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Mechanism of KATP hyperactivity and sulfonylurea tolerance due to a diabetogenic mutation in L0 helix of sulfonylurea receptor 1 (ABCC8)

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ABSTRACT

Activating mutations in different domains of the ABCC8 gene-coded sulfonylurea receptor 1 (SUR1) cause neonatal diabetes. Here we show that a diabetogenic mutation in an unexplored helix preceding the ABC core of SUR1 dramatically increases open probability of $(SUR1/Kir6.2)_4$ channel (KATP) by reciprocally changing rates of its transitions to and from the long-lived, inhibitory ligand-stabilized closed state. This kinetic mechanism attenuates ATP and sulfonylurea inhibition, but not Mg-nucleotide stimulation, of SUR1/Kir6.2. The results suggest a key role for L0 helix in KATP gating and together with previous findings from mutant KATP clarify why many patients with neonatal diabetes require high doses of sulfonylureas.

1. Introduction

ABCC8/KCNJ11-coded KATP [1], stimulated by MgATP/ADP at the ABC ATPase and inhibited by ATP at the K^+ inward rectifier [2,3], control the metabolism-dependent excitability of pancreatic β-cells and certain neurons [4,5]. Activating mutations in either KATP gene cause neonatal diabetes (ND), including severe ND with neurological abnormalities (see [6–8] and many additional reports reviewed in [9,10]). How ND mutations in different domains of SUR1 affect KATP open probability (P_O) and inhibition by sulfonylureas (SU) needs to be better understood.

The first functional analysis of ND mutations in the canonical TMD1-NBD1-TMD2-NBD2 core of SUR1 defined its Mg-nucleotide-dependent hyperstimulation as the first, or A type, mechanism of pathogenic KATP overactivity [7]. Additional tests [11] uncovered that hyperstimulated KATP can show reduced responsiveness to sulfonylureas (SU), thus partly explaining why many ND patients need SU dozes exceeding those recommended by the FDA for treatment of adult-onset (type 2) diabetes (discussed in [9,10]).

A strikingly high percentage of ND mutations map to the L0 linker of the TMD0-L0 gatekeeper module (Fig. 1) that couples the SUR1 core with the KATP pore and controls its nucleotide-independent P_{Omax} [12,13]. The first ND mutation found in the L0 linker, L213R, is in the middle of the hotspot region in the putative interface helix [14] and causes severe ND with neurological abnormalities [7]. We hypothesized that the mutation in the domain not required for nucleotide binding to SUR1 [12] hyperactivates KATP through a mechanism that is different from A type mechanism. Consistent with our hypothesis, the diabetogenic F132L in TMD0 of SUR1 increased KATP activity in the absence of nucleotides [15].

The present study validates the hypothesis and establishes the kinetic mechanism of KATP hyperactivity and reduced SU responsiveness.

2. Materials and methods

Genetic and clinical testing showing that the mutation caused ND by compromising insulin release was described elsewhere [7].

Homology models of the Sav1866-like human SUR1 core and the KirBac/KirChimera-like human $K_{IR}6.2$ pore were built as described previously [11,13]. The mean helical hydrophobic moment was computed as described earlier [16].

Mutagenesis, sequencing, cell culture and transfections were done as described earlier [7,11]. Leu-213 is conserved in all SURs. L213R mutation was introduced into hamster SUR1 cDNA. $SUR1_{L213R}$ or

Abbreviations: SUR1, sulfonylurea receptor 1; KATP, $(SUR1/Kir6.2)_4$ channel; ND, neonatal diabetes

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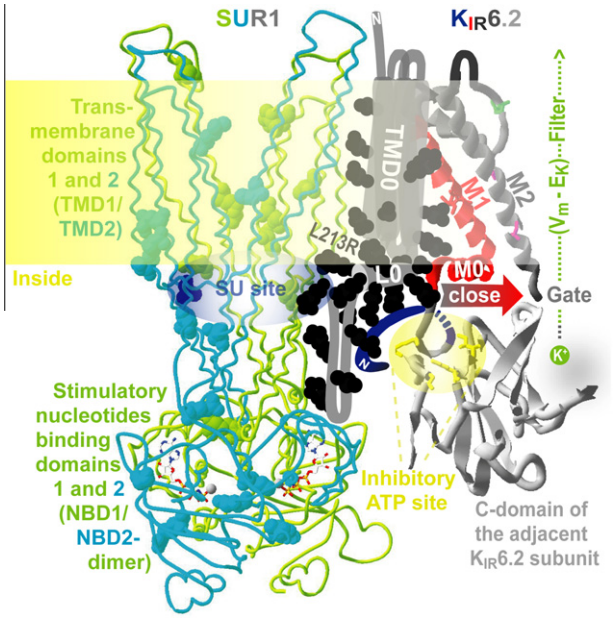

Fig. 1. A working model of a KATP gating unit. One quarter of KATP complex is shown for clarity. ND ABCC8 mutations are mapped as balls. The SUR1 core is shown in a backbone wire presentation. Nucleotides are rendered in licorice style. K_{IR}6.2 pore-forming domains are presented as ribbons. No high resolution structural template is available for the N-terminal domain of SUR1 or the K_{IR} N-tail. L213R and other elements of the model discussed in the text are identified using color-coded labels. The model of SUR1/K_{IR}6.2 coupling predicts the K_{IR}-closing movement of L0 and M0 helices colocalized at the membrane-cytosol interface.

WT receptor was expressed with human K_{IR}6.2 and enhanced green fluorescent protein (a transfection marker) in COSm6 cells lacking any endogenous SUR or K_{IR}.

Expression and SU binding/labeling of mature (complex-glycosylated, ~170 kDa) and immature (core-glycosylated, ~140 kDa) receptors were compared in $SUR1_{L213R}$/K_{IR}6.2- *vs* SUR1/K_{IR}6.2-expressing cells' membranes isolated and photolabeled with ^{125}I-azidoglibenclamide as described previously [17]. The incorporation of ^{125}I-azidoglibenclamide into different bands was estimated by densitometry of the autoradiographs and normalized to the membrane protein concentrations as described earlier [18].

Patch-clamp recording and single-channel kinetics analysis were done as described previously [7,11,19–21]. The pipette solution contained (in mM): 145 KCl; 1 $MgCl_2$; 1 $CaCl_2$; 10 HEPES; pH 7.4 (KOH). The multivalent cation-free 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 $MgCl_2$; 5 EGTA; 5 HEPES; 10 KOH; pH 7.2 (KOH). The $[Mg^{2+}]_i$ in nucleotide-containing solutions was kept at ~0.7 mM. The holding potential was −40 mV. COSm6 cells have negligible background currents, permitting measurements of virtually any low mean KATP currents, *I*, in the native-like environment of mammalian cell membranes. Analysis of currents allowed to verify the unitary current amplitude (*i*) in the cell-attached mode from all-points current amplitude histograms and determine the on-cell activity of *N* identical channels with the mean P_O, $N \times P_O = I \times i^{-1}$. The Colquhoun–Hawkes test was used to evaluate the channel singularity. P_{Omax} determined from single-channel and multi-channel currents were similar. *N* from macrocurrent noise analysis reflected the density of functional channels in the plasma membrane. All-points dwell time distributions were used to determine mean life times of all kinetic states and rates of burst–interburst gating transitions.

Ligand responses of KATP currents were obtained using an automated multi-channel rapid solution changer as described previously [7,11,19–22]. To correct the ATP dose responses for partial rundown and/or refreshment of KATP currents 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 steady-state activity in the presence of other ligands.

Statistical data analysis and curve fitting were done using OriginPro 8 (OriginLab Corporation, Northampton, MA) as described previously [11,22]. Averaged data were expressed as mean ± S.E. for $n \geqslant 5$ with error bars equal to S.E. unless otherwise noted. Significance was evaluated using the unpaired *t* test. Differences with values of $p < 0.05$ were considered to be significant.

3. Results and discussion

Fig. 2 shows that L213R dramatically increases the *Po* in intact cells while not affecting *i* and slightly decreasing *N*. The latter effect is consistent with the small negative effect of L213R on the amount of mature receptor, which is in line with observations that a comparable amphipathic L0 helix of ABCC1 attaches to the membrane [23] and L225P in a less conserved portion of the cytoplasmic linker of SUR1 does not affect *N* [24] or surface expression of SUR1 in the same cell line [25]. The results show that L213R can induce pathogenic currents in intact cells by hyperactivating KATP and support the notion that possible negative effects of some ND mutations on *N* (see also [25]) are overridden by their much stronger effect on P_O.

To establish the principal mechanism of pathogenic increase in on-cell P_O it is essential to determine if and how the mutation

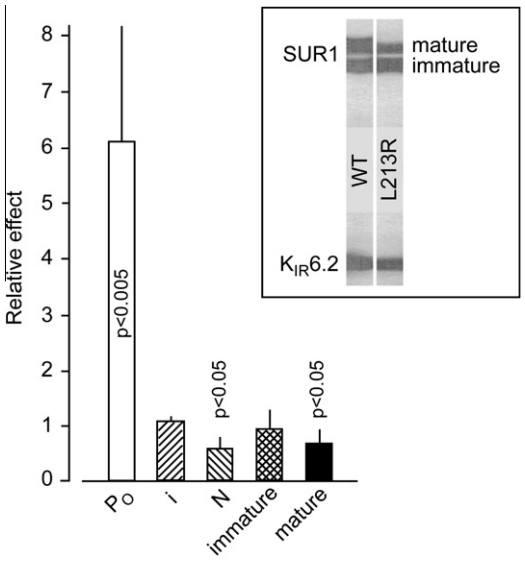

Fig. 2. L213R markedly elevates on-cell P_O, does not affect the unitary conductance, and slightly decreases functional expression of $(SUR1/K_{IR}6.2)_4$ complexes without altering their labeling with 1 nM ^{125}I-azidoglibenclamide. Inset shows similar affinity photolabeling of immature, core-glycosylated $SUR1_{L213R}$ *vs* SUR1 with the second generation SU, and modestly reduced amount of mature, complex-glycosylated mutants trafficking with colabeled K_{IR}6.2. As was reported earlier, nanomolar glibenclamide or micromolar tolbutamide abolish labelling by displacing the photoreactive analogue of glibenclamide which does not label K_{IR}6.2 expressed without SUR1 [17,18,26], the L0 loop apparently coordinates the photoreactive group-containing non-sulfonylurea moiety of the drug [22,27], and deleting the N-terminus of K_{IR}6.2 eliminates its colabeling without disrupting KATP assembly [18] required for trafficking of either SUR1 or glycosylation site-free K_{IR}6.2 to the plasma membrane [26,28]. Therefore, normal affinity labeling of $SUR1_{L213R}$ and K_{IR}6.2, while slightly reduced amount of mature $SUR1_{L213R}$, suggest that the mutation does not alter the SU binding site or the direct proximity of L0 to the K_{IR} N-terminal helix, while slightly reducing KATP trafficking to the plasma membrane; $n \geqslant 5$ for each bar.