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Review

Sulfonylurea receptors: ABC transporters that regulate ATP-sensitive K^+ channels

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Abstract

The association of sulfonylurea receptors (SURs) with $K_{IR}6.x$ subunits to form ATP-sensitive K^+ channels presents perhaps the most unusual function known for members of the transport ATPase family. The integration of these two protein subunits extends well beyond conferring sensitivity to sulfonylureas. Recent studies indicate SUR-$K_{IR}6.x$ interactions are critical for all of the properties associated with native K_{ATP} channels including quality control over surface expression, channel kinetics, inhibition and stimulation by Mg-nucleotides and response both to channel blockers like sulfonylureas and to potassium channel openers. K_{ATP} channels are a unique example of the physiologic and medical importance of a transport ATPase and provide a paradigm for how other members of the family may interact with other ion channels.

Keywords: Sulfonylurea receptor; K_{IR}; ATP-sensitive potassium channels; Tolbutamide; Diazoxide

Contents

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1. Introduction

K_{ATP} channels are a unique combination of transport ATPases complexed with potassium ion channel subunits. While the majority of proteins discussed in this BBA issue on Biomembranes couple the hydrolysis of ATP to movement of molecules across a membrane against a concentration gradient, K_{ATP} channels, the subject of this article, control the flux of potassium ions driven by an electrochemical potential. Control is exerted in response to changes in the concentrations of cytosolic ADP and ATP that result from ATP synthesis and hydrolysis within the cell. This is illustrated diagrammatically in Fig. 1. ATP hydrolysis is believed to occur as an integral part of the regulation of K_{ATP} channels, but does not power K^+ movement.

K_{ATP} channels are comprised of sulfonylurea receptors, SURs, and potassium inward rectifiers, $K_{IR}6.1$ and $K_{IR}6.2$, that assemble to form a large octameric channel with a $(SUR/K_{IR}6.x)_4$ stoichiometry ([1–3], reviewed in [4]). Some of the evidence supporting this stoichiometric model is given in Fig. 2. It is now clear that two gene clusters on the short arms of human chromosomes 11 and 12 encode the SUR1 and $K_{IR}6.2$ subunits, and the SUR2 and $K_{IR}6.1$ subunits, respectively. Differential usage of two terminal exons in the SUR2 gene specifies the two major SUR2A and SUR2B splice variants (reviewed in [4,5]). Additional minor splice variants have been described [6,7]. Based on extensive evidence (reviewed in [4,5,8]) the $SUR1/K_{IR}6.2$ pairs are known to comprise the K_{ATP} channels of pancreatic β-cells, and are found in neurons and neuroendocrine cells. The $SUR2A/K_{IR}6.2$ pair assembles the channels in cardiac ventricular myocytes and skeletal muscle [9,10], while SUR2B pairs with $K_{IR}6.1$ and $K_{IR}6.2$ to form the K_{ATP} channels found in vascular and non-vascular smooth muscle [11,12]. Recent reports have suggested that mixed channels comprised of $SUR1/SUR2/K_{IR}6.1$ or $SUR1/SUR2/K_{IR}6.2$ subunits may exist [13–16].

The β-cell K_{ATP} channels are key players in the ionic pathway that regulates insulin release in response to glucose metabolism. Openings of these channels can set the resting membrane potential of β-cells below the threshold for activation of voltage-gated Ca^{2+} channels when plasma glucose levels are low, thus reducing insulin secretion (reviewed in [5,17]). β-Cell K_{ATP} channels are the target for sulfonylureas, 1st and 2nd generation hypoglycemic agents, e.g., tolbutamide, glipizide, and glibenclamide, used in the treatment of non-insulin dependent diabetes mellitus (NIDDM) to control hyperglycemia, and for potassium channel openers like diazoxide which is used to control hyperinsulinemic states. Mutations in both of the β-cell K_{ATP} channel subunits, SUR1 and $K_{IR}6.2$, have been shown to cause a recessive form of familial hyperinsulinism or persistent hyperinsulinemic hypoglycemia of infancy, PHHI ([18–21], reviewed in [5,17]). The SUR2-based channels are the targets for a variety of potassium channel openers of potential therapeutic importance (reviewed in [22–25]). The $SUR2/K_{IR}6.1$ gene cluster has not been linked to a disease at this time.

The high-affinity sulfonylurea receptor, SUR1, was the first SUR to be cloned [26]. A biochemical approach was used to label and purify SUR1 and obtain peptide sequence for cloning. Homology cloning techniques were then used to obtain SUR1 and SUR2 cDNAs from various tissues and species. $K_{IR}6.1$ was obtained by screening a rat library at low stringency using a fragment of GIRK1 as a probe [27]. $K_{IR}6.2$ was obtained by screening with the $K_{IR}6.1$ cDNA as a probe [28]. The predicted topologies of these subunits are shown in Fig. 2. The K_{IR} subunits have two transmembrane domains (TMDs), termed M1 and M2, and it is assumed that the structure of the pore region will closely resemble that of the KcsA potassium channel from

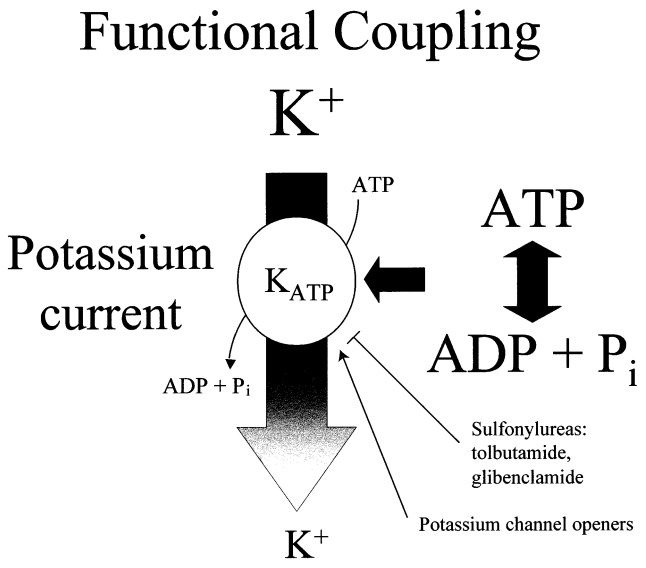

Fig. 1. K_{ATP} channels respond to changes in ATP/ADP to regulate K^+ currents driven by a concentration gradient.

Streptomyces lividans [29], although the precise packing of the M1 and M2 helices in the K_{IR} family may differ from the bacterial model (see [30]). SUR1 is an ATP-binding cassette (ABC) protein or transport ATPase which closely resembles members of the multidrug resistance associated protein, or MRP, family with 17 predicted TMDs and two nucleotide binding folds as illustrated in Fig. 2 ([26,31], reviewed in [4,5,8,32]).

Neither SUR1 nor $K_{IR}6.2$ cDNAs produced recognizable K_{ATP} channel activity when expressed alone, but co-expression generated currents characteristic of native β-cell channels [28,33]. The reason(s) for this strong co-dependence were not immediately clear, but did imply either a very strong integration of the functions of the two subunits, or that parts of both subunits were required to form the conducting pathway. Subsequent work has eliminated the latter possibility, while it has become increasingly clear that the characteristics of the native channel depend on subunit-subunit interactions.

Mutations in the M2 helix suggested that $K_{IR}6.2$ formed the pore [1,34]. A suggestion which was nicely proven by the demonstration that truncation of as few as 16 and up to 36 amino acids from the C-terminus enabled expression of homomeric $K_{IR}6.2$ channels with the same ion conductance as their heteromeric SUR1/$K_{IR}6.2$ counterparts [35]. Why deletion of a small segment enabled expression was not clear at the time, but as discussed below, it is now understood that the truncations removed a critical trafficking signal.

2. Channel composition and stoichiometry

We established the octameric stoichiometry of SUR1/$K_{IR}6.2$ K_{ATP} channels, $(SUR1/K_{IR}6.2)_4$ (see Fig. 2), using biochemical and biophysical methods ([1], reviewed in [4]). Subunits were labeled with a radioiodinated derivative of glibenclamide, [^{125}I]iodoazidoglibenclamide [36], and were shown to be associated with each other by co-purification on lectin columns or by using histidine tagged subunits and Ni^{2+}-agarose [1]. The mature glycosylated form of SUR1 was observed to be preferentially associated with $K_{IR}6.2$, and the molecular mass of the labeled SUR1/$K_{IR}6.2$ complex was estimated to be approx. 950 kDa, matching what was expected for an octameric $(SUR1/K_{IR}6.2)_4$ complex. One-to-one SUR1∼$K_{IR}6.2$ fusion constructs generated complexes with the same mass that produced active channels, but 'triple' fusion constructs, SUR1∼$(K_{IR}6.2)_2$, did not unless they were co-expressed with monomeric SUR1. Co-expression of triple fusion constructs carrying the N160D mutation with the wild-type triple fusion constructs produced three types of channels distinguished by their rectification properties, the parental moderate and strong rectifiers plus an intermediate class as expected from a tetrameric pore. The data were consistent with an octameric structure as illustrated in Fig. 2. Shyng and Nichols [3] and Inagaki et al. [2] arrived at the same structure based on electrophysiological experiments using the same, or similar, single and triple-fused K_{ATP} channel subunits, respectively.

3. Quality control, subunit trafficking and ER retention signals

A report by Zerangue et al. [37] has provided insight into how cells maintain quality control over the assembly of complex proteins like K_{ATP} channels. These authors have shown that protein trafficking signals, specifically an *–RKR–* motif, can function

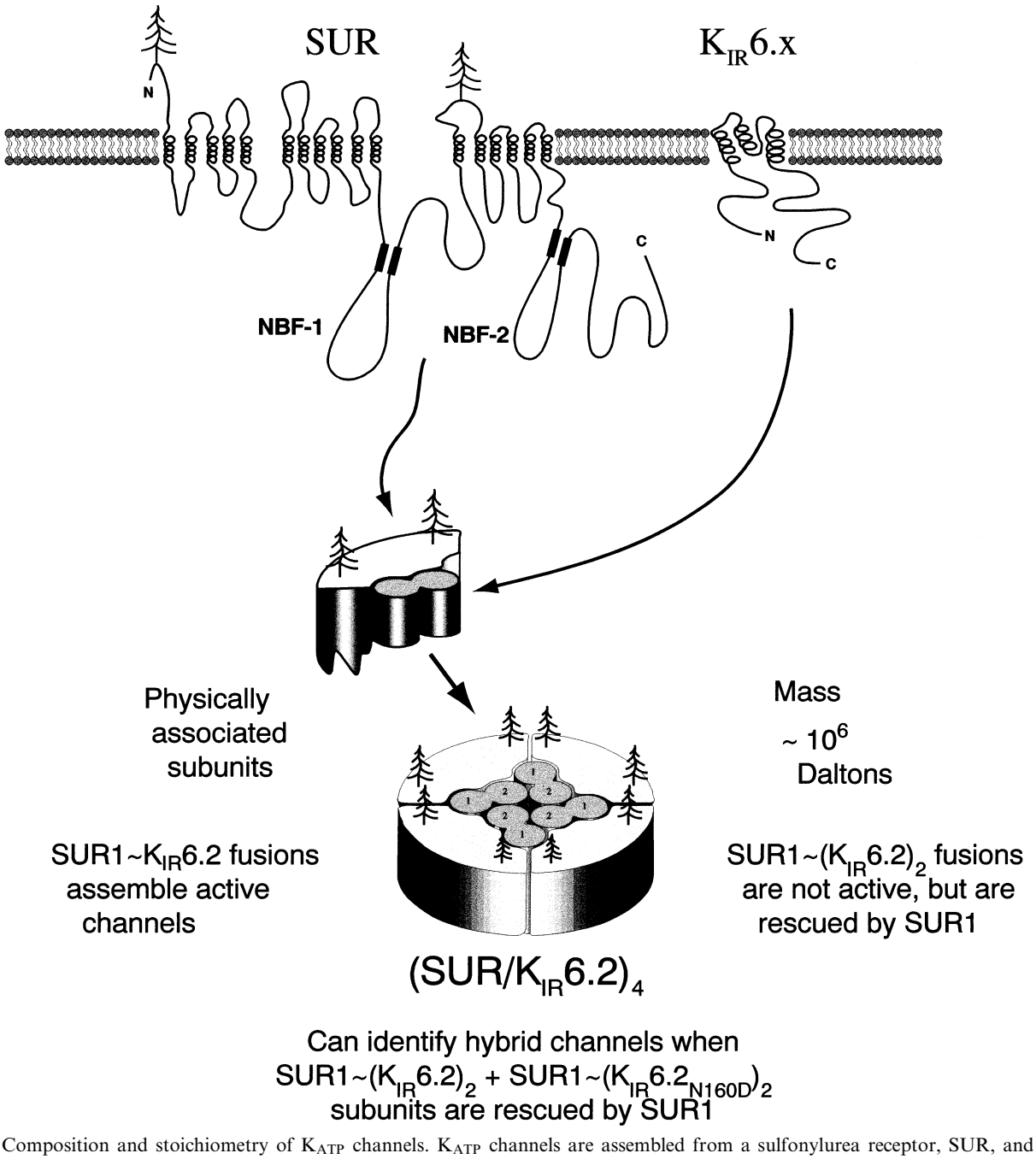

Fig. 2. Composition and stoichiometry of K_{ATP} channels. K_{ATP} channels are assembled from a sulfonylurea receptor, SUR, and either $K_{IR}6.1$ or $K_{IR}6.2$ in an octameric stoichiometry. Some of the results which support an octameric model are outlined.

to keep incompletely assembled K_{ATP} channels from reaching the cell surface. This motif is found in the C-terminus of $K_{IR}6.2$, the region deleted by Tucker et al. [35], and in SUR1 upstream from NBF1 (see the summary illustration in Fig. 14 for the approximate positioning of these signals). A slightly different motif, *–RKQ–*, is found at the equivalent position in SUR2. Extensive mutational analysis indicated that changing the *–RKR–* motif to *–AAA–* in either SUR1 or $K_{IR}6.2$ allowed their surface expression in the absence of the partner subunit. Furthermore, swapping the C-terminus of $K_{IR}6.2$ onto $K_{IR}2.1$, a channel subunit that normally assembles and traffics to the plasma membrane, reduced its surface expres-

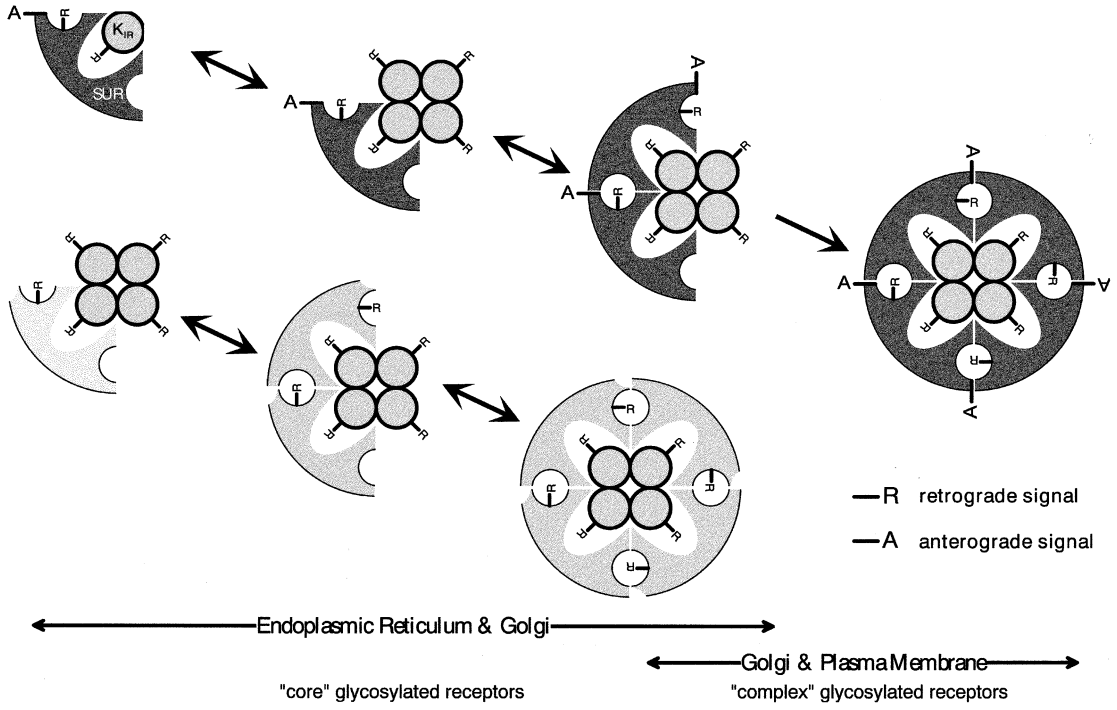

Fig. 3. Schematic diagram summarizing the assembly and maturation of K_{ATP} channels. The scheme for masking of the *–RKR–* ER retention signals is based on Zerangue et al. [37]. The C-terminus of SUR, designated here as an anterograde signal, is required for the channels to exit the ER/*cis*-Golgi compartment as indicated by surface expression and the appearance of complex glycosylated SUR1 [39]. Deletion of the C-terminus of SUR1 inhibits transit from the ER/*cis*-Golgi compartment. (Reproduced from [39] with permission.)

sion. Zerangue et al. [37] proposed that the ER retention signals were masked during assembly of the octamer and that only fully assembled channels were able to reach the plasma membrane thus providing quality control. These findings provided an explanation for the earlier observation that the mature glycosylated form of SUR1 was only present when the receptor was co-expressed with $K_{IR}6.1$ or $K_{IR}6.2$ and that the mature receptor co-purified with the K_{IR} subunit [1]. The results also provide a rationale for the observation by John et al. [38] that high level over-expression of $K_{IR}6.2$ alone can give channel activity, presumably because the ER retention mechanism is saturable.

4. Anterograde signals and PHHI

In addition to an ER retention signal, the last amino acids of SUR1 appear to be required for surface expression of β-cell K_{ATP} channels. Deletion of as few as 7 amino acids from the C-terminus of SUR1 causes a parallel loss of channel activity and surface expression, although the subunits do associate [39]. We have proposed that nonsense and splice-site mutations in SUR1 give rise to PHHI because the resulting channels never reach the plasma membrane [39]. The ER retention mechanism and requirement for an intact SUR C-terminus are summarized schematically in Fig. 3. This model raises interesting questions about how the retention and anterograde signals work and how they are covered and uncovered during assembly of the channel. In addition, the understanding that trafficking plays a major role in K_{ATP} channel assembly and function has given insight into PHHI and has provided an experimental means to compare the properties of the pore with and without SUR.

5. Description of channel activity

The conductance properties and kinetic behavior of K_{ATP} channels have been characterized in some

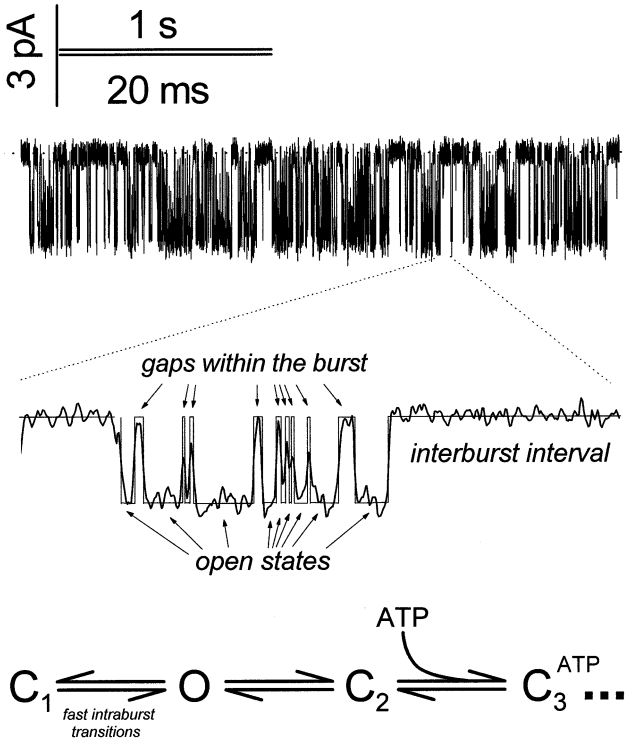

Fig. 4. Electrical activity of a human SUR1/K_{IR}6.2 ATP-sensitive K^+ channel. Recordings from a membrane patch excised from a COSm6 cell expressing human SUR1 and human K_{IR}6.2 illustrate spontaneous channel activity in the absence of nucleotides. The middle single-channel traces, plotted over a shorter time interval, show open and closed states within a burst. The single channel conductance is approx. 75 pS. The kinetic model is based on one originally proposed by Gillis et al. [49].

detail [40–42]. As illustrated in Fig. 4, recombinant SUR1/K_{IR}6.2 channels display a characteristic 'bursting' pattern in which the channel rapidly 'flickers' between open and closed states equivalent to those observed in wild-type channels. The exact mechanism which gives rise to the fast transitions within a burst is not known, but based on the characterization of the structure and the dynamics of the K^+ channel, KcsA, from *S. lividans* by crystallographic[29] and spectroscopic methods [43–46] the short open and closed states are thought to arise from stochastic movements of the M2 helices that form the pore and channel gate. Silent interburst intervals delineate the bursts. As described elsewhere, expression of the SUR1 and SUR2A subtypes with K_{IR}6.2 produces channels with different bursting properties [4,47]. The data indicate the inward rectifier determines the mean duration of the fast openings and closures within a burst, while the SUR subtype determines the rate of transition between bursts and interburst closed states. The application of ATP, with or without Mg^{2+}, reduces the probability of finding the channel in the open state by prolonging the lifetime of the interburst closed states and by shortening the duration of the bursts [10,41,48]. ATP seems to have little effect on the mean duration of the fast open and closed states within a burst [40], consistent with SUR modulating the transition from the bursting to the non-bursting state. Gillis et al. [49] have proposed a simple kinetic scheme to model these characteristics (see Fig. 4). In this model, changes in the apparent sensitivity to ATP could result from modifications that alter the affinity of the C_2, C_3, etc. microstates for nucleotide or from modifications that alter the dwell time in these microstates.

6. Nucleotide effects

The sine qua non of K_{ATP} channels is their response to nucleotides. Fig. 5 summarizes the actions of nucleotides on homomeric $(K_{IR}6.2\Delta C)_4$ and heteromeric $(SUR1/K_{IR}6.2)_4$ channels. (The interactions of nucleotides with SUR and K_{IR} subunits and the importance of these interactions in the actions of glibenclamide are also covered in the article by Ueda et al. in this volume.) The conductance of both channels is equivalent indicating SUR does not contribute to assembly of the pore [35,47,50]. In the absence of Mg^{2+}, conditions under which ATP will bind tightly to SUR1 [51] but presumably not undergo hydrolysis, the application of ATP to either the homomeric or heteromeric configuration results in the inhibition of the spontaneous channel activity observed in the absence of nucleotides. The $IC_{50(ATP)}$ values for inhibition are markedly different with the heteromeric channels being approx. 20–40-fold more sensitive to inhibition by nucleotides [35,47,50]. In the absence of Mg^{2+}, ADP has a similar, but less potent inhibitory action on both configurations. The Hill coefficients for the nucleotide dose-response curves are consistently near one implying one bound nucleotide is enough to inhibit the passage of K^+. This effect does not require intact

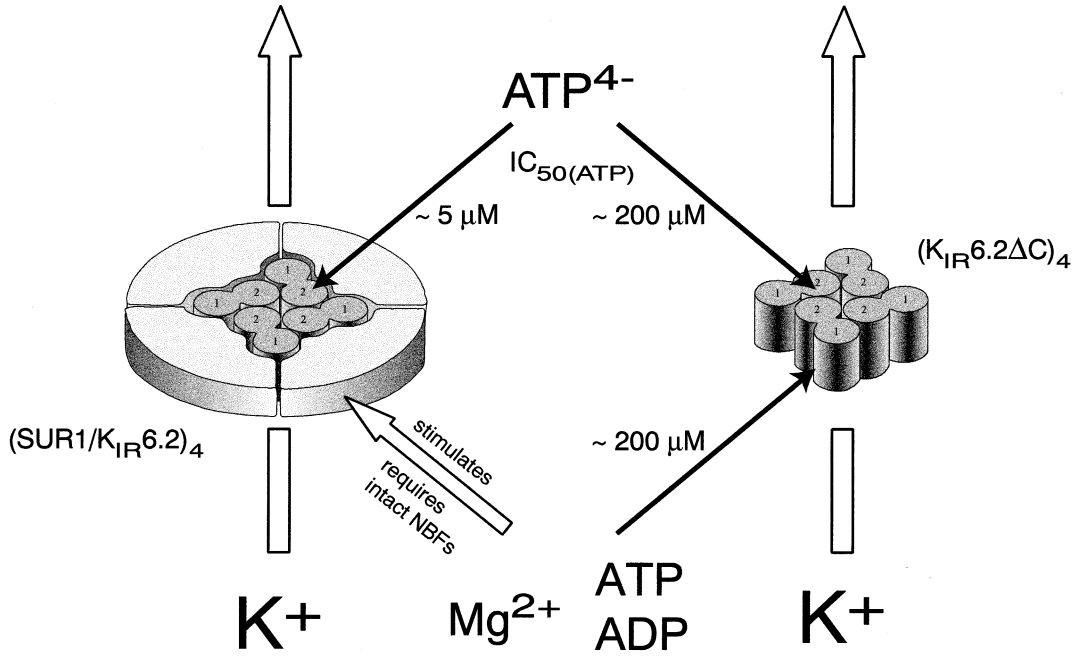

Fig. 5. Nucleotide effects on heteromeric and homomeric K_{ATP} channels. The open vertical arrows symbolize K^+ currents which are equivalent for both configurations. The inhibitory effects of nucleotides acting directly on $K_{IR}6.2$ are indicated by the solid arrows. Comparison of the IC_{50} values implies SUR increases the affinity of $K_{IR}6.2$ for nucleotides. The open arrow symbolizes stimulation of channel activity by Mg-nucleotides acting through SUR via a mechanism that requires intact nucleotide binding folds.

nucleotide binding folds as mutations in the Walker A and B motifs that prevent labeling with 8-azidoATP [51] do not affect the $IC_{50(ATP)}$ values [52,53].

Mg-nucleotides have markedly different effects on the two configurations, inhibiting homomeric channels [35,47,50], while stimulating the heteromeric channels. Stimulation requires receptors with intact NBFs; mutations in the Walker A and B motifs strongly attenuate the effects of MgADP [52,53]. Together these results show that assembly with SUR1 increases the affinity of the nucleotide binding site on $K_{IR}6.2$ for ATP, that nucleotide binding to the K_{IR} inhibits the channel, and that the interaction(s) of Mg-nucleotides with SUR1 can antagonize this inhibition.

7. The cytoplasmic domains of $K_{IR}6.2$ are important for nucleotide binding and control of bursting

In addition to their differential responses to nucleotides, the homo- and heteromeric channels differ markedly in their kinetic properties [35,47,50]. Fig. 6 illustrates this point for human $(SUR1/K_{IR}6.2)_4$ vs. $(K_{IR}6.2\Delta C35)_4$ channels. The single-channel conductance is the same, but the bursting of the homomeric channels is qualitatively different than that of the heteromeric channels. The 'bursts' are extremely short and the homomeric channels spend far less time in open states that the heteromeric channels. In terms of the simple kinetic model in Fig. 4, the affinity of the long-lived closed state for ATP must be markedly reduced as the homomeric channels spend more time in this state, but their $IC_{50(ATP)}$ is 20–40-fold higher than that of the heteromeric channels.

The nature of the nucleotide binding site within $K_{IR}6.2$ is not clear, but recent efforts at its definition illustrate the complexity of the problem and underscore the interdependence of the dwell time in a particular microstate vs. its affinity for nucleotides. Mutations in $K_{IR}6.2$ which, on the basis of the KcsA structure, are predicted to be near the mouth of the pore, for example, K185Q [35], will increase the $IC_{50(ATP)}$ values for both homo- and heteromeric channels without significantly affecting the P_o of the resulting channels [54]. The K185Q mutation may therefore be in the nucleotide binding site and

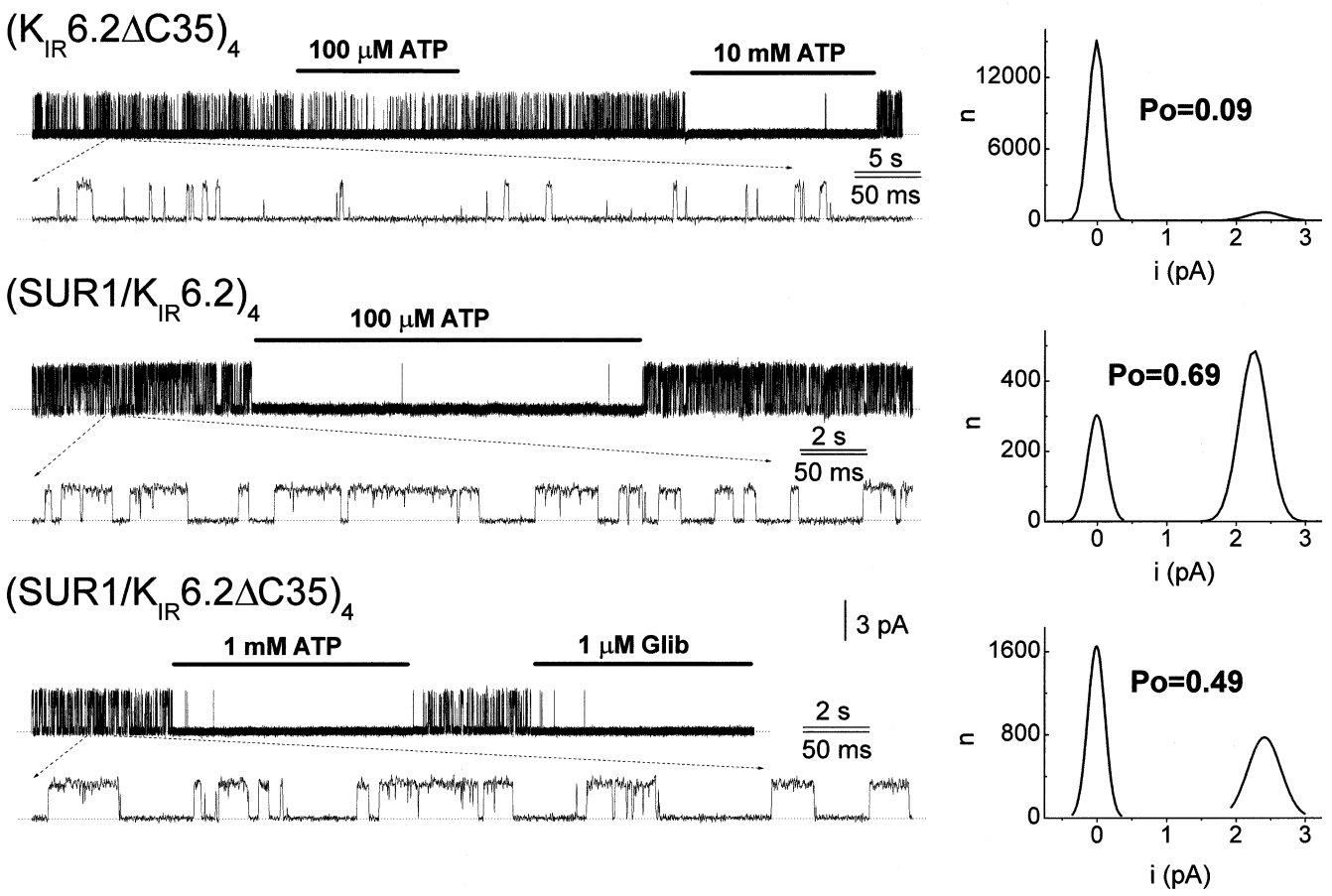

Fig. 6. Comparison of wild-type and truncated K_{ATP} channels. Single channel records from homomeric channels, $K_{IR}6.2\Delta C35$, obtained by deleting 35 amino acids, containing the *–RKR–* ER retention motif from the C-terminus of $K_{IR}6.2$, from heteromeric SUR1/$K_{IR}6.2$ channels and from SUR1/$K_{IR}6.2\Delta C35$ channels are presented. The $(K_{IR}6.2\Delta C35)_4$ channels show abnormal bursting and have a markedly reduced probability of being in an open state. The maximal open probability, P_o, estimated from the all points histogram, is approx. 8-fold lower than the wild-type channels. Co-expression of $K_{IR}6.2\Delta C35$ with SUR1 restores normal bursting, increasing the maximal P_o. The horizontal dotted lines give the zero-current level; the upward deflections correspond to outwardly directed currents. (Reproduced from [54] with permission.)

reduce its affinity for ATP; however, it has been difficult to test the possibility that this mutation is in a linker between a binding site and the channel gate. Other mutations, for example an arginine residue, R50, in the N-terminus of $K_{IR}6.2$ [55] and the N-terminal deletions described below [56–58] markedly increase the P_o by reducing the dwell time in long-lived closed states. The resulting channels also show reduced nucleotide sensitivity, but it is unnecessary to assume the affinity of the microstate(s) for ATP has changed. Interestingly, experiments with 8-azidoATP indicate that despite their observed weak association with ATP, the homomeric channels can be photolabeled [59] and should provide a means to determine what amino acids are in proximity to the adenine ring. Drain [50] has presented evidence suggesting the sequence from 333 to 338 which resembles the F-X_4-K sequence found in ion-motive ATPases is a candidate ATP binding site.

Modifications of the N-terminus of $K_{IR}6.2$ have marked effects on K_{ATP} channel kinetics [56–58]. We have shown that deletion of 5–44 amino acids affects the maximum open probability, P_{omax}, of the SUR1/$\Delta NK_{IR}6.2$ channels as illustrated in Fig. 7. The estimated P_{omax} is that expected if the SUR1/$\Delta NK_{IR}6.2$ channels are in an infinitely long burst having the same intraburst kinetics as the wild-type channels. As would be expected from the reduced

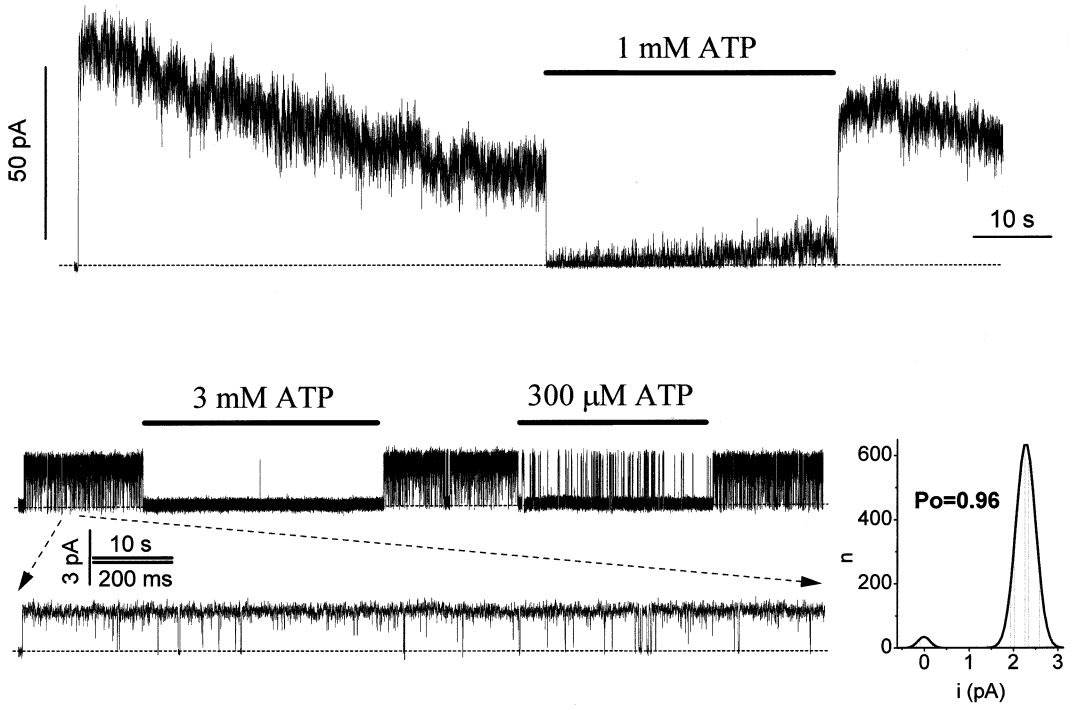

Fig. 7. Amino terminal deletion of $K_{IR}6.2$ generates K_{ATP} channels that burst continuously. The upper panel shows an example of the macro-currents activated upon excision of an inside-out patch taken from a COSm6 cell co-transfected with SUR1 and $\Delta N44K_{IR}6.2$. The single channel recordings in the two lower panels show these channels are less sensitive to ATP and burst nearly continuously with the P_o value expected for an unmodified wild-type channel in an infinitely long burst. We have proposed the lower ATP sensitivity of these channels is the result of their spending less time in interburst closed states able to bind ATP.

occupancy of the nucleotide-binding interburst closed state, the $IC_{50(ATP)}$ for these channels is increased approx. 20-fold, as discussed above. The data are consistent with the idea that the affinity of the closed state for ATP need not be altered. We have proposed that the N-terminus of K_{IR} normally limits the duration of a burst and suggested it could couple conformational changes in SUR to the pore/gate. The structural question of how the N-terminus actually limits bursting and what part(s) of the gate are involved is unanswered.

8. Chimeric K_{ATP} channels are revealing functionally important regions of the receptors

The β-cell and cardiac channels differ in their kinetics and sensitivity to modulators including ATP, sulfonylureas and potassium channel openers. Advantage is being taken of these differences to delineate the regions of the receptors that specify the isoform differences. We have used SUR1∼SUR2A chimeras to define the regions which specify the isoform differences in channel kinetics and sensitivity to ATP [47]. An example is given in Fig. 8. The longer burst duration and greater P_{omax} of the cardiac $SUR2A/K_{IR}6.2$ channels is specified by an N-terminal segment of the SUR2A receptor, while the lower $IC_{50(ATP)}$ of the β-cell channel is specified by a C-terminal segment. Using a matched set of chimeric constructs, the N-terminal segment was identified as the SUR2 TMD1-5 region while the C-terminal segment was identified as the last 42 amino acids of SUR1 [47]. Interestingly, the isoform differences in kinetics and ATP sensitivity were separable indicating that the difference in ATP sensitivity is not due to a decreased occupation of the interburst closed state. These results imply that the SUR TMD1-5 segment is in close proximity to $K_{IR}6.2$ and is in a position to modulate bursting. We have speculated this could result from interactions with the K_{IR} N-terminus [47].

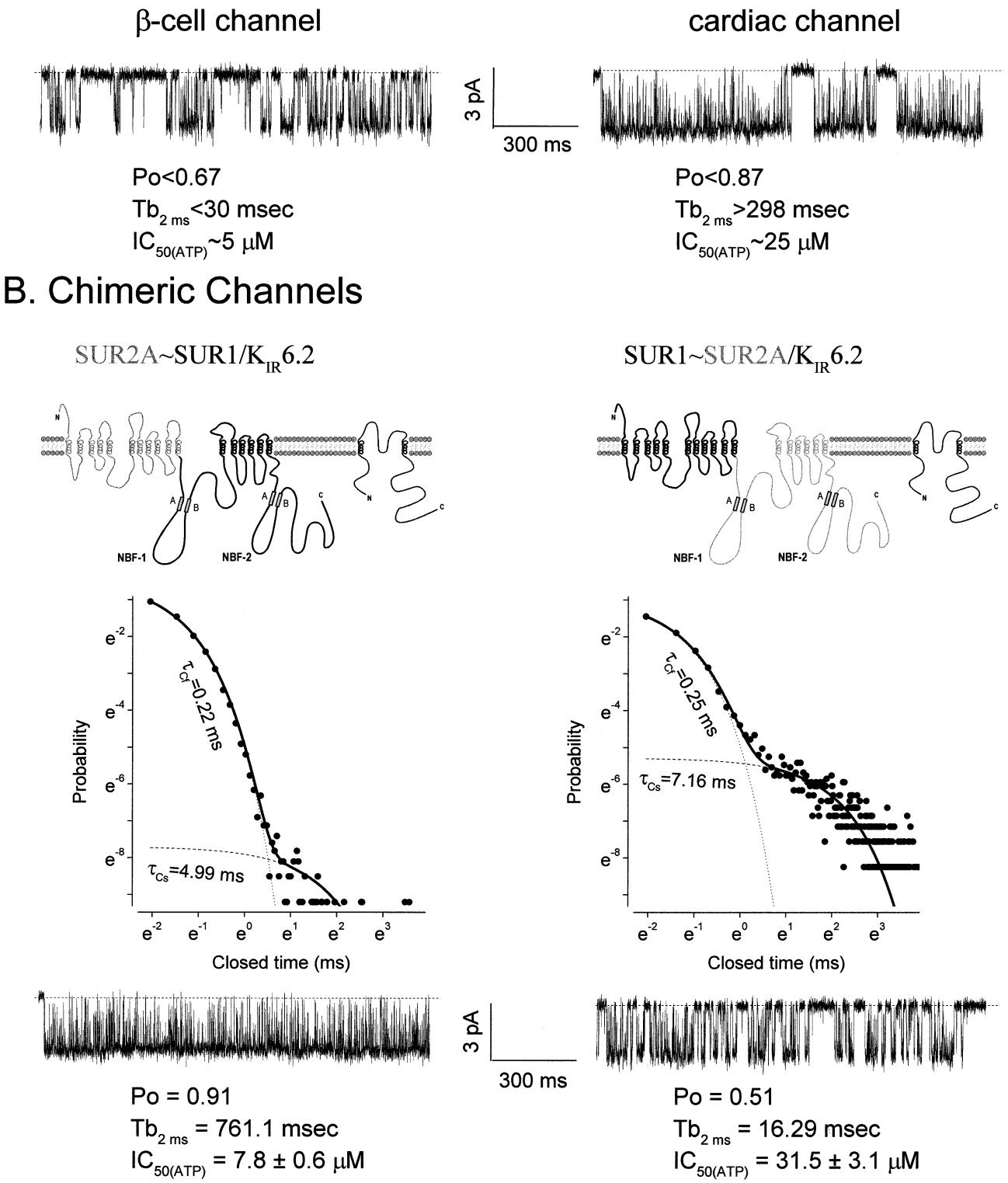
A. Parent Channels
β-cell channel
cardiac channel
3 pA
300 ms
Po<0.67
Tb2 ms<30 msec
IC50(ATP)~5 μM
Po<0.87
Tb2 ms>298 msec
IC50(ATP)~25 μM
B. Chimeric Channels
SUR2A~SUR1/KIR6.2
SUR1~SUR2A/KIR6.2
NBF-1
NBF-2
Probability
τCf=0.22 ms
τCs=4.99 ms
τCf=0.25 ms
τCs=7.16 ms
Closed time (ms)
3 pA
300 ms
Po = 0.91
Tb2 ms = 761.1 msec
IC50(ATP) = 7.8 ± 0.6 μM
Po = 0.51
Tb2 ms = 16.29 msec
IC50(ATP) = 31.5 ± 3.1 μM

Fig. 8. Chimeric SUR/K_{IR}6.2 channels have the combined properties of β-cell and cardiac K_{ATP} channels. The upper set of panels (A) compares the properties of the parental SUR1/K_{IR}6.2 β-cell and SUR2A/K_{IR}6.2 cardiac K_{ATP} channels. The two channel isoforms are distinguishable by their P_o values, the average burst duration (Tb_{2ms}) and $IC_{50(ATP)}$ values. The lower set of panels (B) illustrates the mixed properties of one pair of matched chimeric channels. The swapped segments from the β-cell and cardiac receptors are shown in black and gray, respectively. The intraburst kinetics are not affected by these swaps. The SUR2A∼SUR1/K_{IR}6.2 channels exhibit the higher P_o and longer burst duration of the cardiac channel with the lower $IC_{50(ATP)}$ of the β-cell channel. The SUR1∼SUR2A/K_{IR}6.2 channels exhibit the reciprocal characteristics. Analysis of a more extensive collection of SUR chimeras identifies the SUR TMD1–5 segment as critical for specification of the isoform kinetics, while the last 42 amino acids specify the $IC_{50(ATP)}$ values.
←

9. How do potassium channel openers work?

Potassium channel openers (KCOs) increase the P_o of heteromeric, but not homomeric K_{ATP} channels (see Fig. 9). Recent studies using [^{3}H]P1075, a potent activator of vascular smooth muscle K_{ATP} channels [60,61], suggest that KCOs bind to a specific conformation of sulfonylurea receptors. Switching SURs into this conformation requires a divalent cation (Mg^{2+} or Mn^{2+}) and a hydrolyzable nucleotide [62–65]. Mutations in the NBFs strongly inhibit [^{3}H]P1075 binding which, along with the requirement for divalent cations and a hydrolyzable nucleotide, strongly suggest hydrolysis is required for the conformational change in the receptor. Glibenclamide inhibits [^{3}H]P1075 binding by increasing its rate of dissociation from SURs [62–64]. KCO binding is also inhibited by thiol-modifying reagents [66], perhaps by reaction with the cysteine in NBF1. Competition studies using [^{3}H]P1075 and a variety of other KCOs show rank order of potency consistent with physiologic observations, while binding to the different receptor isoforms indicates the affinity of SUR2B is greatest, consistent with this receptor being a subunit of vascular smooth muscle K_{ATP} channels [11, 63,65].

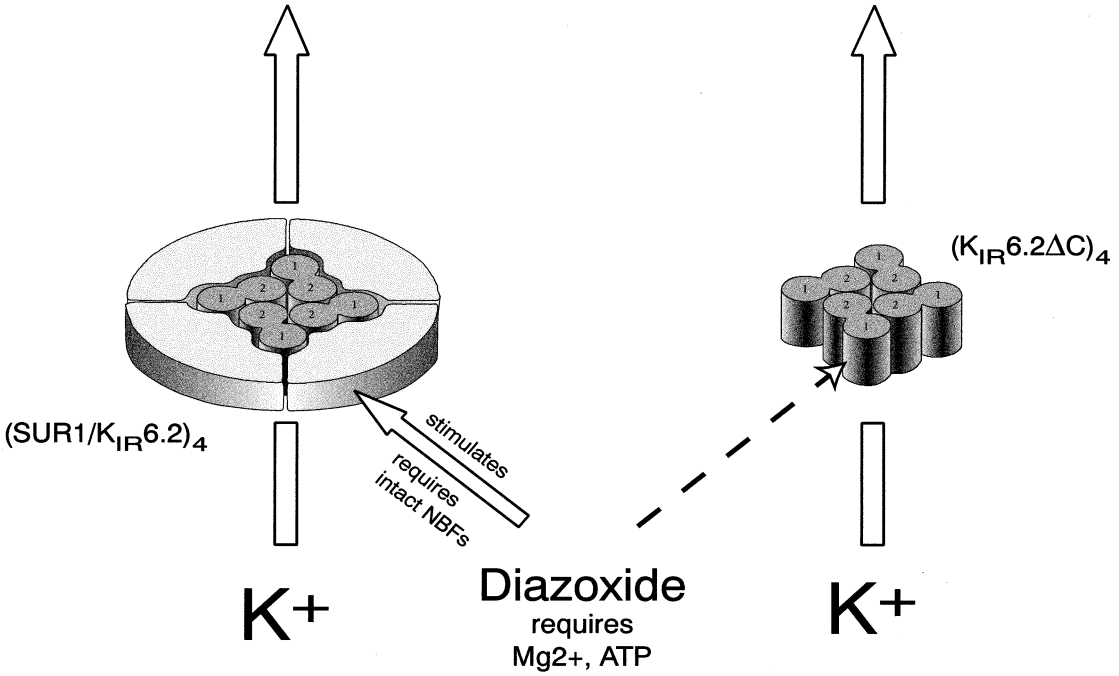

Fig. 9. Diazoxide stimulation of channel activity requires SUR. The open vertical arrows symbolize K^+ currents. Diazoxide and other potassium channel openers do not affect the homomeric channels. Stimulation of heteromeric channels requires divalent cations, hydrolyzable nucleotides and intact nucleotide binding folds on the SUR.

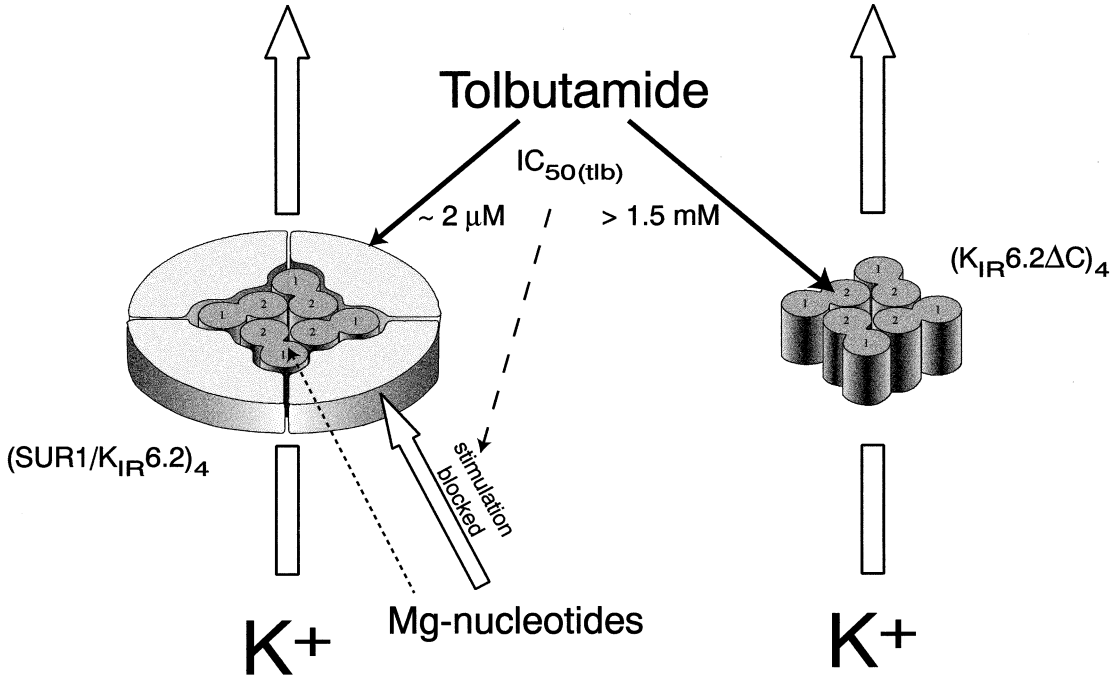

Fig. 10. Sulfonylureas have multiple inhibitory effects on K_{ATP} channels. The effects of the readily reversible 1st generation sulfonylurea, tolbutamide, are illustrated. The solid arrows indicate direct inhibitory actions on the homomeric and heteromeric channels in the absence of the stimulatory effects of Mg-nucleotides, respectively. The IC_{50} values differ markedly; the high-affinity effect on the heteromeric channel requires SUR1, or a segment of SUR1, and an intact N-terminus on $K_{IR}6.2$. The binding of tolbutamide to a K_{ATP} channel in vivo causes a conformational change that blocks the stimulatory action of Mg-nucleotides as symbolized by the dashed arrow. This stimulatory action normally antagonizes the inhibitory effects of nucleotides on $K_{IR}6.2$, thus its elimination augments the effect of tolbutamide. The overall effect is additive, the sum of the direct inhibitory effect of tolbutamide and the inhibitory effect of Mg-nucleotides on $K_{IR}6.2$ (symbolized by the dotted arrow) which are no longer antagonized by SUR1.

10. A two-step mechanism for the action of sulfonylureas

Sulfonylureas are well known to bind to receptors in a variety of tissues (see [5,8,67] for reviews). The K_d values for binding to the high-affinity receptor identified in pancreatic β-cells and in brain are in the low μM range for tolbutamide and in the low nM for glibenclamide. The estimated K_d values for sulfonylurea binding to the SUR2 receptors show their affinities are typically about a thousand-fold weaker than SUR1 for these compounds (see for example a recent comparison of binding data from expressed recombinant receptors [68]). Fig. 10 summarizes the multi-step nature of the effects of sulfonylureas on β-cell K_{ATP} channel activity. High concentrations of tolbutamide, the 1st generation of hypoglycemic sulfonylureas whose actions are rapidly reversible, will inhibit homomeric channels, but this effect is of unknown physiologic importance as concentrations of this compound are never expected to reach these levels [69]. The heteromeric channels are approximately a thousand-fold more sensitive to inhibition by tolbutamide. The dose-response curves for tolbutamide inhibition of SUR1/$K_{IR}6.2$ vs. SUR2A/$K_{IR}6.2$ channels show a large spread in IC_{50} values and demonstrate that the β-cell channels have two components of inhibition, one of high affinity, which is saturated at a value near 100 μM, the other of low affinity, $IC_{50} > 1.5$ mM ([69,70], illustrated in Fig. 11). The low-affinity component has been attributed to a direct action on $K_{IR}6.2$ [69]. The situation is not clear for the SUR2A/$K_{IR}6.2$ channels since the estimated K_d for binding of tolbutamide to SUR2A is approx. 0.3 mM [68] and simulations using two components with K_d values of 0.3 and 1.5 mM indicate the available data are not sufficient to eliminate a contribution of SUR2A in the inhibition of the cardiac channel.

The high-affinity inhibition seen in the β-cell chan-

nels, which is the basis for their selective response to sulfonylureas, is a two-step process. In excised patches, in the *absence* of stimulation by Mg-nucleotides, tolbutamide reduces channel activity by approx. 50–60% (Fig. 11). This inhibition involves the N-terminus of $K_{IR}6.2$ since deletion of 5 amino acids from $K_{IR}6.2$ reduces the effect of tolbutamide while deletion of ten or more residues essentially eliminates the inhibitory effect [58,70]. The results suggest a mechanism in which the binding of tolbutamide to SUR1 results in a conformational change that is linked to, and closes the pore by a process that must involve the N-terminus of $K_{IR}6.2$.

The second effect of the tolbutamide-induced conformational change is the elimination of the stimulatory effect of Mg-nucleotides. The inhibition of β-cell K_{ATP} channels by sulfonylureas has been reported to be 'enhanced' by the presence of Mg-nucleotides [71–73]. This effect is illustrated in Fig. 12. Note that the activity (the relative NP_o values) of channels in the presence of ADP and ATP (at approximately intracellular concentrations, c in Fig. 12) is reduced $>80\%$ by tolbutamide under stimulatory conditions with Mg^{2+} present, but is less than 40% inhibited when Mg^{2+} is absent. These experiments were done using 200 μM tolbutamide, a concentration sufficient to saturate the high-affinity binding site on SUR1. The final NP_o values, in the presence of tolbutamide, were essentially the same whether the channels were stimulated or not. These results are consistent with the idea that tolbutamide acts to disrupt the stimulation of the β-cell channels by Mg-nucleotides. As indicated above, the stimulatory action of nucleotides, through their interactions with SUR, normally antagonize their own inhibitory action on the K_{IR}.

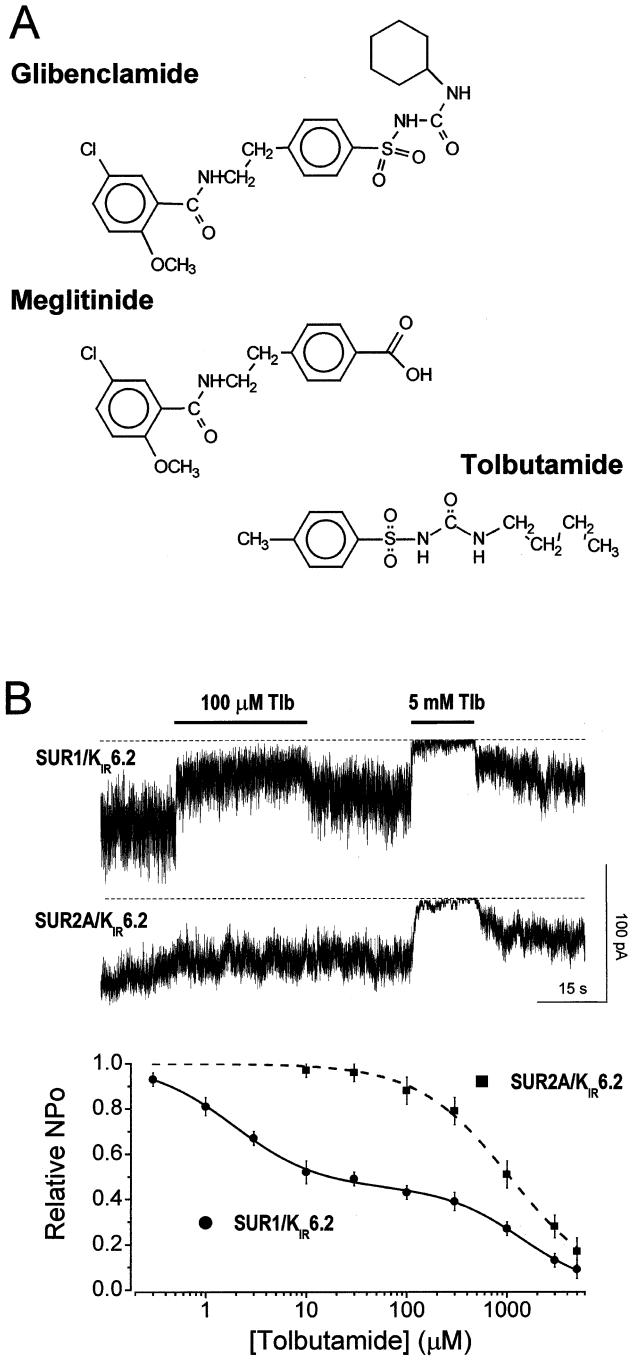

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Fig. 11. SUR1 specifies the high-affinity tolbutamide inhibition of K_{ATP} channels. A comparison of the currents, and dose-response curves, from SUR1/$K_{IR}6.2$ vs. SUR2A/$K_{IR}6.2$ K_{ATP} channels in response to the application of tolbutamide indicates the β-cell channel has two components, while inhibition of the cardiac channel can be described by a single component. The high-affinity component of the SUR1/$K_{IR}6.2$ channels, which accounts for approx. 55% of the total current, is saturated near 100 μM. The solid curve through the SUR1/$K_{IR}6.2$ data is the best fit to a two component pseudo-Hill equation giving $IC_{50H} = 1.9 \pm 0.2$ μM, $h_H = 1.04 \pm 0.08$, $IC_{50L} = 1399 \pm 137.3$ μM, $h_L = 1.13 \pm 0.1$. The dashed line through the SUR2A/$K_{IR}6.2$ data is the best fit to a single component pseudo-Hill equation giving $IC_{50} = 1057.4 \pm 52.1$ μM, and $h = 0.95 \pm 0.04$. These experiments were done without nucleotides in a Mg^{2+}-containing internal solution [70]. Tlb, tolbutamide. (B) Structures of glibenclamide, meglitinide and tolbutamide drawn to illustrate that glibenclamide contains two moieties both of which can bind to SURs and inhibit K_{ATP} channel activity. (Adapted from [70] with permission.)

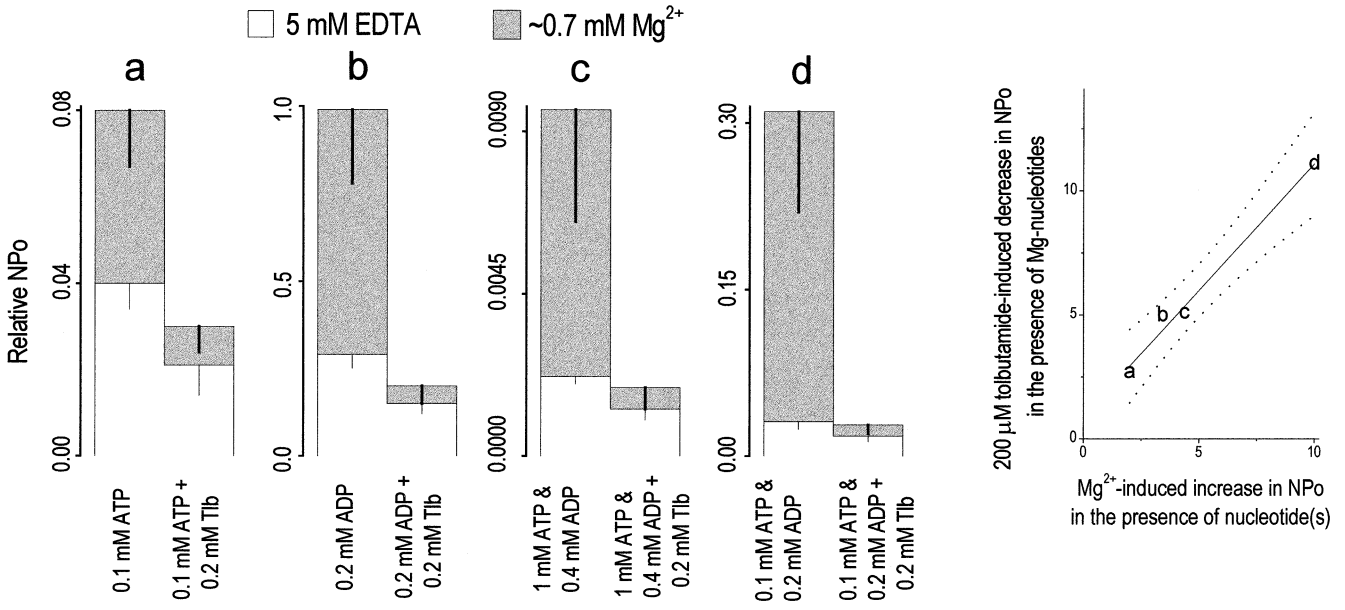

Fig. 12. Tolbutamide binding uncouples the Mg-nucleotide dependent stimulatory action of SUR1 on $K_{IR}6.2$. A concentration of tolbutamide sufficient to saturate the high-affinity component was used to probe conventional inside-out patches containing approximately a thousand K_{ATP} channels. Current measurements were made at the indicated Mg-nucleotide concentrations to provide varying degrees of stimulation (shaded bars). Mg^{2+} was removed with EDTA to eliminate stimulation (open bars). The degree of stimulation can be estimated from the heights of the shaded vs. open bars in the absence of tolbutamide. The 'enhancement' of tolbutamide inhibition (see also [72,73]) by nucleotides can be judged by comparing the degree of inhibition under stimulated (shaded bars) and unstimulated (open bars) conditions. The apparent increase in efficacy is the additive effect of inhibition by tolbutamide and by Mg-nucleotides after eliminating the nucleotide stimulatory effect. Note the scales of the relative NP_o values are different. The relative NP_o values after tolbutamide inhibition in Mg^{2+} containing and Mg^{2+}-free conditions are not significantly different as judged by Student's *t*-test. A plot of the tolbutamide-induced decrease in NP_o in the presence of Mg-nucleotide(s) vs. the Mg-nucleotide stimulated increase in NP_o shows a correlation. The alphabetical symbols correspond to the individual experiments. The solid line is a linear regression, the dashed lines give the 95% confidence limits. (Adapted from [70] with permission.)

Disrupting stimulation thus reveals the inhibitory effects of nucleotides which then become additive with the direct tolbutamide effect observed when nucleotides are absent. This results in the IC_{50} for sulfonylurea inhibition appearing to be a function of nucleotide concentration, and underlies the increased efficacy of sulfonylureas seen in intact cells (for example [74]).

The segment of SUR1 that is necessary for high-affinity tolbutamide inhibition has been identified using chimeric SURs [70,75]. Fig. 13 illustrates this localization using our matched set of SUR chimeras. Inclusion of the SUR1 TMD12–17 segment in a SUR2A background is sufficient to confer high affinity inhibition by tolbutamide. Inclusion of a smaller segment, TMD12–15, ChimXVIII, confers inhibition, but the IC_{50} for tolbutamide is increased, consistent with the TMD12–17 segment containing the sulfonylurea binding site. Comparison of their P_{omax} values demonstrates that swapping the TMD12–17 segments does not affect the kinetics of the chimeric channels indicating that the increased sensitivity to tolbutamide is not the result of increased occupation of a kinetic state that binds sulfonylureas.

Fig. 14 provides a summary. The top panel summarizes the studies aimed at mapping the structural determinants of various characteristics of K_{ATP} channels. The model in the lower panel incorporates many of these features of K_{ATP} channels and speculates on potential interactions between the SUR and $K_{IR}6.x$ subunits. Two pairs of SUR/$K_{IR}6.2$ subunits are illustrated centered around the pore. The SURs are depicted surrounding $K_{IR}6.2$ consistent with there being no indication that SURs contribute to the permeation pathway. We have used our chimeric channel data to position the TMD1–5 and 12–17 segments. As TMD1–5 specifies the isoform differences in gating kinetics we assume this requires close

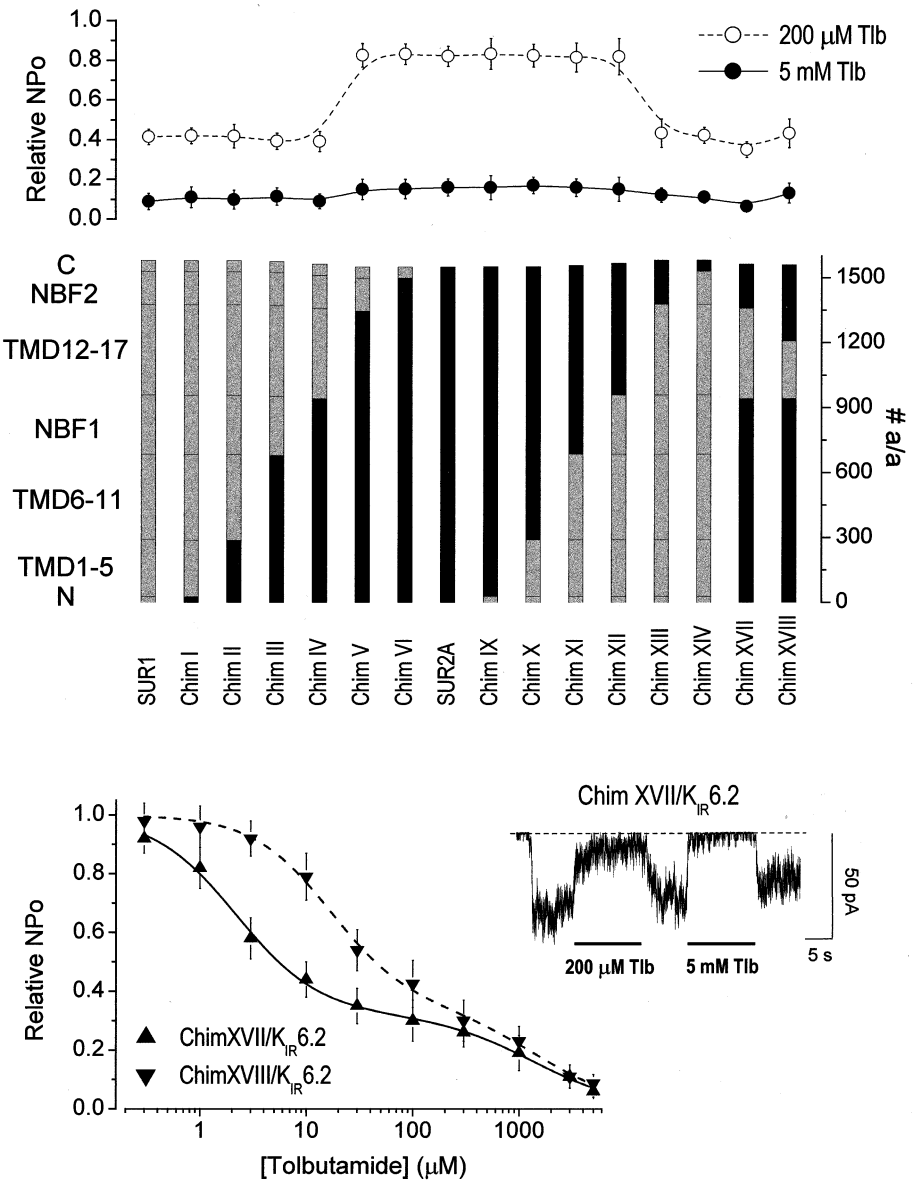

Fig. 13. Chimeric SURs define the SUR1 segment required for high-affinity tolbutamide inhibition of K_{ATP} channels. The top panel shows tolbutamide inhibition of channels assembled from $K_{IR}6.2$ and the parental and chimeric SURs illustrated. The matched chimeras I through VI and IX through XIV were described in a previous report [47]. Segments from SUR1 and SUR2A are shown in black and gray respectively; topological features are indicated on the left axis, amino acid residues are on the right. The presence of the TMD12–17 segment of SUR1 is sufficient to confer a high-affinity interaction between tolbutamide and the chimeric receptor. The recording shows an example of inhibition of the ChimXVII/$K_{IR}6.2$ channels which have the SUR2 TMD1–5 segment and display the high P_{omax} characteristic of cardiac K_{ATP} channels. The dose-response curves for the Chim XVII/$K_{IR}6.2$ and Chim XVIII/$K_{IR}6.2$ channels show that the presence of the TMD12–17 segment of SUR1 is sufficient to specify a high affinity interaction with tolbutamide ($IC_{50H} = 2.2 \pm 0.3$ μM, $h_H = 1.11 \pm 0.11$, $IC_{50L} = 1374.1 \pm 393.2$ μM, $h_L = 0.97 \pm 0.21$, compare with the values for SUR1 in Fig. 11). Reducing the size of the swapped segment reduces the apparent affinity of the chimeric channel for tolbutamide ($IC_{50H} = 16.7 \pm 4.3$ μM, $h_H = 1.18 \pm 0.19$, $IC_{50L} = 1504.6 \pm 914.5$ μM, and $h_L = 1.01 \pm 0.37$) without significantly affecting the apparent efficacy. (Adapted from [70] with permission.)

association with the M2 helix. Similarly we have positioned the C-terminus of SUR near the C-terminal cytoplasmic segment of $K_{IR}6.2$ based on the general idea that the K_{IR} C-terminus carries the low affinity nucleotide binding site and that the SUR C-terminus specifies the isoform differences in $IC_{50(ATP)}$ [70]. The structure of the K_{IR} pore is modeled on the KcsA channel described by Doyle et al.

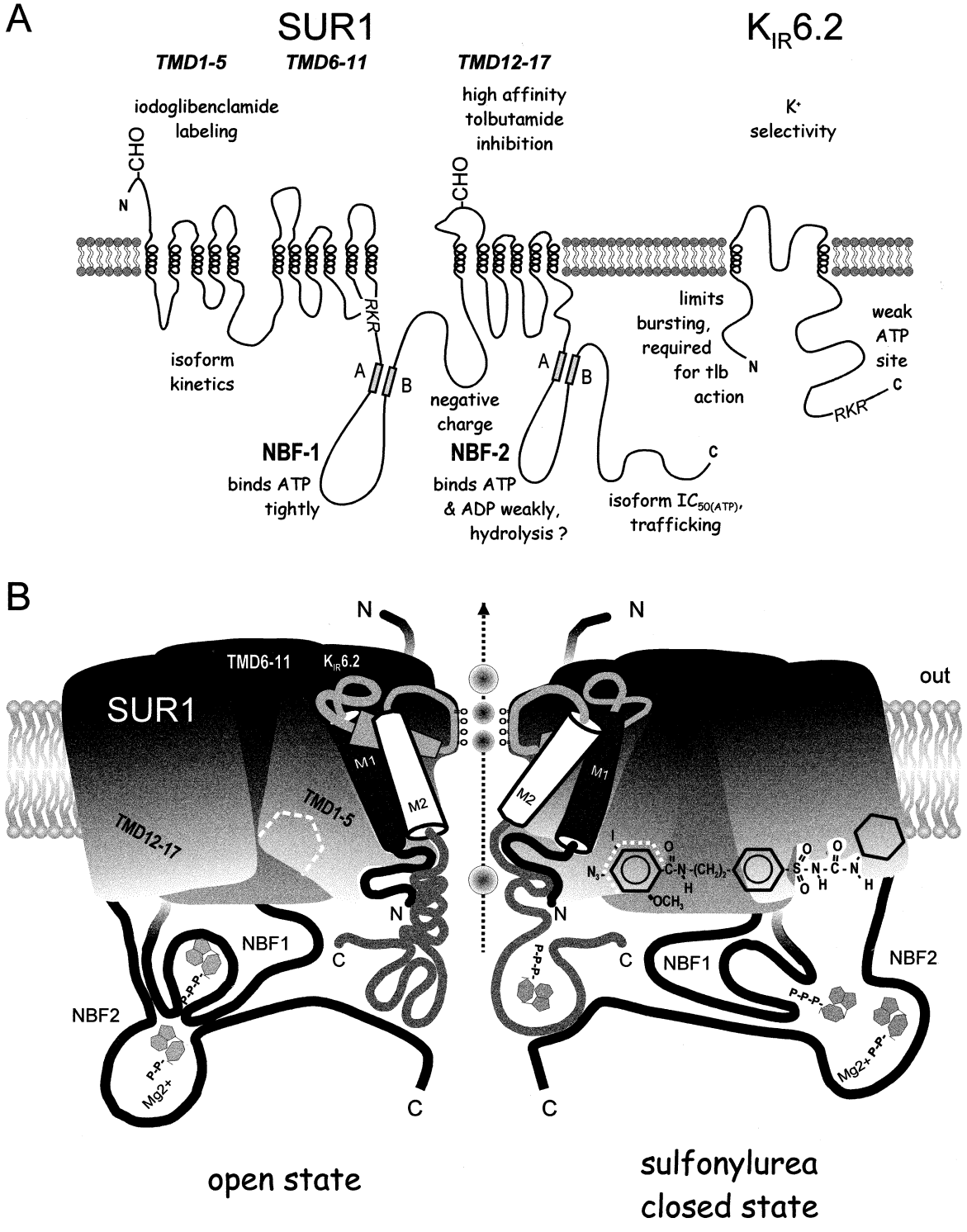

Fig. 14. Summary models. Panel A provides a summary diagram mapping the known properties of K_{ATP} channels to segments and structural features of SUR and $K_{IR}6.2$. Panel B is a schematic illustrating open and sulfonylurea induced closed states of a K_{ATP} channel. The left half of the channel is shown with the M2 helix of $K_{IR}6.2$ in an open orientation allowing K^+ access to the pore. Both nucleotide folds of SUR1 are illustrated with bound nucleotides as suggested by Ueda et al. [51,76]. The right half of the channel is shown in a closed state with glibenclamide bound. The identification of the benzamido (meglitinide) binding site in TMD1–5 is based on photolabeling studies with iodinated glibenclamide [26,77]; the localization of the sulfonylurea binding site is based on the studies with chimeric receptors reviewed here [70,78]. Inhibitory ATP is shown bound to $K_{IR}6.2$, while nucleotide binding to the NBFs is disrupted as suggested by Ueda et al. [76]. The positioning of the SUR segments vis-à-vis $K_{IR}6.2$ is discussed in the text. The representation of $K_{IR}6.2$ is based on the KcsA channel [29]. (Adapted from [70] with permission.)
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[29] without implying an exact packing of M1 and M2: the K^+ selectivity filter is formed by the backbone carbonyl groups (=O) of the *–GFG–* motif. Two dehydrated K^+ ions (smaller spheres) occupy the two sites within the filter; larger hydrated K^+ ions are positioned on either side of the filter. The M2 helices line the aqueous pore within the transmembrane electrical field. 'Gated access' [79] to the pore is believed to be controlled by rigid movements of the M2 helix and possibly an adjacent segment of the C-terminus [43,46]. We have indicated movement of the internal helices by their different positions in the right vs. left halves of the channel. The left pair is illustrated in an open state with the M2 helix positioned to allow access to the pore. No nucleotide is shown on $K_{IR}6.2$ in the open pore state, although there are no data on this point; both NBFs on SUR are occupied, and are shown to be interacting, as they presumably are in vivo. The right pair illustrates a glibenclamide-induced closed state. The glibenclamide binding site is proposed to consist of a benzamido (meglitinide) binding site (dotted ring) on TMD1–5 and the sulfonylurea (tolbutamide) binding site on TMD12–17 consistent with earlier observations on photolabeling with [^{125}I]iodoglibenclamide [26] and the data presented here and in [75]. Interaction with both the benzamido and sulfonylurea binding sites could account for the several thousand-fold increase in affinity of glibenclamide vs. tolbutamide. Cooperative interactions between NBF1 and NBF2 are shown as disrupted by the conformational change (shown here for illustrative purposes as a change in the orientation of the TMD1–5 and 12–17 segments) induced by sulfonylurea binding, consistent with the observations of Ueda et al. [76]. This conformational change repositions the M2 helix to a closed state via a mechanism that requires an intact N-terminus on $K_{IR}6.2$.

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