Brief Review

Gating Mechanism of K_{ATP} Channels: Function Fits Form

D. Enkvetchakul and C.G. Nichols

Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO 63110

Based initially on their prominence in the squid giant axon, their useful pharmacology, and subsequently on being the first cloned K channels, voltage-gated K (Kv) channels have received the lion's share of attention to their biophysical properties. Kv and Ca-activated K channel kinetics and gating mechanisms have been analyzed, and modeled, in exquisite detail (Hille, 2001). Inward rectifier K (Kir) channels have received less attention, and perusal of the literature indicates that there is still no common understanding of kinetic mechanisms in Kir channels. It is our contention, however, that one is in reach. Amongst Kir channels, ATP-sensitive (K_{ATP}) channels are uniquely regulated by cytoplasmic nucleotides and specific pharmacological agents. As such, they play a critical role in coupling cellular metabolism to electrical activity, and are major drug targets in pancreatic, vascular smooth muscle and cardiac muscle (Ashcroft, 1988; Nichols and Lederer, 1991). We would argue that these unique properties also permit elucidation of important features of channel gating that are relevant to the whole class of Kir channels. The molecular mechanisms of K_{ATP} channel regulation have occupied many groups for the last twenty years. Kinetic measurements have led to mathematical models of gating, mutagenesis has indicated relevant molecular elements, and crystallization of various K channel subunits and domains now provides templates for the channel structure. Distilling a consistent model of channel activity and regulation from this broth of data is the challenge for the field, and the topic of this Brief Review.

The K_{ATP} channel is formed from four Kir6.2 pore–forming subunits, and four regulatory sulfonylurea receptor (SUR) subunits (Clement et al., 1997; Inagaki et al., 1997; Shyng and Nichols, 1997) (Fig. 1). Activity is modulated by voltage and by multiple ligands, including ATP and PIP_2, which act on the Kir6.2 subunits themselves, as well as sulfonylureas, potassium channel openers, and Mg-nucleotides, which act on the SUR subunit. Inhibitory ATP binds to the Kir6.2 subunit, while MgATP- and ADP-activation results from interaction with the SUR subunits (Matsuo et al., 1999, 2000; Tanabe et al., 1999; Ueda et al., 1999; MacGregor et al., 2002; Vanoye et al., 2002). K_{ATP} channel behavior is undoubtedly complex, and at present a complete kinetic

model of channel activity, including pharmacological regulation through the SUR subunits is impossible. However, we will argue that a consistent model of Kir6.2 channel activity does arise, and that from this model, the additional complexity of heteromeric complexes will ultimately emerge. We will first consider how thermodynamic and kinetic measurements lead to a model that can explain gating, then consider the structural basis of this behavior.

A Consistent Kinetic Model for Channel Gating

Gating of the K_{ATP} channel: a tetrameric model for Kir channel gating in the absence of ATP. Even in the absence of ATP, single K_{ATP} channel kinetics are complex, and different
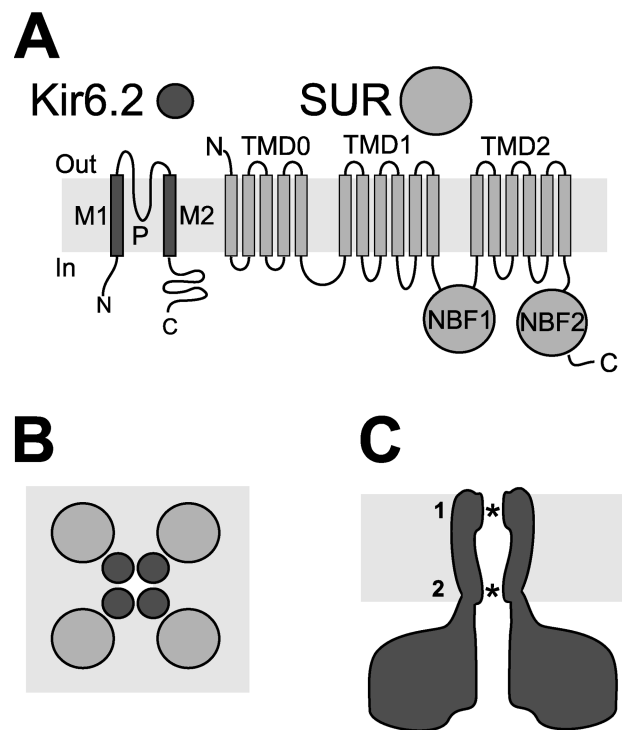

FIGURE 1. The complex K_{ATP} channel. (A) The channel is formed from two dissimilar subunits: Kir6.2 subunits generate the channel pore, SUR subunits generate the regulatory subunit. (B) Each channel is a functional octamer of four Kir6.2 subunits, each associated with four SUR subunits. (C) Likely locations of Kir6.2 channel gating are at the selectivity filter (1) or at the lower end of the inner cavity formed by the M2 helices (2).

laboratories report quantitatively widely differing lifetimes. Nevertheless, certain kinetic components are clearly distinguishable. Single-channel analyses consistently reveal a single-exponential open lifetime distribution, and a multiexponential closed lifetime distribution (Alekseev et al., 1998; Drain et al., 1998; Fan and Makielski, 1999; Enkvetchakul et al., 2000, 2001; Proks et al., 2001). There is invariably a predominant short closed time and one open time (Alekseev et al., 1998; Drain et al., 1998). These short open and closed times, frequently analyzed as the "intraburst" events, are voltage dependent, and are affected by mutations of residues in or near the selectivity filter of the channel (Proks et al., 2001). In addition, there are always several longer closed times that comprise "interburst" closures. As considered below, similar kinetic properties are replicated in other Kir channel family members (e.g., Choe et al., 1999; Bard et al., 2000), consistent with a common underlying gating mechanism.

What kind of model can accommodate these kinetic properties? Numerous schemes have been proposed, based on time-homogeneous Markov models, to describe the open-closed behavior of K_{ATP} channels (Qin et al., 1989; Nichols et al., 1991; Shyng et al., 1997; Alekseev et al., 1998; Enkvetchakul et al., 2000, 2001; Proks et al., 2001; Li et al., 2002). Unfortunately, many models focus on describing limited aspects of channel behavior and do not account for other critical features, which severely limits their predictive utility. Kir channels are tetramers (Glowatzki et al., 1995; Shyng and Nichols, 1997; Doyle et al., 1998; Nishida and MacKinnon, 2002) (see below) and we would strongly argue that relatively simple, tetrameric kinetic models not only replicate all essential features of K_{ATP} channel gating but also have important predictive properties. As illustrated in Fig. 2 A, the assumption that each of the four subunits can be in an open or closed conformation (in the absence of ligand), and that the channel conducts only if all subunits are in the open conformation, automatically produces multiple closed states (Enkvetchakul et al., 2000). Assuming that the open channel can close as a concerted event (i.e., "fast" gating), or by individual subunit closure, such a model (Scheme 0, see Fig. 2 A) will produce one short and five (or four disregarding order of the subunits) "long" closed states, but only one open state, consistent with observed lifetime distributions (Enkvetchakul et al., 2000). This simple tetrameric model will produce bursts of openings, with the intraburst events dominated by the "fast" gating transitions, and interburst closures dominated by subunit closures (Enkvetchakul et al., 2000). It is important to note that even if the subunit open- and closed-durations overlap with the "fast" events, there will still be "bursting" (since there will still be long multi-subunit closures), although a significant number
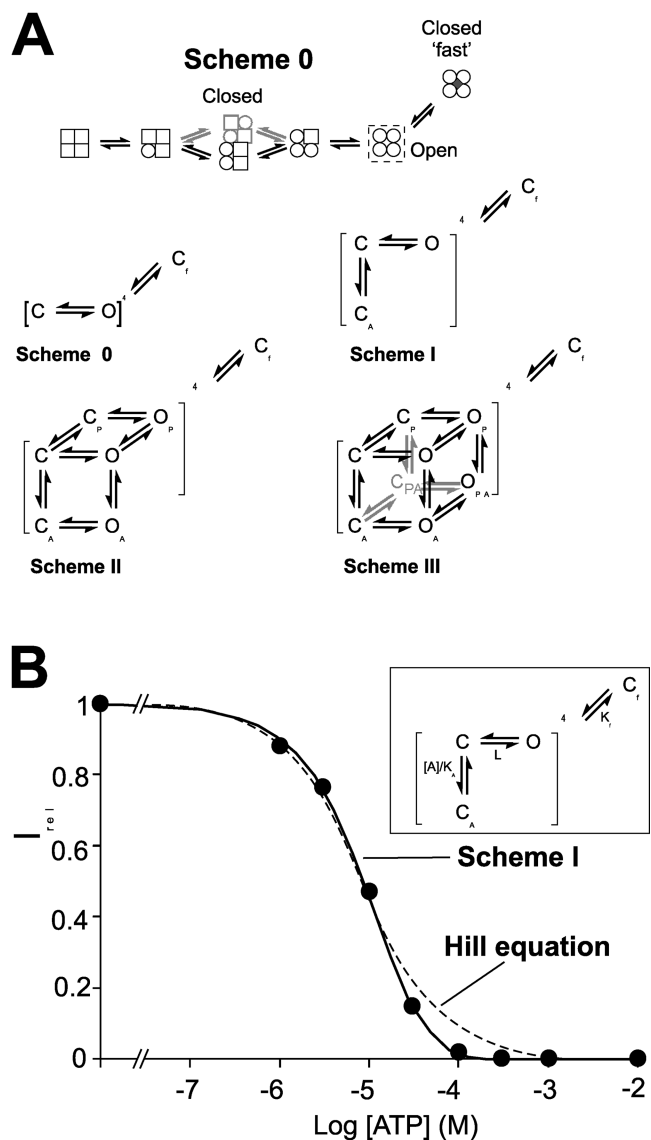

FIGURE 2. Tetrameric-allosteric gating models for K_{ATP} channels. (A) Gating models for unliganded channels (Scheme 0) and subsets (Schemes I and II) of the fully allosteric model (Scheme III) considered in the text. The cartoon (above) illustrates the multiple closed and single open states in scheme 0. (B) For wild-type K_{ATP} (Kir6.2+SUR1) channels, the [ATP]-channel activity relationship is not well fit by a Hill equation ($I = 1/[1 + ([ATP]/K_{1/2})^H]$, where $K_{1/2} = 10\ \mu M$, $H = 1$), but is steeper at higher [ATP] as predicted by Scheme I (Equilibrium constant − L = 10, $K_A = 6.67\ \mu M$, $K_f = 0.136$, data and fits (model V) are from (Enkvetchakul et al., 2000).

of subunit closures will now be included within bursts in any burst-discriminator analysis.

ATP Interactions with the K_{ATP} Channel—Implicating ATP Interaction with Each Subunit

The major consistently reported kinetic feature of ATP gating is that "long" closed lifetime distributions get progressively longer in the presence of inhibitory ATP
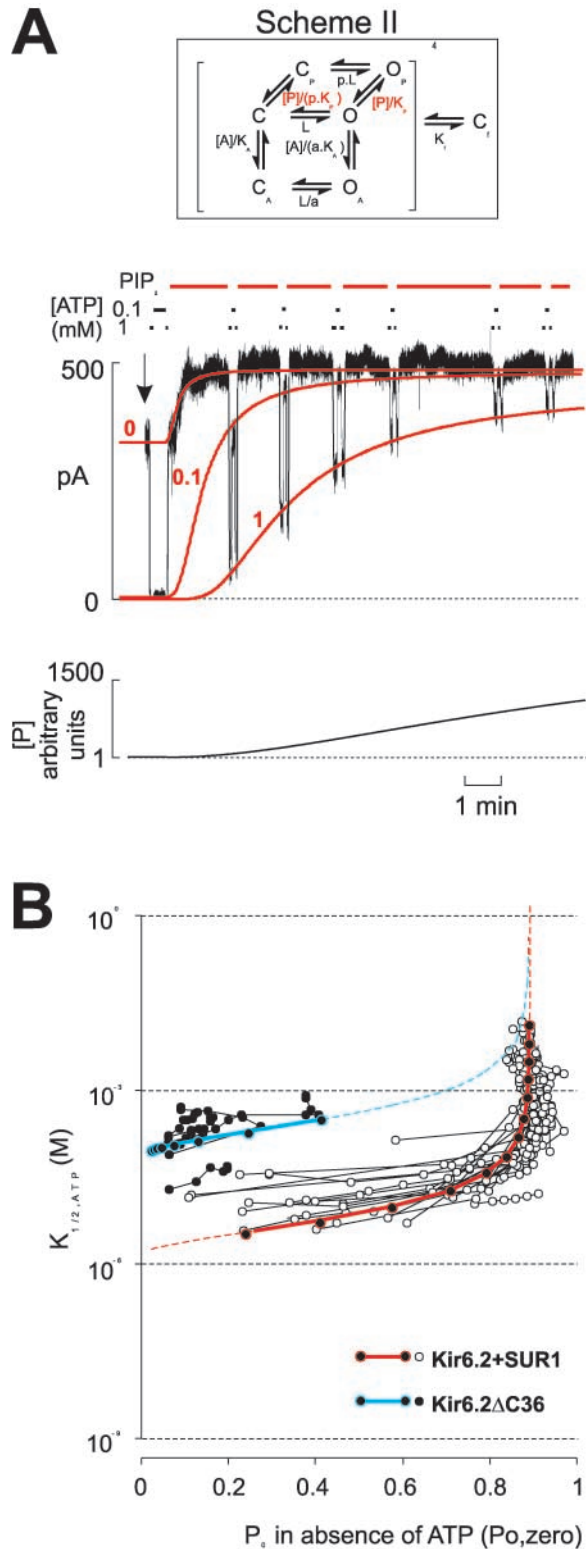
A

Scheme II

B

FIGURE 3. The 6-state tetrameric-allosteric model (Scheme II) predicts complex quantitative dependence of channel activity and ATP sensitivity on membrane PIP_2. (A). Time course of K_{ATP} (Kir6.2+SUR1) channel activity (in [ATP] as indicated) after application of PIP_2 to inside-out membrane patch (data from Shyng and Nichols, 1998). Model simulations are superimposed for the

(Alekseev et al., 1997, 1998; Drain et al., 1998; Enkvetchakul et al., 2000, 2001). Tetrameric models (Enkvetchakul et al., 2000, 2001; Markworth et al., 2000) not only replicate these qualitative features of channel gating, but provide quantitative agreement with additional critical features. While multiple closed lifetimes that all lengthen as ATP increases would require multiple unbound closed states in a linear model, a tetrameric model with only a single ATP-bound subunit conformation (e.g., Scheme I) automatically generates multiple overlapping lifetimes that progressively lengthen as [ATP] (and hence occupancy of C_A state in Scheme I, Fig. 2 A) increases (Enkvetchakul et al., 2000, 2001). Second, steady-state dose–response curves for ATP inhibition of K_{ATP} channels are not well fitted by a symmetrical Hill relationship, since they are considerably steeper at higher [ATP] than they are at lower [ATP] (Fig. 2 B) (Ashcroft and Gribble, 1998; Enkvetchakul et al., 2000; Nichols et al., 1991). Such an asymmetric dose–response curve is automatically generated by a tetrameric subunit model, since only a single subunit transition into a C state is necessary to close the channel (and allow access to a long-lived ATP-bound state) but, at saturating [ATP] concentrations (i.e., with each of the four subunits in the C_A state), four ATP molecules must dissociate for the channel to open (Ashcroft and Gribble, 1998; Enkvetchakul et al., 2000; Markworth et al., 2000).

Antagonistic Behavior of PIP_2 and Open State Stability Mutants on ATP Inhibition: Implicating an Allosteric Four Subunit Model

In the absence of ATP, application of negatively charged phospholipids (in particular PIP_2) to K_{ATP} channels results in an increased open probability

time course of PIP_2 (PIP_2 = 20 + 30,000*[1 − exp(−time/tau)]$^{1.5}$, tau = 5 min), and the predicted current in 0, 0.1, and 1 mM ATP. Equilibrium constants used to simulate the model in this and subsequent figures are given in Table I (slightly modified from those calculated using the rate constants given in Enkvetchakul et al., 2001). L defines the intrinsic open-closed equilibrium in the unliganded subunit. p and 1/a define the relative stabilization of open/closed state ratios, when the subunit is PIP_2 bound, or ATP bound, respectively. (B). Relationship between $K_{1/2,ATP}$ ([ATP] causing half-maximal inhibition) and Po,zero for wild-type K_{ATP} (Kir6.2 + SUR1) channels after application of PIP_2 from patches like that shown in A (data from Enkvetchakul et al., 2000). The red dashed and solid lines are the predictions of the model, the limits of the solid line correspond to PIP_2 = 4 and 8,192 arbitrary units, points indicate the predictions for each doubling of PIP_2. Also shown are measured data and the predicted relationship between $K_{1/2,ATP}$ and Po for truncated Kir6.2ΔC36 channels expressed without SUR1 (see Table I) after application of PIP_2 (from Enkvetchakul et al., 2000). The blue dashed and solid lines are the predictions of the model, the limits of the solid line again corresponding to PIP_2 = 4 and 8,192 a.u., points indicating the predictions for each doubling of PIP_2.

(Po,zero) (Hilgemann and Ball, 1996; Fan and Makielski, 1997). Since these first reports on K_{ATP} channels, a similar action has been demonstrated on all Kir channels (Liou et al., 1999; Rohacs et al., 1999, 2003; Zhang et al., 1999; Lopes et al., 2002). Importantly, PIP_2 has also been shown to be synergistic to activating ligands (e.g., G-proteins on Kir channels) and antagonistic to inhibitory ATP on Kir6.2 channels (Baukrowitz et al., 1998; Shyng and Nichols, 1998; Fan and Makielski, 1999; Kobrinsky et al., 2000; Okamura et al., 2001; Sadja et al., 2001). It has been suggested that the two effects on K_{ATP} channels (i.e., to increase Po,zero and to reduce ATP-sensitivity) may reflect distinct processes (Fan and Makielski, 1999; Okamura et al., 2001), based primarily on the argument that increase of Po,zero is detectable before loss of ATP sensitivity is. However, such a temporal disparity also follows directly from the tetrameric model (Fig. 3 A, for modeling details see figure legends and original publications; Enkvetchakul et al., 2000, 2001). To explicitly incorporate PIP_2 binding steps, Scheme I must be extended, e.g., to Scheme II (Enkvetchakul et al., 2001), which is a restricted case of the fully allosteric model (Scheme III, Fig. 2). The essence of such models is that each subunit can exist in open or closed states whether or not any particular ligand is bound. The intrinsic "open state stability" is a useful descriptor of the intrinsic open/closed equilibrium of the unliganded subunit (denoted by the equilibrium constant L in the modeling below). The open/closed equilibrium is then weighted by some factor (a or p in the modeling), when ATP or PIP_2 is bound (so that although L describes the unliganded open/closed equilibrium, it will reflect the equilibrium distribution at any given $[PIP_2]$). The restricted scheme II may be a reasonable approximation, consistent with the likely structural basis of ligand sensitivity, since PIP_2 and ATP binding may be mutually exclusive (MacGregor et al., 2002; Vanoye et al., 2002).

Fig. 3 A shows the time course of change in Po,zero and ATP sensitivity from a wild-type Kir6.2+SUR1 channel after exposure to micellar PIP_2 (data from Shyng and Nichols, 1998). PIP_2 incorporates into the cytoplasmic leaflet of the membrane, and the PIP_2 sensed by the channel is modeled as an arbitrary "apparent" concentration of PIP_2 in the membrane. Assuming the time course of change of the "apparent" $[PIP_2]$ to be a saturating function, Scheme II can accurately predict the time course of change of Po,zero and the delayed change of ATP sensitivity. Fig. 3 B (open symbols) shows the observed relationship between Po,zero and $K_{1/2,ATP}$ (i.e., the [ATP] causing half-maximal inhibition) obtained from multiple similar time-course experiments on Kir6.2+SUR1 channels (Shyng and Nichols, 1998). The red curve represents the relationship predicted by Scheme II. While the

TABLE I

Equilibrium Constants Used in Simulating 6-state Allosteric Tetramer Model (Scheme II)

Wild-type Kir6.2 + SUR1	
K_A	5 μM
K_P	1 au[a]
K_f	0.1
p[c]	10,000
a[c]	200
L	0.5

Adjustments for mutant channels		
Δ36 (-SUR1)	K_P	1000 au
	K_A	250 μM
L164C	L	1,800
M158C	L	200
R176A[b]	K_P	0.0333 au[b]

[a]Arbitrary units.

[b]Parameter adjustments are combined for double mutant (Kir6.2 [L164C,R176A] etc. channels in Fig. 4 C).

[c]p and 1/a are the relative stabilization of open/closed state ratios, when the subunit is PIP_2 bound, or ATP bound, respectively.

measured parameters (Po,zero and $K_{1/2,ATP}$) change with a time course that varies considerably from experiment to experiment, they remain closely correlated to one another, as predicted by Scheme II (Enkvetchakul et al., 2000)

Qualitatively similar behavior is observed for the gating of Kir6.2 in both the presence and absence of SUR subunits (Tucker et al., 1997). In each case, the channels are inhibited by ATP, and activated by PIP_2 (which causes both an increase in Po,zero and decreased sensitivity to inhibitory ATP; Baukrowitz et al., 1998; Enkvetchakul et al., 2000). However, there are significant quantitative differences: the Po,zero is considerably lower, and the stimulatory effect of PIP_2 is weaker, on Kir6.2ΔC36 channels expressed without SUR1 (Fig. 3 B, closed symbols, from Enkvetchakul et al., 2000). Are these features predicted in any straightforward way by the tetrameric-allosteric model? Steady-state relationships are well modeled quantitatively by simply assuming that the effect of removing SUR is to stabilize the ligand-unbound state (Enkvetchakul et al., 2000). As shown in Fig. 3 B, stabilizing the unliganded channel (by increasing K_A and K_P by 50-fold and 1,000-fold, respectively), quantitatively predicts both the steady-state and the time-dependent changes of $K_{1/2,ATP}$-Po,zero relationships after application of PIP_2 for Kir6.2ΔC channels expressed without SUR1. Predicting the behavior of the channel in the absence or presence of SUR1 thus requires no change in L, i.e., no change in the properties of the subunit gate itself.

Mutations which change the binding affinity of ATP per se (i.e., which alter K_A), will not affect current in
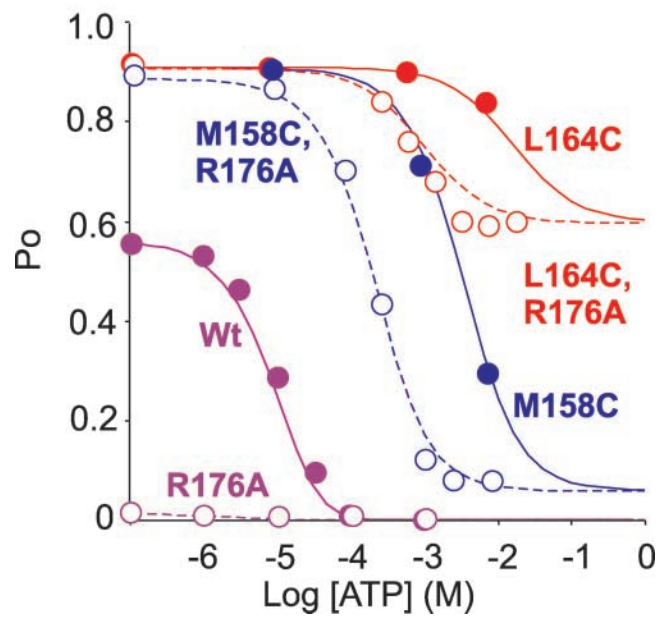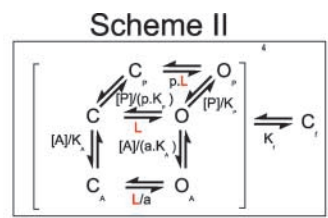
FIGURE 4. The 6-state allosteric model (Scheme II, Table I) also predicts the quantitative consequences of mutations which increase the intrinsic open state stability on channel activity and ATP sensitivity. Increase in open-closed equilibrium (L) leads to a plateau in [ATP]-activity relationship at high [ATP] for high open state stability mutants such as M158C and L164C (from Enkvetchakul et al., 2001).

the absence of ATP. However, many point mutations of Kir6.2 have been shown to cause correlated increase of $P_{o,zero}$ and decrease of ATP sensitivity (Shyng et al., 1997; Drain et al., 1998; Trapp et al., 1998; Tucker et al., 1998; Enkvetchakul et al., 2000). How are these effects incorporated into the gating scheme? Such mutations can change ATP sensitivity and $P_{o,zero}$ by changing the intrinsic equilibrium between the open and closed states of the unliganded subunit (i.e., the intrinsic "open state stability," L) (Enkvetchakul et al., 2001). An important prediction of the model is that in addition to reducing the efficacy of ATP inhibition, a plateau of inhibition will be observed at saturating [ATP] if L is increased sufficiently (i.e., so that L/a becomes large enough, such that the O_A occupancy becomes reasonable at saturating [ATP]). This important prediction is actually met by certain mutations in the lower part of M2 of Kir6.2 (Enkvetchakul et al., 2001). In particular, Kir6.2[L164C]+SUR1 channels are extremely insensitive to ATP, and show very little inhibition by

even 10 mM ATP (Fig. 4). Scheme II predicts that combining this mutation with a mutation to decrease PIP_2 binding (i.e., to decrease Kp) will increase the apparent sensitivity to ATP, and reveal a plateau of inhibition above ~1 mM ATP (Fig. 4). Strikingly, this is indeed observed experimentally for Kir6.2[L164C, R176A] mutations (Fig. 4) (Enkvetchakul et al., 2001).

As discussed above, the major consistently reported kinetic feature of ATP gating is that "long" closed lifetime distributions get progressively longer in the presence of inhibitory ATP, and "burst" durations shorten (Alekseev et al., 1997, 1998; Drain et al., 1998; Enkvetchakul et al., 2000, 2001). The ligand-induced shifts in the distribution of closed state lifetimes are indicative of ATP interaction with the closed state. As discussed above, Scheme II also predicts ATP interaction with the open state. Experimentally this has been more difficult to demonstrate, but in high [ATP] open lifetime distributions clearly shorten in both wild-type (Li et al., 2002) and mutant channels (Enkvetchakul et al., 2001), accounted for in the model by a faster $O_A \rightarrow C_A$ transition than $O_p \rightarrow C_p$ or $O \rightarrow C$ (Enkvetchakul et al., 2001). Thus, ATP can access both open and closed channels, although in wild-type channels, the predominant pathway of gating is predicted to be $O_p \rightarrow O \rightarrow C \rightarrow C_A$. An important caveat of Scheme I (and of this predominant pathway in Scheme II) is that although ATP only (or predominantly) interacts with the closed state (and therefore does not shorten the "real" burst duration, which is determined by subunit closure $O \rightarrow C$), experimentally estimated burst durations can still be shortened if subunit opening ($C \rightarrow O$) rates are similar to the $C_f \leftrightarrow O$ transition rates, since subunit closures will be falsely included in bursts, but will increasingly be correctly excluded as interburst closures, when ATP increases and $C \rightarrow C_A$ transitions become more frequent. In Scheme II, real "burst" durations will additionally shorten as [ATP] increases, since the $O_A \rightarrow C_A$ transition is faster than the $O \rightarrow C$ transition (Enkvetchakul et al., 2001). While it is clearly desirable to know, it is not a trivial procedure to estimate burst durations from Scheme II, and whether the model can adequately account for the experimentally observed shortening of K_{ATP} "burst" durations is not yet clear.

What about other Kir Channels? How Generally Applicable Might the 'Tetrameric-allosteric' Model Be?

Thus far, we have limited discussion to interaction of ligands (ATP, PIP_2) with, and mutations to, the Kir6.2 subunit. In all Kir channels, there are short "intraburst" single closed and open times, modulated by voltage and dependent on the structure of the selectivity filter (Trapp et al., 1998; Choe et al., 1999, 2001; Bard et al., 2000; Lu et al., 2001a,b; Proks et al., 2001; Sadja et al., 2001; Jin et al., 2002). There are also always multiple

"interburst" closed events, and these are invariably the durations that are predominantly modified by regulatory ligands, including ATP (Alekseev et al., 1998; Fan and Makielski, 1999; Enkvetchakul et al., 2000), G-proteins (Bard et al., 2000), and pH (Wu et al., 2002). It is now clear that all Kir channels are activated by PIP$_2$, involving common structural elements (see below) (Hilgemann and Ball, 1996; Fan and Makielski, 1997; Huang et al., 1998; Kim and Bang, 1999; Rohacs et al., 1999, 2003; Sadja et al., 2001; Lopes et al., 2002; Lu et al., 2002; MacGregor et al., 2002; Vanoye et al., 2002; Schulze et al., 2003) to increase the channel open probability. In so doing, PIP$_2$ always reduces sensitivity to inhibitory ligands and enhances sensitivity to activating ligands, consistent with essentially the same allosteric gating mechanism being involved. Thus, although this has not yet been formally considered, the tetrameric-allosteric model is likely to be generally applicable to the gating of other inward rectifiers.

The subunit gating model we propose is essentially the tetrameric subunit gating model proposed fifty years ago by Hodgkin and Huxley (1952) for voltage-gated K channels, each subunit gating equally and independently. More sophisticated models of voltage gating invoke cooperative interactions between subunits to explain gating of Kv channels (Schoppa and Sigworth, 1998; Zagotta et al., 1994) and Ca-activated K channels (Horrigan et al., 1999; Horrigan and Aldrich, 2002; Magleby, 2003), but the essential tetrameric nature of gating seems established. Conceivably, the fundamental subunit mechanism of voltage gating and ligand gating may be common to all K channels, and the recent demonstration that voltage-gated K channels are also activated by PIP$_2$ (Zhang et al., 2003) brings the possibility closer.

The Structural Basis of K$_{ATP}$ Channel Gating

What is the actual gate? Is there a common gate in Kv and Kir channels? What is the subunit "gate" that is stabilized in the closed state by ATP, and in the open state by PIP$_2$? The recent crystal structures of KcsA and MthK demonstrate a plausible gating mechanism for K channels, whereby the M2 helices undergo a rigid body motion to open up the entry to the inner vestibule at the region of the "bundle-crossing" (Fig. 5 B) (Jin et al., 2002; Jiang et al., 2002a,b). Such a model is very consistent with a large body of work demonstrating the ability to trap organic blockers, applied from the cytoplasmic surface, inside Kv channels, when the channels are closed by depolarization, and to block the accessibility of cysteine-reactive molecules to the inner vestibule (Armstrong, 1969; Holmgren et al., 1997; del Camino et al., 2000; del Camino and Yellen, 2001; Shin et al., 2001). Importantly, mutation of M2 residues, particularly residues near the expected bundle crossing (Enk-
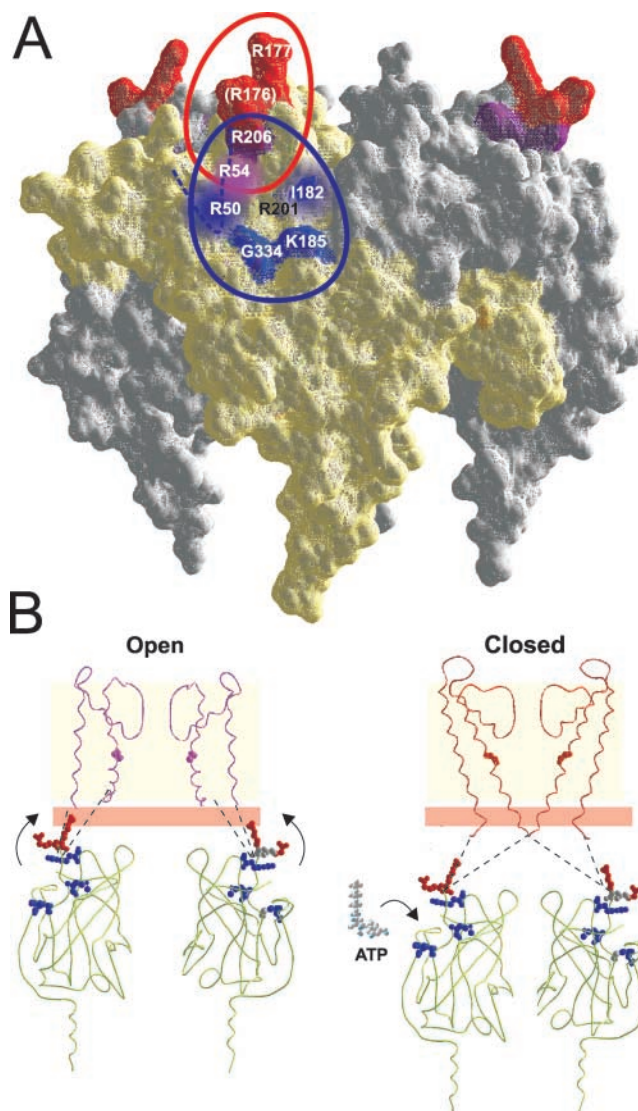

FIGURE 5. The structural basis of ATP gating. (A) Proposed location of the ligand binding domains of Kir6.2. The Kir3.1-NC structure with mutations of residues corresponding to putative ATP- and PIP$_2$-binding residues in Kir6.2 is shown as space filling representation (SwissProt). Putative ATP-binding residues are colored blue. Putative PIP$_2$ binding residues are colored red, and residues proposed to be involved in the binding of both are colored purple. Unresolved NH$_2$-terminal residues are proposed to fold over the ligand binding domain (dashed line) and the suggested location of missing critical residues are shown as colored patches. (B) Proposed model for ligand gating in Kir6.2 channels. The open channel (left), based on the MthK structure, is stabilized by membrane phospholipids binding to the cytoplasmic domain (red residues). The closed channel (right) is stabilized by ATP binding to the cytoplasmic domain (blue residues).

vetchakul et al., 2000, 2001; Sadja et al., 2001; Yi et al., 2001) (and moreover at equivalent residues to those that lead to voltage-independent opening of Kv channels; Espinosa et al., 2001; Hackos et al., 2002) can have very profound effects on ligand gating in Kir channels, indicating a clear involvement of M2 in coupling the

gating sensors to the gates and leading to the implicit supposition that M2 helices themselves act as the subunit gates to pinch off the permeation pathway in the closed state (Enkvetchakul et al., 2000; Loussouarn et al., 2001; Jin et al., 2002).

Significant evidence has been marshaled in support of the idea that a very tight steric closure of Kv and HCN channels does occur at the level of the M2 (S6) "bundle crossing" (Liu et al., 1997; del Camino et al., 2000; del Camino and Yellen, 2001; Shin et al., 2001; Rothberg et al., 2002). Quantitative analysis of the dependence of reagent accessibility on open probability now indicates a critical caveat to interpretation of experiments using accessibility measurements to assess the position of the channel gate in Kir channels (Phillips et al., 2003). With both rapid gating and rapid reagent access, relative to modification rates, the problem of reagent "trapping" can obscure a gated access, unless the degree of closure is very high. For Kir6.2 channels, the data indicate that access of the sulfhydryl reactive MTSEA to the inner vestibule is gated by ATP (Phillips et al., 2003). While we thus favor the interpretation that the "gate" lies at the "bundle crossing" (Jin et al., 2002), the results of reagent accessibility studies in several ligand-gated cation channels, including Kir channels, have been more ambiguous (Flynn and Zagotta, 2001; Bruening-Wright et al., 2002; Xiao and Yang, 2002; Proks et al., 2003), and there remains considerable controversy on this point.

The Structure of the K_{ATP} Ligand-binding Domains?

Many studies have sought to localize the ATP binding site on Kir6.2 by mutagenesis (Shyng et al., 1997, 2000; Tucker et al., 1997, 1998; Shyng and Nichols, 1998; Trapp et al., 1998; Koster et al., 1999; Proks et al., 1999; Reimann et al., 1999; Tanabe et al., 1999; Enkvetchakul et al., 2000; Xu et al., 2001; Cukras et al., 2002a,b). Much evidence has indicated a structurally conserved Kir cytoplasmic domain (Durell and Guy, 2001; Cukras et al., 2002b), containing both NH_2 terminus and COOH terminus contributions (Tucker and Ashcroft, 1999; Jones et al., 2001), and this is now confirmed by the recent crystallization of the linked NH_2 and COOH termini of Kir3.1 (GIRK-NC) (Nishida and MacKinnon, 2002), which generates a stable tetramer of globular domains (see also Note added in proof). Most mutations that alter apparent ATP sensitivity of K_{ATP} do so by allosterically altering L (i.e., they affect the intrinsic open-closed equilibrium) and very few residues (R50; Proks et al., 1999; Cukras et al., 2002a) in the NH_2 terminus, and I182 (Li et al., 2000), K185 (Tucker et al., 1997; 1998; Koster et al., 1999), R201 (Cukras et al., 2002b), and G334 (Drain et al., 1998) in the COOH terminus, actually control ATP affinity itself. Even though these residues are well separated in the primary sequence, the GIRK-NC structure (Nishida and MacKinnon, 2002) shows that the corresponding residues are all located in a single patch on the external surface of each subunit (Fig. 5 A). It is particularly satisfying that two residues, K185 (Tucker et al., 1997; Reimann et al., 1999) and G334 (Drain et al., 1998), which have been most carefully and definitively identified as controlling ATP binding rather than allosteric coupling, are predicted to be extremely close in the 3-D structure (Fig. 5 A). Likely PIP_2-interacting residues (R54, R176, R177, R206) (Baukrowitz et al., 1998; Shyng and Nichols, 1998; Shyng et al., 2000; Cukras et al., 2002a,b; Schulze et al., 2003) are also in an overlapping patch, on the top surface of the domain, where they would be expected to be directly opposing lipid headgroups (Fig. 5 A). Since there is an ATP binding site located on each subunit (see also Note added in proof), four separate ATP binding sites per channel are thus established. The overlapping nature of the ATP-binding sites and the PIP_2-interacting sites is very consistent with the proposed "negative heterotropic" interaction of these two groups in channel regulation (Shyng and Nichols, 1998). The apparent competition of the two ligands for the same state (i.e., the nonligand bound state) (MacGregor et al., 2002), but without perfect exclusion of one ligand by the other, is also consistent with the proposed gating model (Scheme II above) (Enkvetchakul et al., 2000, 2001). Important details are of course still missing from this structure. The exact orientation of ATP within its binding site needs to be established (see also Note added in proof) and at least part of the potential PIP_2 binding site is unresolved. Nevertheless, essential structural features are present and provide additional food for thought regarding the nature of the SUR1–Kir6.2 interaction. Part of the crystallized NH_2 terminus is unresolved, a part that likely forms some kind of "lid" over the ATP/ PIP_2 binding sites (Fig. 5 A). Possibly SUR1 helps to stabilize this fold, such an effect could lead to the destabilization of the ligand-unbound states (relative to either the PIP_2, or ATP-bound states; Enkvetchakul et al., 2000).

Putting it All Together. Does Function Meet Form?

Beginning with early analyses (Shyng et al., 1997; Nichols et al., 1991; Alekseev et al., 1998), tetrameric-allosteric models (Enkvetchakul et al., 2001; Enkvetchakul et al., 2000) can reproduce the essential aspects of K_{ATP} channel gating. Adjusting only one or two parameters in such models allows quantitative prediction of the effects of mutations that alter PIP_2 binding, ATP binding, or the intrinsic open state stability on both macro- and microscopic gating. The concepts behind the tetrameric model are not original—both the subunit mechanism and allosteric kinetic modeling

have been used in other channel fields and protein function fields before (Monod et al., 1965; Zagotta et al., 1994; Sunderman and Zagotta, 1999; Magleby, 2003), but the predictive capacity of such models for K_{ATP} channels is quite striking.

How does ligand-binding "gate" Kir channels? The crystallization of open and closed K channel pores and the cytoplasmic domain of a Kir channel now provide dramatic snapshots of the likely structures of both the gating modules and perhaps the gates themselves, in Kir channels. The demonstration of four separated ligand-binding domains per channel (Nishida and MacKinnon, 2002; see Note added in proof) supports a tetrameric subunit model of gating, and provides a plausible physical gating mechanism (Enkvetchakul et al., 2000, 2001; Jiang et al., 2002b) (Fig. 5 B). We propose that ligand-gating of Kir channels occurs by "pinching" of the permeation pathway at the M2 helix bundle crossing (Enkvetchakul et al., 2000; Jiang et al., 2002b; Jin et al., 2002; Phillips et al., 2003). The relatively short distance between the ends of the M1/2 helices and the crystallized GIRK-NC cytoplasmic domain leaves little ambiguity regarding the orientation of the domains relative to one another, and the location of the cytoplasmic domain relative to the membrane. Interaction of residues 54, 176, 177, 206 (in Kir6.2) (Fan and Makielski, 1997; Baukrowitz et al., 1998; Shyng and Nichols, 1998; Shyng et al., 2000; Cukras et al., 2002a; Schulze et al., 2003) with PIP_2 is proposed to "pull" the cytoplasmic domain toward the membrane, thereby stabilizing the open state of the channel. Conversely, interaction of an overlapping set of residues (50, 54, 182, 185, 201, 20b, 334) (Tucker et al., 1997; Drain et al., 1998; Li et al., 2000; Shyng et al., 2000) with ATP, will destabilize/exclude the membrane interaction, "pulling" the cytoplasmic domain away from the membrane and thereby stabilizing the closed state. This model achieves the lofty goal of equating specific physical states of each subunit with specific kinetic states. Of course it suffers from being mere conjecture on our behalf. However, it is an explicit model that can be tested—and one that undoubtedly will be in the near future!

Olaf S. Andersen served as editor.

Note added in proof. The recently described crystal structure of a bacterial Kir channel greatly clarifies the NH_2- and COOH-terminal interactions and the structural link to the transmembrane domains (Kuo, A., J.M. Gulbis, J.F. Antcliffe, T. Rahman, E.D. Lowe, J. Zimmer, J. Cuthbertson, F.M. Ashcroft, T. Ezaki, and D.A. Doyle. 2003. Crystal structure of the potassium channel KirBac1.1 in the closed state. *Science.* 300:1922–1926.). Recent quantitative computer-docking experiments predict ATP binding to essentially the site proposed in Fig. 5 on each Kir6.2 subunit (Trapp, S., S. Haider, P. Jones, M.S. Sansom, F.M. Ashcroft. 2003. Identification of residues contributing to the ATP binding site of Kir6.2. *EMBO J.* 22:2903–2912.).

REFERENCES

Alekseev, A.E., P.A. Brady, and A. Terzic. 1998. Ligand-insensitive state of cardiac ATP-sensitive K+ channels. Basis for channel opening. *J. Gen. Physiol.* 111:381–394.

Alekseev, A.E., M.E. Kennedy, B. Navarro, and A. Terzic. 1997. Burst kinetics of co-expressed Kir6.2/SUR1 clones: comparison of recombinant with native ATP-sensitive K+ channel behavior. *J. Membr. Biol.* 159:161–168.

Armstrong, C.M. 1969. Inactivation of the potassium conductance and related phenomena caused by quaternary ammonium ion injection in squid axons. *J. Gen. Physiol.* 54:553–575.

Ashcroft, F.M. 1988. Adenosine 5′-triphosphate-sensitive potassium channels. *Annu. Rev. Neurosci.* 11:97–118.

Ashcroft, F.M., and F.M. Gribble. 1998. Correlating structure and function in ATP-sensitive K+ channels. *Trends Neurosci.* 21:288–294.

Bard, J., M.T. Kunkel, and E.G. Peralta. 2000. Single channel studies of inward rectifier potassium channel regulation by muscarinic acetylcholine receptors. *J. Gen. Physiol.* 116:645–652.

Baukrowitz, T., U. Schulte, D. Oliver, S. Herlitze, T. Krauter, S.J. Tucker, J.P. Ruppersberg, and B. Fakler. 1998. PIP2 and PIP as determinants for ATP inhibition of KATP channels. *Science.* 282:1141–1144.

Bruening-Wright, A., M.A. Schumacher, J.P. Adelman, and J. Maylie. 2002. Localization of the activation gate for small conductance Ca2+- activated K+ channels. *J. Neurosci.* 22:6499–6506.

Choe, H., L.G. Palmer, and H. Sackin. 1999. Structural determinants of gating in inward-rectifier K+ channels. *Biophys. J.* 76:1988–2003.

Choe, H., H. Sackin, and L.G. Palmer. 2001. Gating properties of inward-rectifier potassium channels: effects of permeant ions. *J. Membr. Biol.* 184:81–89.

Clement, J.P.T., K. Kunjilwar, G. Gonzalez, M. Schwanstecher, U. Panten, L. Aguilar-Bryan, and J. Bryan. 1997. Association and stoichiometry of K(ATP) channel subunits. *Neuron.* 18:827–838.

Cukras, C.A., I. Jeliazkova, and C.G. Nichols. 2002a. The role of NH(2)-terminal positive charges in the activity of inward rectifier K(ATP) channels. *J. Gen. Physiol.* 120:437–446.

Cukras, C.A., I. Jeliazkova, and C.G. Nichols. 2002b. Structural and functional determinants of conserved lipid interaction domains of inward rectifying kir6.2 channels. *J. Gen. Physiol.* 119:581–591.

del Camino, D., M. Holmgren, Y. Liu, and G. Yellen. 2000. Blocker protection in the pore of a voltage-gated K+ channel and its structural implications. *Nature.* 403:321–325.

del Camino, D., and G. Yellen. 2001. Tight steric closure at the intracellular activation gate of a voltage- gated k(+) channel. *Neuron.* 32:649–656.

Doyle, D.A., J. Morais Cabral, R.A. Pfuetzner, A. Kuo, J.M. Gulbis, S.L. Cohen, B.T. Chait, and R. MacKinnon. 1998. The structure of the potassium channel: molecular basis of K+ conduction and selectivity. *Science.* 280:69–77.

Drain, P., L. Li, and J. Wang. 1998. KATP channel inhibition by ATP requires distinct functional domains of the cytoplasmic C terminus of the pore-forming subunit. *Proc. Natl. Acad. Sci. USA.* 95:13953–13958.

Durell, S.R., and H.R. Guy. 2001. A family of putative Kir potassium channels in prokaryotes. *BMC Evol. Biol.* 1:14.

Enkvetchakul, D., G. Loussouarn, E. Makhina, and C.G. Nichols. 2001. ATP interaction with the open state of the K(ATP) channel. *Biophys. J.* 80:719–728.

Enkvetchakul, D., G. Loussouarn, E. Makhina, S.L. Shyng, and C.G. Nichols. 2000. The kinetic and physical basis of K(ATP) channel gating: toward a unified molecular understanding. *Biophys. J.* 78:2334–2348.

Espinosa, F., R. Fleischhauer, A. McMahon, and R.H. Joho. 2001. Dynamic interaction of S5 and S6 during voltage-controlled gating in a potassium channel. *J. Gen. Physiol.* 118:157–170.

Fan, Z., and J.C. Makielski. 1997. Anionic phospholipids activate ATP-sensitive potassium channels. *J. Biol. Chem.* 272:5388–5395.

Fan, Z., and J.C. Makielski. 1999. Phosphoinositides decrease ATP sensitivity of the cardiac ATP-sensitive K(+) channel. A molecular probe for the mechanism of ATP-sensitive inhibition. *J. Gen. Physiol.* 114:251–269.

Flynn, G.E., and W.N. Zagotta. 2001. Conformational changes in S6 coupled to the opening of cyclic nucleotide-gated channels. *Neuron.* 30:689–698.

Glowatzki, E., G. Fakler, U. Brandle, U. Rexhausen, H.P. Zenner, J.P. Ruppersberg, and B. Fakler. 1995. Subunit-dependent assembly of inward-rectifier K+ channels. *Proc. R. Soc. Lond. B. Biol. Sci.* 261:251–261.

Hackos, D.H., T.H. Chang, and K.J. Swartz. 2002. Scanning the intracellular s6 activation gate in the shaker k(+) channel. *J. Gen. Physiol.* 119:521–532.

Hilgemann, D.W., and R. Ball. 1996. Regulation of cardiac Na+, Ca2+ exchange and KATP potassium channels by PIP2. *Science.* 273:956–959.

Hille, B. 2001. Gating mechanisms: kinetic thinking. *In* Ion Channels of Excitable Membranes. Sinauer Associates, Sunderland, MA. 575–602.

Hodgkin, A.L., and A.F. Huxley. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* 117:500–544.

Holmgren, M., P.L. Smith, and G. Yellen. 1997. Trapping of organic blockers by closing of voltage-dependent K+ channels - evidence for a trap door mechanism of activation gating. *J. Gen. Physiol.* 109:527–535.

Horrigan, F.T., and R.W. Aldrich. 2002. Coupling between voltage sensor activation, Ca2+ binding and channel opening in large conductance (BK) potassium channels. *J. Gen. Physiol.* 120:267–305.

Horrigan, F.T., J. Cui, and R.W. Aldrich. 1999. Allosteric voltage gating of potassium channels I. Mslo ionic currents in the absence of Ca(2+). *J. Gen. Physiol.* 114:277–304.

Huang, C.L., S. Feng, and D.W. Hilgemann. 1998. Direct activation of inward rectifier potassium channels by PIP2 and its stabilization by Gbetagamma. *Nature.* 391:803–806.

Inagaki, N., T. Gonoi, and S. Seino. 1997. Subunit stoichiometry of the pancreatic beta-cell ATP-sensitive K+ channel. *FEBS Lett.* 409:232–236.

Jiang, Y., A. Lee, J. Chen, M. Cadene, B.T. Chait, and R. MacKinnon. 2002a. Crystal structure and mechanism of a calcium-gated potassium channel. *Nature.* 417:515–522.

Jiang, Y., A. Lee, J. Chen, M. Cadene, B.T. Chait, and R. MacKinnon. 2002b. The open pore conformation of potassium channels. *Nature.* 417:523–526.

Jin, T., L. Peng, T. Mirshahi, T. Rohacs, K.W. Chan, R. Sanchez, and D.E. Logothetis. 2002. The βγ subunits of G proteins gate a K(+) channel by pivoted bending of a transmembrane segment. *Mol. Cell.* 10:469–481.

Jones, P.A., S.J. Tucker, and F.M. Ashcroft. 2001. Multiple sites of interaction between the intracellular domains of an inwardly rectifying potassium channel, Kir6.2. *FEBS Lett.* 508:85–89.

Kim, D., and H. Bang. 1999. Modulation of rat atrial G protein-coupled K+ channel function by phospholipids. *J. Physiol.* 517:59–74.

Kobrinsky, E., T. Mirshahi, H. Zhang, T. Jin, and D.E. Logothetis. 2000. Receptor-mediated hydrolysis of plasma membrane messenger PIP2 leads to K+-current desensitization. *Nat. Cell Biol.* 2:507–514.

Koster, J.C., Q. Sha, S. Shyng, and C.G. Nichols. 1999. ATP inhibition of KATP channels: control of nucleotide sensitivity by the N-terminal domain of the Kir6.2 subunit. *J. Physiol.* 515:19–30.

Li, L., X. Geng, and P. Drain. 2002. Open state destabilization by ATP occupancy is mechanism speeding burst exit underlying KATP channel inhibition by ATP. *J. Gen. Physiol.* 119:105–116.

Li, L., J. Wang, and P. Drain. 2000. The I182 region of k(ir)6.2 is closely associated with ligand binding in K(ATP) channel inhibition by ATP. *Biophys. J.* 79:841–852.

Liou, H.H., S.S. Zhou, and C.L. Huang. 1999. Regulation of ROMK1 channel by protein kinase A via a phosphatidylinositol 4,5-bisphosphate-dependent mechanism. *Proc. Natl. Acad. Sci. USA.* 96:5820–5825.

Liu, Y., M. Holmgren, M.E. Jurman, and G. Yellen. 1997. Gated access to the pore of a voltage-dependent K+ channel. *Neuron.* 19:175–184.

Lopes, C.M., H. Zhang, T. Rohacs, T. Jin, J. Yang, and D.E. Logothetis. 2002. Alterations in conserved Kir channel-PIP2 interactions underlie channelopathies. *Neuron.* 34:933–944.

Loussouarn, G., L.R. Phillips, R. Masia, T. Rose, and C.G. Nichols. 2001. Flexibility of the Kir6.2 inward rectifier K(+) channel pore. *Proc. Natl. Acad. Sci. USA.* 98:4227–4232.

Lu, M., S.C. Hebert, and G. Giebisch. 2002. Hydrolyzable ATP and PIP(2) modulate the small-conductance K(+) Channel in apical membranes of rat cortical-collecting duct (CCD). *J. Gen. Physiol.* 120:603–615.

Lu, T., A.Y. Ting, J. Mainland, L.Y. Jan, P.G. Schultz, and J. Yang. 2001a. Probing ion permeation and gating in a K+ channel with backbone mutations in the selectivity filter. *Nat. Neurosci.* 4:239–246.

Lu, T., L. Wu, J. Xiao, and J. Yang. 2001b. Permeant ion-dependent changes in gating of Kir2.1 inward rectifier potassium channels. *J. Gen. Physiol.* 118:509–522.

MacGregor, G.G., K. Dong, C.G. Vanoye, L. Tang, G. Giebisch, and S.C. Hebert. 2002. Nucleotides and phospholipids compete for binding to the C terminus of KATP channels. *Proc. Natl. Acad. Sci. USA.* 99:2726–2731.

Magleby, K.L. 2003. Gating mechanism of BK (Slo1) channels: so near, yet so far. *J. Gen. Physiol.* 121:81–96.

Markworth, E., C. Schwanstecher, and M. Schwanstecher. 2000. ATP4 mediates closure of pancreatic beta-cell ATP-sensitive potassium channels by interaction with 1 of 4 identical sites. *Diabetes.* 49:1413–1418.

Matsuo, M., N. Kioka, T. Amachi, and K. Ueda. 1999. ATP binding properties of the nucleotide-binding folds of SUR1. *J. Biol. Chem.* 274:37479–37482.

Matsuo, M., K. Tanabe, N. Kioka, T. Amachi, and K. Ueda. 2000. Different binding properties and affinities for ATP and ADP among sulfonylurea receptor subtypes, SUR1, SUR2A, and SUR2B. *J. Biol. Chem.* 275:28757–28763.

Monod, J., J. Wyman, J.P. Changeux. 1965. On the nature of allosteric transitions: a plausible model. *J. Mol. Biol.* 12:88–118.

Nichols, C.G., and W.J. Lederer. 1991. Adenosine triphosphate-sensitive potassium channels in the cardiovascular system. *Am. J. Physiol.* 261:H1675–H1686.

Nichols, C.G., W.J. Lederer, and M.B. Cannell. 1991. ATP dependence of KATP channel kinetics in isolated membrane patches from rat ventricle. *Biophys. J.* 60:1164–1177.

Nishida, M., and R. MacKinnon. 2002. Structural basis of inward rectification. Cytoplasmic pore of the G protein-gated inward rectifier GIRK1 at 1.8 A resolution. *Cell.* 111:957–965.

Okamura, M., M. Kakei, K. Ichinari, A. Miyamura, N. Oketani, N. Koriyama, and C. Tei. 2001. State-dependent modification of ATP-sensitive K+ channels by phosphatidylinositol 4,5-bisphosphate. *Am. J. Physiol. Cell Physiol.* 280:C303–C308.

Phillips, L.R., D. Enkvetchakul, and C.G. Nichols. 2003. Gating dependence of inner pore access in inward rectifier K(+) channels. *Neuron.* 37:953–962.

Proks, P., J.F. Antcliff, and F.M. Ashcroft. 2003. The ligand-sensitive gate of a potassium channel lies close to the selectivity filter. *EMBO Rep.* 4:70–75.

Proks, P., C.E. Capener, P. Jones, and F.M. Ashcroft. 2001. Mutations within the P-loop of Kir6.2 modulate the intraburst kinetics of the ATP-sensitive potassium channel. *J. Gen. Physiol.* 118:341–353.

Proks, P., F.M. Gribble, R. Adhikari, S.J. Tucker, and F.M. Ashcroft. 1999. Involvement of the N-terminus of Kir6.2 in the inhibition of the KATP channel by ATP. *J. Physiol.* 514:19–25.

Qin, D.Y., M. Takano, and A. Noma. 1989. Kinetics of ATP-sensitive K+ channel revealed with oil-gate concentration jump method. *Am. J. Physiol.* 257:H1624–H1633.

Reimann, F., T.J. Ryder, S.J. Tucker, and F.M. Ashcroft. 1999. The role of lysine 185 in the Kir6.2 subunit of the ATP-sensitive channel in channel inhibition by ATP. *J. Physiol.* 520:661–669.

Rohacs, T., J. Chen, G.D. Prestwich, and D.E. Logothetis. 1999. Distinct specificities of inwardly rectifying K(+) channels for phosphoinositides. *J. Biol. Chem.* 274:36065–36072.

Rohacs, T., C.M. Lopes, T. Jin, P.P. Ramdya, Z. Molnar, and D.E. Logothetis. 2003. Specificity of activation by phosphoinositides determines lipid regulation of Kir channels. *Proc. Natl. Acad. Sci. USA.* 100:745–750.

Rothberg, B.S., K.S. Shin, P.S. Phale, and G. Yellen. 2002. Voltage-controlled gating at the intracellular entrance to a hyperpolarization-activated cation channel. *J. Gen. Physiol.* 119:83–91.

Sadja, R., K. Smadja, N. Alagem, and E. Reuveny. 2001. Coupling Gβγ-dependent activation to channel opening via pore elements in inwardly rectifying potassium channels. *Neuron.* 29:669–680.

Schoppa, N.E., and F.J. Sigworth. 1998. Activation of Shaker potassium channels. III. An activation gating model for wild-type and V2 mutant channels. *J. Gen. Physiol.* 111:313–342.

Schulze, D., T. Krauter, H. Fritzenschaft, M. Soom, and T. Baukrowitz. 2003. Phosphatidylinositol 4,5-bisphosphate (PIP2) modulation of ATP and pH sensitivity in Kir channels. A tale of an active and a silent PIP2 site in the N terminus. *J. Biol. Chem.* 278:10500–10505.

Shin, K.S., B.S. Rothberg, and G. Yellen. 2001. Blocker state dependence and trapping in hyperpolarization-activated cation channels: evidence for an intracellular activation gate. *J. Gen. Physiol.* 117:91–101.

Shyng, S., T. Ferrigni, and C.G. Nichols. 1997. Control of rectification and gating of cloned KATP channels by the Kir6.2 subunit. *J. Gen. Physiol.* 110:141–153.

Shyng, S., and C.G. Nichols. 1997. Octameric stoichiometry of the KATP channel complex. *J. Gen. Physiol.* 110:655–664.

Shyng, S.L., C.A. Cukras, J. Harwood, and C.G. Nichols. 2000. Structural determinants of PIP(2) regulation of inward rectifier K(ATP) channels. *J. Gen. Physiol.* 116:599–608 (In Process Citation).

Shyng, S.L., and C.G. Nichols. 1998. Membrane phospholipid control of nucleotide sensitivity of KATP channels. *Science.* 282:1138–1141.

Sunderman, E.R., and W.N. Zagotta. 1999. Sequence of events underlying the allosteric transition of rod cyclic nucleotide-gated channels. *J. Gen. Physiol.* 113:621–640.

Tanabe, K., S.J. Tucker, M. Matsuo, P. Proks, F.M. Ashcroft, S. Seino, T. Amachi, and K. Ueda. 1999. Direct photoaffinity labeling of the Kir6.2 subunit of the ATP-sensitive K+ channel by 8-azido-ATP. *J. Biol. Chem.* 274:3931–3933.

Trapp, S., P. Proks, S.J. Tucker, and F.M. Ashcroft. 1998. Molecular analysis of ATP-sensitive K channel gating and implications for channel inhibition by ATP. *J. Gen. Physiol.* 112:333–349.

Tucker, S.J., and F.M. Ashcroft. 1999. Mapping of the physical interaction between the intracellular domains of an inwardly rectifying potassium channel, Kir6.2. *J. Biol. Chem.* 274:33393–33397.

Tucker, S.J., F.M. Gribble, P. Proks, S. Trapp, T.J. Ryder, T. Haug, F. Reimann, and F.M. Ashcroft. 1998. Molecular determinants of KATP channel inhibition by ATP. *EMBO J.* 17:3290–3296.

Tucker, S.J., F.M. Gribble, C. Zhao, S. Trapp, and F.M. Ashcroft. 1997. Truncation of Kir6.2 produces ATP-sensitive K+ channels in the absence of the sulphonylurea receptor. *Nature.* 387:179–183.

Ueda, K., J. Komine, M. Matsuo, S. Seino, and T. Amachi. 1999. Co-operative binding of ATP and MgADP in the sulfonylurea receptor is modulated by glibenclamide. *Proc. Natl. Acad. Sci. USA.* 96:1268–1272.

Vanoye, C.G., G.G. MacGregor, K. Dong, L. Tang, A.S. Buschmann, A.E. Hall, M. Lu, G. Giebisch, and S.C. Hebert. 2002. The carboxyl termini of K(ATP) channels bind nucleotides. *J. Biol. Chem.* 277:23260–23270.

Wu, J., H. Xu, Z. Yang, Y. Wang, J. Mao, and C. Jiang. 2002. Protons activate homomeric kir6.2 channels by selective suppression of the long and intermediate closures. *J. Membr. Biol.* 190:105–116.

Xiao, J., and J. Yang. 2002. Where is the activation gate for PIP2 gating of Kir2.1 channels? *Biophys. J.* 82:180a.

Xu, H., J. Wu, N. Cui, L. Abdulkadir, R. Wang, J. Mao, L.R. Giwa, S. Chanchevalap, and C. Jiang. 2001. Distinct histidine residues control the acid-induced activation and inhibition of the cloned katp channel. *J. Biol. Chem.* 276:38690–38696.

Yi, B.A., Y.F. Lin, Y.N. Jan, and L.Y. Jan. 2001. Yeast screen for constitutively active mutant G protein-activated potassium channels. *Neuron.* 29:657–667.

Zagotta, W.N., T. Hoshi, and R.W. Aldrich. 1994. Shaker potassium channel gating. III: Evaluation of kinetic models for activation. *J. Gen. Physiol.* 103:321–362.

Zhang, H., L.C. Craciun, T. Mirshahi, T. Rohacs, C.M. Lopes, T. Jin, and D.E. Logothetis. 2003. PIP(2) activates KCNQ channels, and its hydrolysis underlies receptor-mediated inhibition of M currents. *Neuron.* 37:963–975.

Zhang, H., C. He, X. Yan, T. Mirshahi, and D.E. Logothetis. 1999. Activation of inwardly rectifying K+ channels by distinct Ptd-Ins(4,5)P2 interactions. *Nat. Cell Biol.* 1:183–188.