel ABCC8 (SUR1)-dependent Mechanism of Metabolism-Excitation Uncoupling

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ATP/ADP-sensing (sulfonylurea receptor (SUR)/KIR6)2 KATP channels regulate the excitation of our insulin secreting and other vital cells via the differential MgATP/ADP-dependent stimulatory actions of their tissue-specific ATP-binding cassette regulatory subunits (sulfonylurea receptors), which counterbalance the nearly constant inhibitory action of ATP on the K+ inwardly rectifying pore. Mutations in SUR1 that abolish its stimulation have been found in infants persistently releasing insulin. Activating mutations in SUR1 have been shown to cause neonatal diabetes. Here, analyses of KATP channels with diabetogenic receptors reveal that MgATP-dependent hyperstimulation of mutant SUR can compromise the ability of KATP channels to function as metabolic sensors. I demonstrate that the channel hyperactivity rises exponentially with the number of hyperstimulating subunits, so small subpopulations of channels with more than two mutant SUR can dominate hyperpolarizing currents in heterozygous patients. I uncovered an attenuated tolbutamide inhibition of the hyperstimulated mutant, which is normally sensitive to the drug under non-stimulatory conditions. These findings show the key role of SUR in sensing the metabolic index in humans and urge others to retest mutant SUR/KIR6 channels from probands in physiologic MgATP.

Inborn errors of glucose homeostasis and metabolism (1) have illuminated a vital link between a metabolic index, the ATP/ADP ratio, and cellular excitability that utilizes ABCC8/9 (KCNJ11) encoded KATP channels as metabolic sensors (2, 3). These tetradsimeric channels are proposed to link the cell membrane potential, Vm, with the ATP/ADP ratio via the differential stimulatory actions of Mg2+/ATP/ADP on their cell type-specific regulatory sulfonylurea receptor (SUR)2 subunits (s). Like other members of the largest family of eukaryotic membrane transport proteins (4), SUR possess two non-equivalent nucleotide-binding domains, NBD1 and NBD2. Magnesium-nucleotide-bound NBD1/NBD2 dimers counterbalance a magnesium-independent nucleotide inhibition of the KATP pore, an effect essentially saturated in intact cells by ATP present at >100 times the IC50 (ATP) (5). Consistent with this mechanism, loss-of-stimulation mutations in NBD of SUR1 (ABCC8), the neuroendocrine-type receptor, have been discovered in infants with persistent hyperinsulinemic hypoglycemia (6), whereas mutations in ABCC8 that overactivate KATP in millimolar MgATP have been shown to cause permanent or transient neonatal diabetes, including ND with neurological symptoms (7). Several ND mutations map to the transmembrane (TM) domains of the ABCC8 core, TMD1 and TMD2, whose role in controlling the nucleotide-dependent open channel probability (Po) needs to be understood.

This study is the first analysis of permanent ND currents caused by a heterozygous mutation in a key TMD2-coding region of ABCC8, ABCC8Q1178R (NDSUR1) found in a proband with normal KCNJ11 (8). To understand how ABCC8Q1178R (NDSUR1) hyperactivate the heterozygous KATP ensemble, I used direct approaches, including recordings of single channels with the differentially restricted receptor composition, as well as structural modeling of the receptor core. The results reveal a novel mechanism of channelopathies, explain why the smallest subpopulation of hyperstimulated KATP channels can make a large contribution to the pathogenic conductance in heterozygous patients, and reveal that normal tolbutamide sensitivity of non-stimulated mutant SUR does not guarantee their normal response to the drug in physiologic MgATP.

EXPERIMENTAL PROCEDURES

Mutagenesis, sequencing, cell culture and transfections were done as described previously (7). Gln-1178 is conserved in all SURs. All the described mutations were introduced into hamster SUR1 cDNA (9). Concatemers were engineered as described previously (5); different and similar subunits were fused via –TSGGG– and –SGGGASGGG– linkers, respectively. The cDNA construct(s) were co-expressed in COSm6 cells with enhanced green fluorescent protein as described previously (5); heterozygous cells are cells expressing SUR1 and NDSUR1 (1:1 construct ratio) plus KIR6.2.

Patch clamp recording and current analysis was done as described previously (7). The pipette solution contained (in mM): 145 KCl; 1 MgCl2; 1 CaCl2; 10 HEPES; pH 7.4 (KOH). The bath MgCl2-free ([Mg2+]i < 0.1 mM) internal solution contained (in mM): 140 KCl; 5 EDTA; 5 HEPES; 10 KOH; pH 7.2 (KOH). The bath intracellular solution contained (in mM): 140 KCl; 1 MgCl2; 5 EGTA; 5 HEPES; 10 KOH; pH 7.2 (KOH). The [Mg2+]i, in nucleotide-containing solutions was kept at ~0.7 mM by adding MgCl2. The holding potential was ~40 mV. COSm6 cells have negligible background currents, permitting measurements of virtually any low mean KATP currents, I. I analyzed the inwardly directed currents through <102 KATP channel-containing patches, allowing me to verify the unitary
current amplitude, \( i \), in cell-attached configuration from all-points current amplitude histograms and thus accurately determine the on-cell activity of \( n \) identical channels with the mean open probability \( P_{o,n} \times P_{o} = I \times i^{-1} \). The Colquhoun-Hawkes test was used to evaluate the channel singularity. The ligand-independent \( P_{\max} \) determined from single-channel currents and from macro-current noise were similar. The ligand responses of \( K_{\text{ATP}} \) channels were obtained using a programmed rapid solution changer. To correct the ATP dose responses for run-down and/or refreshment, the \( I \) value in the presence of each ATP concentration was normalized to the arithmetic mean of the \( I \) values before application of each [ATP] and after washout. Similar corrections were applied when estimating the relative steady-state activity in the presence of other ligands. The averaged values were expressed as mean ± S.E. for \( n \geq 5 \); differences were evaluated using unpaired \( t \) test (\( p \) values are given in text or figure legends).

Molecular modeling was done as described previously (10) using Sav1866 coordinates (11) and the sequence alignment shown in supplemental Fig. S1. Supplemental Fig. S2 shows a stereo view of the NDSUR1 core model.

**RESULTS AND DISCUSSION**

Fig. 1 shows that NDSUR1 hyper-activate normal \( K_{\text{ATP}} \) pores in intact cells without changing their ligand-independent activity or apparent \( K_{D} \) for inhibitory ATP. The effect of the mutation on the \( P_{o} \) on-cell can be accounted for by its effect on the MgATP-dependent stimulatory action predicted to saturate at millimolar MgATP (5). The ADP in human insulin-secreting \( \beta \)-cells is unlikely to rise above 0.5 mM, and the intracellular ATP in these and other mammalian cells is unlikely to drop below 0.5 mM (12, 13). Fig. 1A, panel b, shows that in 0.5 mM MgATP + 0.5 mM MgADP, approximating severe catabolic conditions, the activity of the NDSUR1 channel is slightly higher than that of the WT channel. This implies that ADP makes a relatively small contribution to the hyperstimulation of NDSUR1 in vivo, where [ADP] should be lower and [ATP] + [ADP] > 1 mM, even in low glucose (12, 13). In the hyperglycemic NDSUR1 subject, the [ATP]/[ADP] > 1. Therefore I determined the fraction of the maximal activity of the NDSUR1 versus WT channel in 1
MgATP/ADP ratios indicates that the Q1178R markedly significant (for NDSUR1K719R ATP-bound MalKE159Q dimer and open MalFG (18). The pro-
posal passed two complementary tests. First, as Mn2+
ules, the differences between the two channel activities on-cell and in 1 mM MgATP; the fractions of the P_{omax} were 0.003 ± 0.0012
and 0.0034 ± 0.0018 versus 0.0025 ± 0.0001 and 0.0028 ± 0.0011
for NDSUR1K719R+K1384R versus SUR1K719R+K1384R channel,
respectively (n = 3 for each). These activities match the activi-
ties of the NDSUR1 and SUR1 channels in 1 mM ATP without Mg2+
(Fig. 1A, panel b).

Fig. 1A, panel b, also reveals that inhibition of the hyper-
stimulated channels by tolbutamide is attenuated, whereas the
same population of NDSUR1 channels under non-stimulatory
conditions normally responds to the same drug. The IC_{50h} =
1.8 ± 0.3 μM, h_h = 1.01 ± 0.07, IC_{50o} = 1305.5 ± 201.3 μM, h_l =
1.09 ± 0.11 and h_l = 0.438 ± 0.017 describing the two-compo-
ponent tolbutamide dose response of the non-stimulated
NDSUR1 channels were undistinguishable from the corre-
sponding parameters for the WT channels under similar exper-
imental conditions (14). Consistent with the latter report and
my model, I hypothesize that the Q1178R mutation reduces the
ability of the sulfonylurea-binding TM domain to destabilize the
magnesium-nucleotide-bound state of the receptor. This is
in line with several observations. (i) The ND mutation is
within the TM15–16 segment of the TM2, which specifies the
stronger inhibition of SUR1- versus SUR2A-containing
channels by tolbutamide (14, 20). (ii) The coupling helix
between TM15 and TM16 interacts with the canonical ATP-
binding domain of the ABCC8 core (supplemental Fig. S2).
(iii) The sulfonylurea binding releases ATP from ABCC8 and
abolishes its stimulation (14, 21).

To further examine the proposed mechanism of metabo-
lism-excitation uncoupling, I obtained full ATP dose responses of
the ND versus WT metabolic sensors with versus without physiologic [Mg2+], and derived their apparent net stimulation
curves. Essential for this analysis, my assay resolves very low
activities of K_{ATP} channels in supramillimolar ATP. The results (Fig. 1B) show the following. (i) The ATP dose response of both
WT and mutant channels, in intracellular concentrations of
free Mg2+ (~0.7 mM), deviates from an inhibitory Hill function
when the concentration of ATP is greater than either the IC_{50(DATP)} or the K_D for the magnesium-independent binding of
ATP at the first Walker A-based site (~<10 μM (16)). (ii) The
deviation is greater for mutant channels in which the hyper-
stimulating conformer is stabilized. (iii) The net stimulatory
action of ATP, reflected by the ratio of the mean open channel
probabilities in ATP with Mg2+ to ATP without Mg2+, is a
sigmoidal (logistic) function, and C_{50} values for NDSUR1 and
WT SUR1 are comparable with the apparent K_{AP} values
for nucleotide binding, K_{DATP(ADP)} and K_{ATP} for ATP hydrolysis
at the lower affinity, second Walker A-based site of SUR1 and
SUR1-K_{M652} fusion (see Refs. 16 and 22, respectively, and note
that all of the K values for the ABCC7 channel are also between
0.1 and 1 mM (23), whereas biochemical experiments on SUR1
indicate a very low, ADP-insensitive hydrolytic activity (16, 24).
(iv) The net stimulation saturates with a much higher
maximum for the NDSUR1 channel. These findings reinforce
the conclusion that the ND mutation in the TMD2 stabilizes
the magnesium-nucleotide-bound ABCC8 core whose lower
affinity Walker A-based site specifies the stimulatory profile of
the neuroendocrine-type metabolic sensors.

The ND mutation exerts its pathogenic action in the het-
erygous state. To deduce which species of the heterogeneous
ND-K_{ATP} ensemble dominate the pathogenic conductance,
one must determine the effectiveness of NDSUR1 in mixed
K_{ATP} channels. I solved the problem using heteroconcatemers
(Fig. 2A). Fig. 2B shows an exponential dependence of channel
hyperstimulation on NDSUR1 number. Macroscopic I_{max} from
heterozygous, homozygous, and WT cells (10 for each) were
similar, revealing no effect of the ND mutation on the density of
K_{ATP} channels; the N_{Heterozygous}/N_{WT} and N_{Homozygous}/N_{WT}
K\textsubscript{ATP} channels seems to be sufficient to uncouple excitability from the metabolic index in at least two human cell types with high input impedance, e.g. β-cells and neurons, thus illuminating the key role of cell type-specific regulators of ubiquitous K\textsubscript{ATP} in fine-tuning the V\textsubscript{m} response to the tissue-specific dynamic metabolic rate. The regulatory signal (differential stimulation) is specified by the lower affinity, second Walker A-based site of ABCC8. The disease due to the gain in the ABCC8 response to physiologic [MgATP] implies that evolution has optimized SUR/KIR6.6 channels to monitor the submillimolar ADP at this lower affinity site of the NBD1/NBD2 dimer, positioned several nm away from the membrane phospholipids, when all of the other nucleotide-binding sites in the channel are essentially saturated by intracellular [ATP]. ATP-sensitive K\textsuperscript{+} pores themselves cannot function as sensors of the metabolic index. Nature created high fidelity, low noise metabolic sensors by coupling intrinsically low active weak inward rectifiers with ABC-based integral proteins that decrease the IC\textsubscript{50(ATP)} despite increasing the P\textsubscript{omax} (25) and are large enough to encircle and thus shield the K\textsubscript{tr} pore from promiscuous activators.

The attenuated response of NDSUR1 channels to tolbutamide in the presence, but not absence, of MgATP is consistent with the proposed mechanism of hyperstimulation and reveals a potential overlooked mechanism of sulfonylurea tolerance, a compromised inhibition of receptor stimulation (14). Predicting the effectiveness of the sulfonylurea treatment of ND requires testing recombinant ND channels at physiologic [MgATP]. The daily dose of tolbutamide (per kg of body weight) needed to transfer the NDSUR1 patient from insulin therapy to sulfonylurea treatment is predicted to exceed the currently recommended dose for treatment of type 2 diabetes.

The exponential dependence in Fig. 2B is consistent with the view of K\textsubscript{ATP} channels as concerted tetradimers (5) and may close debates on the stoichiometry of K\textsubscript{ATP} stimulation as four NDSUR1s with intact NBDs stimulate more than three K\textsuperscript{+}rectifiers with ABC-based integral proteins, thus illuminating the key role of cell type-specific regulators of ubiquitou...
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