A mutation (R826W) in nucleotide-binding domain 1 of ABCC8 reduces ATPase activity and causes transient neonatal diabetes

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Activating mutations in the pore-forming Kir6.2 (*KCNJ11*) and regulatory sulphonylurea receptor SUR1 (*ABCC8*) subunits of the K_{ATP} channel are a common cause of transient neonatal diabetes mellitus (TNDM). We identified a new TNDM mutation (R826W) in the first nucleotide-binding domain (NBD1) of SUR1. The mutation was found in a region that heterodimerizes with NBD2 to form catalytic site 2. Functional analysis showed that this mutation decreases MgATP hydrolysis by purified maltose-binding protein MBP–NBD1 fusion proteins. Inhibition of ATP hydrolysis by MgADP or BeF was not changed. The results indicate that the ATPase cycle lingers in the post-hydrolytic MgADP·P_i-bound state, which is associated with channel activation. The extent of MgADP-dependent activation of K_{ATP} channel activity was unaffected by the R826W mutation, but the time course of deactivation was slowed. Channel inhibition by MgATP was reduced, leading to an increase in resting whole-cell currents. In pancreatic beta cells, this would lead to less insulin secretion and thereby diabetes.

Keywords: SUR1; *ABCC8*; K_{ATP} channel; nucleotide-binding domain; neonatal diabetes; ATP hydrolysis

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INTRODUCTION

Gain-of-function mutations in *ABCC8* (SUR1) and *KCNJ11* (Kir6.2) are a common cause of both transient (TNDM) and permanent

neonatal diabetes (Hattersley & Ashcroft, 2005; Ashcroft, 2007). The sulphonylurea receptor SUR1 acts as the regulatory subunit of the ATP-sensitive potassium (K_{ATP}) channel and assembles with pore-forming Kir6.2 subunits to form an octameric (4:4) complex. Both Kir6.2 and SUR1 act as metabolic sensors, enabling the K_{ATP} channel to respond to changes in cellular metabolism. Binding of ATP to Kir6.2, in a Mg²⁺-independent manner, closes the channel (Tucker *et al*, 1997), whereas Mg-nucleotide binding/hydrolysis by the nucleotide-binding domains (NBDs) of SUR1 stimulates channel opening (Nichols *et al*, 1996). K_{ATP} channel activity is thus governed by the balance between the inhibitory and stimulatory effects of adenine nucleotides, with low metabolism (low ATP, high MgADP) favouring channel activation and high metabolism (high ATP, low MgADP) favouring inhibition.

SUR1 belongs to the ABCC family of ATP-binding cassette (ABC) proteins. In all ABC proteins studied so far, NBD1 and NBD2 associate in a sandwich dimer conformation to form two catalytic ATP-binding sites (sites 1 and 2) (Hollenstein *et al*, 2007). Each site contains residues from the Walker A (W_A) and Walker B (W_B) motifs of one NBD and from the signature sequence of the other NBD: for example, site 2 comprises the NBD1 linker and the W_A and W_B motifs of NBD2. It is believed that occupancy of site 2 of SUR by MgADP leads to K_{ATP} channel opening and, because the intracellular concentration of ATP is much higher than that of ADP, that this is achieved by binding and hydrolysis of MgATP (Fig 1A; Zingman *et al*, 2001).

Metabolic regulation of K_{ATP} channels is crucial for glucose-stimulated insulin secretion (Ashcroft, 2007). In unstimulated pancreatic beta cells, K_{ATP} channels are open, hyperpolarizing the membrane and keeping voltage-gated Ca²⁺ channels closed. When plasma glucose levels rise, glucose uptake and metabolism are stimulated, leading to K_{ATP} channel closure, membrane depolarization and Ca²⁺ channel opening. The consequent Ca²⁺ influx stimulates insulin release. Activating mutations in *ABCC8* or *KCNJ11* cause neonatal diabetes by preventing metabolically induced channel closure (Hattersley & Ashcroft, 2005; Ashcroft, 2007).

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Fig 1 | Sequence and structure of site 2. (A) The SUR1 ATPase catalytic cycle. E is SUR1; E1, E2, ... are various transitional states during ATP hydrolysis; k_1 , k_{-1} , ... are the rate constants for entering and leaving the different states. BeF is shown as trapping the cycle in the E2 · MgATP state. (B) Signature sequence of NBD1 for various ABCC family proteins (consensus: LSxGQ/E). Residues equivalent to SUR1-Arg 826 are shown in bold. (C) Side view along the plane of the membrane of a homology model of SUR1-NBD1. The catalytic core is shown in grey, the helical subdomain in purple and the signature sequence in yellow. MgADP, bound to site 2, is indicated in green. The R 826–W 778–R 842 interaction helps to hold the helical subdomain together. In this view, the transmembrane domains (TMDs) contact NBD1 from above, indicating that Arg 826 is positioned at the NBD-TMD interface. h, human; MRP, multidrug resistance-associated protein; NBD1, nucleotide-binding domain 1; r, rat; Sav1866, bacterial ABC protein.

Here, we report a new functional mechanism for neonatal diabetes: a reduction in the ATPase activity of SUR1, caused by a mutation (R826W) in NBD1 of SUR1, which lies within site 2. The observation that a decrease in ATP hydrolysis at site 2 results in neonatal diabetes is in marked contrast to a previous study that showed that an increase in ATPase activity caused neonatal diabetes (de Wet *et al*, 2007a).

RESULTS

Patient details

The proband was born at 30 weeks gestation with a birth weight of 990 g (third centile). The premature delivery was attributed to maternal pre-eclampsia. Diabetes was diagnosed at birth and was treated with insulin until 7 weeks of age, when the diabetes remitted. The patient is now 11 years old and remains in remission with a random blood glucose level of 5.5 mM and an HbA1c of 5.2% (reference range 4–6%). He has learning delay and has recently been diagnosed with dyslexia and attention-deficit hyperactivity disorder. There is no family history of diabetes.

Molecular genetic analysis

Sequencing of the patient's *KCNJ11* gene failed to detect a mutation, but analysis of his *ABCC8* gene identified a heterozygous

mutation R826W (around 2476 Cys>Thr) (Flanagan *et al*, 2007). Neither parent carried the mutation, indicating that it had arisen *de novo* in the proband. Microsatellite analysis confirmed the family relationships.

Location of the mutation

Arg 826 lies immediately amino terminal to the signature sequence of NBD1 and is conserved across most ABCC proteins (Fig 1B). We created a homology model of SUR1 using the 3 Å resolution structure of the ABC transporter Sav1866 (Dawson & Locher, 2006) as a template. In our model (Fig 1C), the signature sequence of NBD1 associates with the W_A and W_B motifs of NBD2 to form nucleotide-binding site 2. Arg 826 does not seem to be directly involved in nucleotide binding but instead lies close to the interface with the transmembrane domains (TMDs).

ATPase activity

ATPase activity was measured on isolated NBD1 fused to maltose-binding protein (MBP) to enhance solubility (de Wet *et al*, 2007b): we assume that NBD1 operates as a homodimer. Wild-type NBD1 hydrolysed ATP with a turnover rate (k_{cat}) of 0.026 s^{−1} and a K_m of 0.39 mM (Fig 2; Table 1), values similar to those reported earlier (Masia *et al*, 2005; de Wet *et al*, 2007a,b). Mutation of Arg 826 to

Fig 2 | ATPase activity is decreased by the R826W mutation. (A) ATPase activity of isolated MBP-NBD1. Wild type (WT): circles, $n = 7$; R826W (RW): triangles, $n = 7$. The lines are the best fit of the Michaelis-Menten equation to the mean data. MBP, maltose-binding protein; NBD1, nucleotide-binding domain 1.

Table 1 | ATPase activities and kinetic constants

Construct	Turnover rate (s^{-1})	V_{max} (nmol P_i /min/mg)	K_m (mM)	n
NBD1-WT	0.026 ± 0.002	17.65 ± 1.21	0.39 ± 0.03	7
NBD1-R826W	$0.016 \pm 0.001^{***}$	$11.33 \pm 0.89^{***}$	0.45 ± 0.04	7

WT, wild type. Significance against NBD1-WT: $***P < 0.001$.

Trp decreased k_{cat} about 1.6-fold ($P < 0.001$) without altering the K_m (Fig 2; Table 1).

As found previously (de Wet et al, 2007a,b), the ATPase activity of an equimolar mixture of wild-type NBD1 and NBD2 was intermediate between that of NBD1 and NBD2 (supplementary Table S1 online). The R826W mutation decreased the ATPase activity of the NBD1-NBD2 mixture by the amount expected from the lower activity of the mutant NBD1 (supplementary Fig S1 and Table S1 online).

Inhibition by BeF was unaltered, suggesting that the affinity for inorganic phosphate (P_i) is not affected (supplementary Fig S2 and Table S2 online). Product-dependent inhibition of ATPase activity of the R826W mutant by MgADP ($K_{i(MgADP)}$) seemed to be unchanged in either homodimers or equimolar mixtures of the NBDs (supplementary Fig S3A,B and supplementary Table S2 online), suggesting that MgADP binding is unimpaired. However, as the affinity for MgADP is very low, modest changes would be difficult to detect.

ATP inhibition of K_{ATP} currents

To examine the effect of the R826W mutation on the nucleotide sensitivity of the K_{ATP} channel, we expressed wild-type or mutant SUR1 with Kir6.2 and applied nucleotides to the intracellular surface of inside-out membrane patches.

In the presence of Mg^{2+} , mutant channels were about 3.5-fold less sensitive to ATP than wild-type channels: half-maximal block (IC_{50}) was produced by 17 and 57 μM ATP for wild-type and mutant channels, respectively (Fig 3A,C; Table 2; $P < 0.001$). The amount of current remaining unblocked at physiological MgATP concentrations (3 mM) also increased (Table 2).

When Mg^{2+} is absent, ATP is thought to influence K_{ATP} channel activity only through Kir6.2 (Gribble et al, 1998). There was a small difference in the ATP sensitivity of wild-type ($IC_{50} = 10 \mu M$) and mutant ($IC_{50} = 14 \mu M$) channels in Mg^{2+} -free solution (Fig 3B,D; Table 2; $P < 0.01$). However, the data are consistent with the idea that the primary effect of the R826W mutation is to modulate the ability of Mg-nucleotides to stimulate channel activity through SUR1.

In support of this idea, there was no obvious effect of the R826W mutation on the single-channel kinetics (supplementary Table S3 online) or the intrinsic open probability (P_o): in the absence of ATP and Mg^{2+} , P_o was 0.43 ± 0.09 ($n = 5$) for wild-type channels and 0.48 ± 0.12 ($n = 5$) for R826W channels.

Channel activation by MgADP

One explanation for the shift in the MgATP concentration-inhibition curve is that the R826W mutation enhances the ability of bound MgADP to transduce a conformational change that results in pore opening. However, no increase in the extent of channel activation by 100 μM MgADP was observed in either the presence or absence of MgATP (Fig 4A; Table 2).

We tested the effect of the K_{ATP} channel opener diazoxide in the presence of 100 μM MgATP (Fig 4B). R826W currents were larger than wild-type currents in 100 μM MgATP in both the presence and absence of diazoxide. However, the extent of diazoxide activation was reduced, being sixfold for wild-type channels and twofold for R826W channels.

For 100 μM MgADP, the rates of activation and deactivation were reasonably well fit by single exponentials. The activation time constant was similar for wild-type (0.78 ± 0.10 s, $n = 10$) and R826W (0.68 ± 0.09 s, $n = 10$) channels. However, deactivation time constants were about threefold slower for R826W (9.7 ± 1.1 s, $n = 10$) than for wild-type (3.3 ± 0.4 s, $n = 10$) channels ($P < 0.001$; Fig 4C).

Effect on the whole-cell K_{ATP} current

Wild-type whole-cell K_{ATP} currents are very small under resting conditions, due to the high intracellular ATP concentration, but are markedly increased by sodium azide, which inhibits mitochondrial metabolism (Fig 4D). Resting R826W currents were about twofold larger than wild-type currents ($P < 0.05$), consistent with their reduced block by MgATP: they were further increased by metabolic inhibition, indicating that the channel is only partly closed at resting ATP. Sulphonylureas are selective inhibitors of K_{ATP} channels that act by binding to SUR: the sulphonylurea tolbutamide blocked both wild-type currents and R826W currents by about 94% ($n = 10$).

DISCUSSION

Our data show that the R826W mutation in SUR1 reduces K_{ATP} channel inhibition by MgATP, thereby increasing the resting K_{ATP} current about twofold. An enhanced K_{ATP} current would be expected to hyperpolarize the pancreatic beta cell, even in the

Fig 3 | ATP block of wild-type and mutant K_{ATP} channels. (A,B) Macroscopic wild-type and R826W K_{ATP} currents recorded at -60 mV in an excised patch in the (A) presence and (B) absence of Mg^{2+} . The dashed line indicates the zero current level. Application of $10\text{ }\mu\text{M}$ ATP is indicated by the bar. (C,D) Mean relationship between (C) $[MgATP]$ or (D) $[ATP]$ and K_{ATP} current (I), expressed relative to that in the absence of nucleotide (I_c), for wild-type (open circles, $n=5$) and R826W (filled circles, $n=5$) channels. Membrane potential, -60 mV. In (C) and (D), the lines are the best fit of the Hill equation to the mean data: (C) wild type, $IC_{50}=17\text{ }\mu\text{M}$, $h=0.97$; R826W, $IC_{50}=48\text{ }\mu\text{M}$, $h=0.82$, $a=0.02$. (D) Wild type, $IC_{50}=9.5\text{ }\mu\text{M}$, $h=1.2$; R826W, $IC_{50}=14.5\text{ }\mu\text{M}$, $h=1.2$.

Table 2 | Nucleotide modulation of wild-type and mutant channels

Parameter	Wild type	R826W
IC_{50} (Mg^{2+} solution)	$17 \pm 3\text{ }\mu\text{M}$ ($n=5$)	$57 \pm 4\text{ }\mu\text{M}$ ($n=5$)***
IC_{50} (Mg^{2+} -free solution)	$10 \pm 1\text{ }\mu\text{M}$ ($n=6$)	$14 \pm 1\text{ }\mu\text{M}$ ($n=6$)**
Current remaining in 3 mM $MgATP$	$1.0 \pm 0.1\%$ ($n=6$)	$6.4 \pm 1.1\%$ ($n=6$)*
Activation: 0.1 mM $MgADP$	2.8 ± 0.2	2.5 ± 0.5
Activation: 0.1 mM $MgATP$	0.27 ± 0.02	$0.43 \pm 0.06^*$
Activation: 0.1 mM $MgADP + 0.1\text{ mM}$ $MgATP$	1.1 ± 0.2	1.2 ± 0.5

Activation: fold increase in current produced by $MgATP$ (0.1 mM) or $MgADP$ (0.1 mM) expressed relative to that in the absence of nucleotide, or by 0.1 mM $MgADP$ added in the presence of 0.1 mM $MgATP$, expressed relative to that in 0.1 mM $MgATP$ alone; IC_{50} , half-maximal block produced by ATP (no Mg^{2+}) or $MgATP$; * $P \leq 0.05$, ** $P \leq 0.01$; *** $P \leq 0.001$ against wild type.

presence of glucose, thereby reducing insulin secretion and accounting for the diabetes of patients carrying the R826W mutation. Why the diabetes is transient is unclear but might be related to the fact that, similar to other TNDM mutations, the mutation causes only a small increase in K_{ATP} current (Ashcroft, 2007). The efficacy of tolbutamide in closing mutant K_{ATP}

channels suggests that if the patient's diabetes relapses, it should respond to sulphonylurea therapy, as found for many *KCNJ11* and *ABCC8* mutations (Pearson *et al*, 2006).

Molecular mechanism: MgADP activation

As the R826W mutation has only a small effect on ATP block in the absence of Mg^{2+} (which is mediated solely through Kir6.2), the shift in the $MgATP$ dose-inhibition curve must be mainly due to enhanced Mg -nucleotide activation at SUR1.

Mutant and wild-type currents were activated to a similar extent by $MgADP$. Mutant channels were slower to deactivate following $MgADP$ removal but showed equally fast activation. This would be expected to enhance $MgADP$ activation, which was not observed. Thus, we postulate that the activation rate was also decreased by the R826W mutation but was not measurable experimentally: this is reasonable, as $MgADP$ activation rates were similar to those of solution exchange. We conclude that the slower $MgADP$ deactivation of R826W currents is unlikely to be responsible for the shifted $MgATP$ dose-inhibition curve, as $MgADP$ activation is unaffected. Similarly, mutation of Gly827 caused a marked slowdown of both $MgADP$ deactivation and activation, but did not alter $MgATP$ inhibition (Shyng *et al*, 1997).

Molecular mechanism: MgATP activation

Channel activation by $MgATP$ requires binding/hydrolysis of the nucleotide at the NBDs of SUR1, with bound $MgADP$ stimulating channel opening. Assay of the ATPase activity of the isolated NBDs showed that the rate of P_i release is reduced by the R826W

Fig 4 | Nucleotide and metabolic regulation of wild-type and mutant channels. (A) Mean K_{ATP} current recorded in the presence of 100 μ M MgADP, 100 μ M MgATP or 100 μ M MgATP + 100 μ M MgADP ($n = 5$). Current (I) is expressed relative to that in control solution (I_c). (B) Mean K_{ATP} current recorded in the presence of 100 μ M MgATP or 100 μ M MgATP + 100 μ M diazoxide ($n = 6$). Current (I) is expressed relative to that in control solution (I_c). Scale bar is different from that in (A). (C) Rate of current deactivation following removal of 100 μ M MgADP for wild-type (open circles) and R826W (filled circles, $n = 10$) channels. (D) Mean steady-state whole-cell K_{ATP} currents evoked by a voltage step from -10 to -30 mV before (control, grey bars) and after (white bars) the application of 3 mM azide, and in the presence of 3 mM azide plus 0.5 mM tolbutamide (striped bars) for wild-type ($n = 10$) and R826W channels ($n = 10$). * $P \leq 0.05$ against wild type (t -test).

mutation, indicating that the ATPase cycle is slowed (Fig 1A). The K_m for MgATP catalysis and the $K_{i(BeF_3)}$, however, were unchanged. Thus, it seems that the mutant ATPase lingers in the post-hydrolytic state ($E3 \cdot MgADP \cdot P_i$; Fig 1A). This would imply that the MgADP $\cdot P_i$ -bound state ($E3$), similar to the MgADP-bound state ($E4$), must transduce a conformational change that causes channel activation (and thereby decreases MgATP inhibition).

Both the R826W and R1380C mutations cause neonatal diabetes, yet R1380C enhances ATPase activity (de Wet *et al*, 2007a) and, as shown here, R826W reduces ATPase activity. This paradox is resolved by the fact that both mutations increase the time spent in the MgADP-bound states $E3$ and $E4$, which are associated with channel activation.

Comparison with other inherited SUR NBD mutations

This is the first report of a naturally occurring mutation in NBD1 of SUR that affects ATPase activity. Two detailed studies of the effects of inherited mutations in NBD2 of SUR on ATPase activity have been published so far. The first is the R1380C mutation in SUR1, discussed above, that enhances ATPase activity (de Wet *et al*, 2007a). The other study focused on two mutations in NBD2 of SUR2A, A1513T and L1524fs, that cause dilated cardiomyopathy (Bienengraeber *et al*, 2004). Both mutant MBP-NBD2s had

lower ATPase activity than that of wild type: the A1513T mutation decreased the off rate of MgADP (k_{-4}), whereas L1524fs decreased k_3 (Fig 1A).

Structural considerations

In a homology model of SUR1, Arg826 forms a putative cation- π interaction with Trp778, which in turn forms a second possible cation- π interaction with Arg842 on the opposite side of the ring (Fig 1C). Interestingly, R826W causes TNDM, whereas R842G is a known congenital hyperinsulinaemia mutation (Fournet *et al*, 2001).

Trp778 lies close to the conserved Q-loop glutamine (Gln775), which is located at the N-terminal end of the helical subdomain (HD) of NBD1 (Fig 1C). Crystallographic and molecular dynamic simulation studies of the NBDs of other ABC transporters suggest that, during the ATPase catalytic cycle, the HD rotates as a rigid body with respect to the catalytic core of the NBD (Zaitseva *et al*, 2006; Jones & George, 2007). The hinge region for the HD rotation has been proposed to lie close to the Q-loop. It seems possible that the R826-W778 interaction might contribute to the stability of the HD and that disruption of this interaction could impair movement of the HD as a rigid body. As a consequence, the signature sequence might not rotate away with the rest of the HD, thus slowing down the dissociation of MgADP

and prolonging the respective conformational change in SUR1. Comparative molecular dynamic simulations of wild-type and mutant NBD heterodimer models suggest that the R826W mutation alters the structure and dynamics of the HD and D-loop of NBD1 and might thereby affect ATP hydrolysis at site 2 (supplementary information online).

Owing to its location on the membrane-facing side of NBD1, Arg826 might participate in interactions across the NBD–transmembrane domain interface. In our model, Arg826 lies close to Glu1209 and Glu1206 in cytosolic loop 7 of SUR1 (supplementary Fig S5 online). Mutation of Arg826 might therefore not only (allosterically) influence nucleotide binding/hydrolysis at site 2 but also affect the mechanism by which this is transduced into changes in the opening and closing of the Kir6.2 pore.

Speculation

Caution is needed when comparing measurements of Kir6.2/SUR1 channel activity with ATPase assays of isolated NBD1. Nevertheless, our results indicate that the reduced MgATP inhibition of R826W currents might be the consequence of stabilization of the post-hydrolytic ($E_3 \cdot \text{MgADP} \cdot P_i$) state at site 2. The observation that the extent of current activation by MgADP is unaltered is explained by the fact that the direct binding of MgADP leads to the E_4 rather than the E_3 state (Fig 1A).

METHODS

Molecular genetic analysis. Genomic DNA was extracted from peripheral lymphocytes using standard procedures. The *KCNJ11* and *ABCC8* genes were amplified and sequenced as described previously (Flanagan *et al*, 2007). *ABCC8* sequences were compared with the published sequence NM_000352.2, which incorporates the alternatively spliced residue in exon 17 (L78208 and L78224). Microsatellite analysis of three markers on chromosome 11 (D11S1901, D11S1902 and D11S921) confirmed the family relationships.

Nucleotide hydrolysis by NBD1. The carboxyl terminus of MBP was fused to the N terminus of the first NBD of rat SUR1 (residues Val 618–Leu 1004) using the pMal-c2 vector system (New England Biolabs Inc., Ipswich, MA, USA) and expressed as a fusion protein (abbreviated as NBD1). Site-directed mutagenesis was carried out according to the manufacturer's instructions (QuickChange SDM kit, Stratagene, La Jolla, CA, USA). Wild-type and mutant proteins were purified, and purity and integrity were confirmed, as described previously (de Wet *et al*, 2007b; supplementary Fig S6 online).

ATPase activity was measured using a colorimetric assay for liberated P_i at 37 °C (de Wet *et al*, 2007b). Experimental repeats (n) refer to separate protein preparations. All protein preparations were assayed in duplicate. Values are mean $\pm$ s.e.m. The Michaelis–Menten equation was fitted to concentration–activity relationships and used to obtain the K_m . Turnover rate (nmol P_i released/s/nmol protein) was calculated using molecular masses of 87 kDa for MBP–NBD1 and 74 kDa for MBP–NBD2. The IC_{50} values for BeF inhibition were calculated by fitting the data to the Langmuir equation: $y = B + \{1/[1 + ([I]/IC_{50})]\}$, where y is the ATP hydrolysis rate, IC_{50} is the concentration of the inhibitor (I) at half-maximal inhibition and B is the level of remaining ATPase activity at maximal inhibition (where $B = 0$ for complete inhibition).

K_i values were calculated from IC_{50} using $K_i = IC_{50}/(1 + \{[ATP]/K_m(ATP)\})$ (Cheng & Prusoff, 1973).

Electrophysiology. Wild-type or mutant rat SUR1 was expressed with human Kir6.2 in *Xenopus* oocytes. Whole-cell currents, as well as macroscopic and single-channel currents from inside-out membrane patches, were recorded (de Wet *et al*, 2007a; supplementary information online). Nucleotide-containing solutions were applied using a rapid solution changer (RSC-200, BioLogic Science Instruments, Claix, France). Current (I) is expressed as a fraction of the mean of that in control solution (I_c) before and after the application of nucleotide-containing solution. ATP dose–response curves were fitted with

$$I/I_c = a + (1 - a)/(1 + ([ATP]/IC_{50})^h)$$

where IC_{50} is the ATP concentration at which the inhibitory effect of ATP is half-maximal. h is the Hill coefficient and a is the fraction of unblocked current ($a = 0$ except for R826W in Fig 3C). Deactivation of K_{ATP} currents after MgADP activation was fitted with a monoexponential function:

$$I_{KATP}(t) = A \times \exp(-t/\tau) + B$$

Data are given as mean $\pm$ s.e.m. Significance was evaluated using Student's t -test.

Homology modelling. The sequence of the SUR1 NBDs and transmembrane domains 1 and 2 (residues 301–1,581) was aligned with two copies of the Sav1866 monomer sequence (1,152 residues), and an SUR1 homology model was generated on the basis of this alignment and the dimeric Sav1866 structure (Protein Data Bank entry 2HYD; 3.0 Å resolution). The NBD model comprised SUR1 residues 691–752, 768–931 and 1,345–1,581. See the supplementary information online for details.

Supplementary information is available at *EMBO reports* online (<http://www.emboreports.org>).

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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