How ATP Inhibits the Open $K_{\text{ATP}}$ Channel

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ATP-sensitive potassium ($K_{\text{ATP}}$) channels are composed of four pore-forming Kir6.2 subunits and four regulatory SUR1 subunits. Binding of ATP to Kir6.2 leads to inhibition of channel activity. Because there are four subunits and thus four ATP-binding sites, four binding events are possible. ATP binds to both the open and closed states of the channel and produces a decrease in the mean open time, a reduction in the mean burst duration, and an increase in the frequency and duration of the interburst closed states. Here, we investigate the mechanism of interaction of ATP with the open state of the channel by analyzing the single-channel kinetics of concatenated Kir6.2 tetramers containing from zero to four mutated Kir6.2 subunits that possess an impaired ATP-binding site. We show that the ATP-dependent decrease in the mean burst duration is well described by a Monod-Wyman-Changeux model in which channel closing is produced by all four subunits acting in a single concerted step. The data are inconsistent with a Hodgkin-Huxley model (four independent steps) or a dimer model (two independent dimers). When the channel is open, ATP binds to a single ATP-binding site with a dissociation constant of 300 μM.

INTRODUCTION

ATP-sensitive potassium ($K_{\text{ATP}}$) channels couple cell metabolism to transmembrane potassium fluxes in many cell types (Ashcroft, 2005). They are important in the regulation of neuronal excitability (Liss et al., 1999; Hernandez-Sanchez et al., 2001; Miki et al., 2001; Yamada et al., 2001), neuronal degeneration (Liss et al., 2005), cardiac ischaemia and stress adaptation (Zingman et al., 2002; Liu et al., 2004; Kane et al., 2005), vascular smooth muscle tone (Miki et al., 2002), skeletal muscle fatigue (Gong et al., 2000), and hormonal secretion (Ashcroft et al., 1984; Reimann and Gribble, 2002; MacDonald et al., 2007). In pancreatic β cells, for example, closure of $K_{\text{ATP}}$ channels in response to glucose metabolism depolarizes the plasma membrane, thereby triggering calcium influx and insulin release (Ashcroft et al., 1984). Accordingly, gain-of-function mutations in the genes encoding the β-cell $K_{\text{ATP}}$ channel cause neonatal diabetes and loss-of-function mutations cause the converse disorder, congenital hyperinsulinism (Ashcroft, 2005; Hattersley and Ashcroft, 2005).

The $K_{\text{ATP}}$ channel is an octameric complex of two types of membrane-protein subunit (Inagaki et al., 1995; Sakura et al., 1995). Kir6.2, a member of inwardly rectifying potassium channel family, forms a central tetrameric pore. Each Kir6.2 subunit associates with a regulatory protein, the sulphonylurea receptor (SUR1), in 4:4 stoichiometry (Clement et al., 1997; Mikhailov et al., 2005).

Metabolism is believed to regulate $K_{\text{ATP}}$ channel activity primarily via changes in the concentrations of intracellular adenine nucleotides. Channel inhibition is produced by binding of ATP to Kir6.2 in a Mg-independent manner (Tucker et al., 1997; Gribble et al., 1998). Conversely, occupancy of the nucleotide-binding domains of SUR1 by MgADP stimulates channel activity, a circumstance that can be achieved either by direct binding of MgADP or by binding and subsequent hydrolysis of MgATP (Nichols et al., 1996; Tucker et al., 1997; Gribble et al., 1998; Zingman et al., 2001). Identification of the molecular mechanism by which the $K_{\text{ATP}}$ channel is gated by ATP and MgATP/MgADP is therefore crucial for understanding how cell metabolism is linked to channel activity and thereby to the plasma membrane potential. This paper focuses on the mechanism of inhibition by ATP. This can be studied in isolation from nucleotide interactions with SUR1 by using Mg$^{2+}$-free solutions.

The $K_{\text{ATP}}$ channel is spontaneously active in the absence of ATP: we refer to this as intrinsic gating (sometimes it is also referred to as ligand-independent gating). The single-channel kinetics are characterized by bursts of short openings and closings separated by long, non-conducting interburst intervals. Addition of ATP causes a marked reduction in burst duration and pronounced prolongation of the long closed states. ATP therefore interacts with all these states.

Analysis of ATP inhibition of macroscopic currents does not enable the interaction of ATP with the different states of the channel to be distinguished. It also cannot be reliably used to determine the stoichiometry of ATP inhibition, as many different models can be easily fit to the ATP concentration–response relationship.

Abbreviations used in this paper: BR, burst ratio; D, dimer; HH, Hodgkin-Huxley; MWC, Monod-Wyman-Changeux; WT, wild type.
Although single-channel analysis overcomes some of these problems, analysis of the interaction of ATP with the long closed states of the channel remains a difficulty because there are multiple intrinsic long closed states whose distribution varies between individual channels; and because ATP-dependent stabilization of the closed states is otherwise too complex to model adequately at present. In this paper, we therefore focus on the interaction of ATP with the channel open state.

Several previous single-channel studies have examined the interaction of ATP with the channel open state and have concluded that ATP destabilizes the channel open state and thus promotes channel closure (Gillis et al., 1989; Nichols et al., 1991; Davies et al., 1992; Drain et al., 1998; Trapp et al., 1998; Fan and Makielski, 1999; Li et al., 2002). They have also shown that there is very little effect of ATP on the short closed state (Aleksiev et al., 1998; Trapp et al., 1998; Li et al., 2002), indicating that the reduction in the burst duration is primarily due to the interaction of ATP with the channel open state. Consequently, the burst duration can be used to analyze how ATP interacts with the open state.

Single-channel analysis in several previous studies made the assumption that channel closure resulted from interaction of one ATP molecule with the open state (Trapp et al., 1998; Li et al., 2002). However, since each Kir6.2 subunit possesses an inhibitory ATP-binding site (Markworth et al., 2000; Antcliff et al., 2005) and the \( K_{ATP} \) channel has four Kir6.2 subunits (Clement et al., 1997), up to four ATP molecules could bind to the open state. The relationship between the number of ATP molecules bound and pore closure is unclear. More recently, Abraham et al. (2002) and Selivanov et al. (2004) assumed a kinetic model with four independent binding sites for ATP; despite the assumption of independence, the best fit of ATP dose–response curves required that more than one (at least two out of four) binding sites had to be occupied to induce channel closure.

In this paper, we describe a method for analyzing the single-channel kinetics of \( K_{ATP} \) channels containing different numbers of wild-type subunits and subunits with a mutated ATP-binding site. We use this method to determine the mechanism by which ATP interacts with the open state and thereby facilitates the closure of the channel pore. We conclude that the data can be described well by the Monod-Wyman-Changeux model.

**MATERIALS AND METHODS**

Molecular Biology

Concatenated Kir6.2 tetramers were engineered by stepwise addition of monomeric subunits, using the compatibility of BglIII and BamHI restriction endonuclease sites. Wild-type Kir6.2 monomers were produced by standard PCR techniques. The Kir6.2ΔC monomer had a C-terminal deletion of 36 amino acids (ΔC36) (Tucker et al., 1997), a BamHI site at the 5’ end, and a BglII site at the 3’ end. For the first (N-terminal) Kir6.2 subunit, a Kozak ribosome binding sequence was engineered at the 5’ end, and for the final (C-terminal) subunit, a stop codon was inserted at the 3’ end. Glycine residues were also engineered into the subunits to extend the linker region between subunits: two glycines before the 3’ restriction site in the first subunit; two glycines after the 5’ restriction site and two glycines before the 3’ restriction site in the central subunits; two glycines after the 5’ restriction site in the final subunit. This results in an intersubunit linker of GGRRGSGG. All tetramers were constructed using the cloning vector pGEMT-Easy, before being subcloned into pBF vector for cRNA production.

To construct the Kir6.2 tetramer, the first subunit was inserted into pGEMT-Easy using the 5’-A overhangs, according to the vector protocol (Promega). For each subsequent subunit addition, the construct was cut with BglIII, and the subunit, cut with BamHI and BglIII, was ligated in to the BglIII-cut construct. If the subunit is inserted in the correct orientation, the 5’ BamHI site is lost in the ligation process, leaving a 3’ BglIII site, thus allowing addition of the next subunit in the same way. Mutant K185E subunits were produced by standard site-directed mutagenesis and inserted into the tetramer at the desired points by the same technique. A similar approach was taken by Wang et al. (2007).

Electrophysiology

Patch electrodes were pulled from thick-walled borosilicate glass (GC-150; Harvard Electronics). Single-channel activity of 1–20 min duration was recorded at −60 mV, filtered at 5 kHz, and digitized at 50 kHz using an Axopatch 200B patch-clamp amplifier (Axon Instruments) and pClamp software (Axon Instruments). All experiments were done at room temperature (20–22 °C). The pipette solution contained (in mM) 140 KCl, 1.2 MgCl₂, 2.6 CaCl₂, 10 HEPES (pH 7.4 with KOH). The internal (bath) solution contained (in mM) 107 KCl, 1 K₂SO₄, 10 EGTA, 10 HEPES (pH 7.2 with KOH) and \( K_{ATP} \) as indicated. Before and after application of ATP, the patch was exposed to ATP-free (control) solution for 1 min. During long recordings in high ATP solution, if channel activity was very low, ATP-free solution was applied at intervals to check for channel rundown.

The data were analyzed using a combination of Clampfit, Origin (OriginLab Corporation) and in-house software. Dwell-time histograms were constructed and analyzed at a resolution of 0.15 ms as described previously (Davies et al., 1992). Open time distributions were fit with a single exponential; up to four exponentials were fitted to dwell-time distributions. Burst durations were determined using the criterion for the critical time described by Magleby and Pallotta (1983):

\[
a_i e^{-t_{i}/\tau_i} = \sum_i a_i (1 - e^{-t_{i}/\tau_i})
\]

where \( a_i \) and \( \tau_i \) are the area and the mean lifetime of the intra-burst state (which corresponds to the component with the shortest mean lifetime in the distribution of closed times), and \( a_i \) and
in the presence of ATP. To assess the accuracy of the mean BR obtained by 
Magleby and Pallotta’s method, BR was also determined by an 
independent method, described by Fang et al. (2006). There was no 
significant difference between the mean BRs obtained by these 
two methods (see online supplemental material, http://www.jgp. 
.org/cgi/content/full/jgp.200709874/DC1).
The mean burst duration in ATP-free solution ($\tau_{0}$) was 
taken as an average of that measured before and after application 
of ATP. In this paper, we calculate the mean burst duration as fol-
 lows: first we measured the mean burst duration in three to five 
single-channel patches, and then we calculated the average of 
these mean burst durations.

The value of the mean burst duration obtained by averaging all 
burst events can be distorted by a “missed events” error, which re-
sults from missing very short solitary openings (as these also con-
tribute to the burst pdf, e.g., Shelley and Colquhoun, 2005). In 
this study, however, such errors will be small. Both homomeric 
and heteromeric K$_{ATP}$ channels exhibited quasi-monoe
donential distributions of open times over the range of ATP concen-
trations of up to 5 mM. A similar result for homomeric wild-type K$_{ATP}$ 
channels was previously obtained by Li et al. (2002). The mean open 
time ranged from 0.4 to 2 ms, which is well above the limit of de-
tectability in our system. Thus the number of very brief openings 
that go undetected is likely to be very small compared with the 
total number of bursts. The largest error can be expected for uncon-
nected wild-type channels, for which the reduction in open time 
by ATP is largest (Fig. S13 C). However, the value of $K_{O}$ (or 
$K_{O}$(0)) is the ATP 

$$I(\text{ATP}) = I(0) + \frac{1}{1 + \frac{[\text{ATP}]}{K_{C_0}}} \tau_{c},$$

where $I(\text{ATP})$ and $I(0)$ are the mean values of the K$_{ATP}$ current in 
the presence and absence of the nucleotide, $[\text{ATP}]$ is the ATP 
concentration, $IC_{50}$ is the ATP concentration at which inhibition 
is half maximal, $h$ is the slope factor (Hill coefficient), and $p$ is the 
fraction of unblocked current (pedestal) at saturating ATP concen-
trations (i.e., where all ATP-binding sites are occupied by nu-
cleotide). For most types of K$_{ATP}$ channel, channel activity at ATP 
concentrations >1 mM was determined by single-channel record-
ing at high gain, where the background leak current could be 
clearly distinguished. Background currents determined in this 
way were extremely small. Thus they were regarded as negligible 
in those cases where the fraction of ATP-resistant current was too 
large to employ single-channel analysis (for example, tetrameric 
constructs with four or three mutant subunits). To control for 
possible rundown of channel activity, $I(0)$ was taken as the mean of 
the conductance in control solution before and after ATP applica-
tion. Each data point represents the mean ± SEM of n patches.

Online Supplemental Material
The online supplemental material is divided into four parts (avail-
able at http://www.jgp.org/cgi/content/full/jgp.200709874/DC1).
In the first part, which includes Figs. S1–S10 and Table S1, we 
compare the analysis of the mean burst ratios determined by the 
Magleby and Pallotta method (1985) with that of Fang et al. 
(2006). In the second part, we compare the ATP sensitivity of con-
nected tetramers in macroscopic and single-channel recordings 
(Fig. S11). In the third part, we present the data on the ATP de-
pendence of the burst ratio for K$_{ATP}$ channels containing uncon-
nected Kir6.2 subunits (Fig. S12). In the fourth part, we compare 
the macroscopic and single-channel properties of homomeric 
wild-type K$_{ATP}$ subunits with those of homomeric K$_{ATP}$ channels 
formed from connected and separate Kir6.2 subunits (Fig. S13).

RESULTS

Connected Kir6.2 Tetramers
To assess how ATP interacts with the open state of the 
K$_{ATP}$ channel, we studied channels containing various 
combinations of wild-type (WT) and mutant (M) Kir6.2 
subunits. The K185E mutation was selected because it 
has been extensively characterized in previous studies 
(Reimann et al., 1999; John et al., 2003; Ribalet et al., 
2003; Wang et al., 2007) and it severely reduces ATP in-
hibition without altering intrinsic gating (i.e., that in the 
absence of ATP). Residue K185 is predicted to reside 
within the ATP-binding site based on molecular model-
ing studies (Trapp et al., 2003; Antcliff et al., 2005).

Coinjection of a mixture of wild-type and mutant 
Kir6.2 mRNAs produces a mixed population of channels 
containing variable numbers of wild-type and mutant 
subunits. However, it does not constrain the subunit stoici-
ometry or position. We therefore constructed connected 
tetramers. As Fig. 1 shows, the tetramers contained 
between zero and four mutant subunits. To address the 
possibility of asymmetry in intersubunit interactions, we 
made two constructs with 2WT:2M stoichiometry: cis, in 
which wild-type and mutant subunits reside next to each 
other and trans, in which wild-type and mutant subunits 
face one another across in the assembled tetramer.

Three Major Types of Gating Models
ATP binds to the cytosolic domain of Kir6.2 (Drain et al., 
1998; Tucker et al., 1998) and induces a conformational
change(s) that leads to stabilization of the closed conformation. Because there are four subunits, there are four ATP-binding sites per tetrameric channel. These could operate independently or in concert. Thus three types of models could describe the gating of the \( K_{\text{ATP}} \) channel (Fig. 2), in which the “gating unit” has one, two, or four ATP-binding sites and each \( K_{\text{ATP}} \) channel tetramer has four (4GU\(_1\)), two (2GU\(_2\)), or one (GU\(_4\)) gating unit, respectively (the subscript in each term refers to the number of ATP-binding sites within the gating unit). In all models, the gating units are considered to alternate between “active” (open) and “inactive” (closed) conformations. We further assume that transition of any one gating unit to its “inactive” conformation (strongly promoted by ATP binding to that unit) closes the pore. This terminates the burst and facilitates entry into the long closed states.

First, it is possible that each cytosolic domain of the Kir6.2 tetramer operates independently so that the channel may be considered to have four gating units (4GU\(_1\)). Thus, ATP binding to a single Kir6.2 subunit promotes channel closure (Fig. 2 A). In the simplest case, each Kir6.2 subunit behaves independently: i.e., ATP binds independently to the cytosolic domain of each Kir6.2 subunit, producing a conformational change that is independent of the ligand-binding state of all other subunits, and leads to closure of the channel pore. The channel will be closed as long as one gating unit is in the “closed” conformation; all four gating units must be in “open” conformation for the channel to be open. This mechanism is similar to that originally described by Hodgkin and Huxley for gating of voltage-dependent channels (Hodgkin and Huxley, 1952). It has also been proposed previously for \( K_{\text{ATP}} \) channels (Enkvetchakul et al., 2000).

However, studies of the intrinsic burst duration (Drain et al., 2004) and intrinsic interburst intervals (Fang et al., 2006) are inconsistent with this idea. Instead the data suggest that the four cytosolic domains of the channel may not function independently but operate together as a single gating unit (GU\(_1\)). In this case, we will assume that ATP binds to each subunit independently and makes an additive contribution to the free energy of the open or closed states (Fig. 2 B). This is known as the Monod-Wyman-Changeux model (Monod et al., 1965).

Finally, molecular dynamics simulations suggest that the \( K_{\text{ATP}} \) channel may operate as a dimer of dimers (Haider et al., 2005a). In this case, the channel would have two gating units formed by two opposing (or adjacent) Kir6.2 subunits (2GU\(_2\)). In the simplest case, binding of ATP to all four sites is independent; the effects of binding of two ATP molecules to one gating unit are additive; and the movements of the two gating units are independent. The channel will be closed as long as one gating unit (i.e., one dimer) is in the closed conformation; both dimers must be in the open conformation for the channel to be open. We refer to this as the dimer model.

For all models, the effects of ATP binding on the free energy of the open state are additive. However, binding of a single ATP will have the greatest effect in the case of the Hodgkin-Huxley (HH) model, and the least effect in the case of the MWC model.

To determine which model most closely describes the effect of ATP on the open state of the channel, it is necessary to have an ATP-dependent parameter that can be reliably determined from the experiment, and whose ATP dependence differs between the three models described above. Previous single-channel studies have characterized the ATP dependence of the mean open time and the mean burst duration (Davies et al., 1992; Trapp et al., 1998; Fan and Makielski, 1999; Li et al., 2002). Of these two parameters, the mean burst duration shows a dramatically greater dependence on ATP than the mean open time (e.g., 0.6 mM ATP reduces the former \( \sim 32\)-fold and the latter \( \sim 2.5\)-fold; Li et al., 2002). We therefore used the mean burst duration and its dependence on ATP as a probe to determine which kinetic model best describes ATP-dependent closure of the \( K_{\text{ATP}} \) channel.

The gating of the \( \beta \)-cell \( K_{\text{ATP}} \) channel can be well described by a kinetic scheme with a single open state (O), one intraburst closed state (C\(_{C} \)), and several interburst closed states (e.g., Alekseev et al., 1998; Trapp et al., 1998; Enkvetchakul et al., 2000; Fang et al., 2006). In this study, we use a simplified kinetic scheme with a single interburst closed state (C\(_{\xi} \)), as our analysis is not concerned with transitions between interburst closed states:

\[
\begin{align*}
C_F & \xrightarrow{a} O \quad \xleftarrow{c} C_{\xi} \\
& \xrightarrow{b} \quad \xleftarrow{d}
\end{align*}
\]  
(Scheme 1)
where $K_O$ is the equilibrium binding constant for ATP binding to intraburst states (O, C_F) and $K_C$ is the equilibrium binding constant for ATP binding to the interburst long closed states (C_S). Because the effect of ATP on intraburst closed states is very small (Alekseev et al., 1998; Trapp et al., 1998; Li et al., 2002), for simplicity, we assume that intraburst transitions (i.e., rate constants $a, b$) are unaffected by the nucleotide; we also assume they are the same for all three models shown in Fig. 2. In contrast, ATP substantially affects the rates of transitions between open and interburst closed states. Thus the rate constants governing transitions between the ATP-bound open state $O_A$ and ATP-bound interburst closed state $C_SA$ must include proportionality coefficients ($m, n$) that describe how much the rate of closure, or opening, respectively, is altered when ATP is bound (i.e., $c*m, d*n$).

The right side of the Scheme 2 corresponds to the allosteric cycle for binding of one ATP molecule in Fig. 2, where $E_O (c/d)$ is the equilibrium gating constant for transitions between states O and C_S and $t (m/n)$ is the transduction coefficient for the effect of ATP binding on this reaction.

Since ATP is a K_ATP channel antagonist, it must bind with higher affinity to the interburst closed state (Cs in Scheme 2) than to the open state ($K_C > K_O$). From the principle of microscopic reversibility $K_C = t*K_O$, and thus $t > 1$.

The mean burst durations for the models shown in Fig. 2 can now be fully determined from the rate constants in Scheme 2 and the bulk ATP concentration ([ATP]). The general formula for calculation of the mean burst duration $\tau_B$ is (Colquhoun and Hawkes, 1982):

$$\tau_B = \varphi_B (1 - G_{AB} G_{BA})^{-1} (-Q_A)^{-1} (1 - Q_{AB} Q_{BB}^{-1} G_{BA}) u_A.$$  \hspace{1cm} (6)

Using Eq. 6, the mean burst durations for the three gating models shown in Fig. 2 (which are expanded from Scheme 2) can be expressed as:

$$\frac{1}{\tau_{B,HH} ([ATP])} = \frac{4*c*(1 + m_{HH} * K_{OHH} *[ATP])}{(1 + F) * (1 + K_{OHH} *[ATP])}$$  \hspace{1cm} (7)

$$\frac{1}{\tau_{B,MWC} ([ATP])} = \frac{c*(1 + m_{MWC} * K_{OMWC} *[ATP])^4}{(1 + F) * (1 + K_{OMWC} *[ATP])^4}$$  \hspace{1cm} (8)

$$\frac{1}{\tau_{B,D} ([ATP])} = \frac{2*c*(1 + m_{D} * K_{OD} *[ATP])^2}{(1 + F) * (1 + K_{OD} *[ATP])^2},$$  \hspace{1cm} (9)
where \( \tau_{B,HH}(ATP) \), \( \tau_{B,MWC}(ATP) \), and \( \tau_{B,D}(ATP) \) are the mean burst durations given by the HH, the MWC, and the dimer (D) models, respectively. \( F = a/b \) is the equilibrium constant for intraburst closures, \( \epsilon \) is the rate of closure of the slow gate in the absence of ATP, and \( m \) is a proportionality coefficient that determines how much the rate of slow gate closure is increased when ATP is bound. Note that the values of \( K_0 \) and \( m \) vary with the different models, as indicated by the subscripts \( HH \), \( MWC \), and \( D \).

Inspection of these equations reveals that, due to the imposed symmetry, they simply reflect the properties of the individual gating units in each model (4GU\(_1\), 2GU\(_2\), 1GU\(_4\)). Thus for the Hodgkin-Huxley model, there is one ATP-binding site per gating unit, for the dimer model there are two ATP-binding sites, and for the MWC model there are four ATP-binding sites. The coefficients 4, 1, and 2 in front of Eqs. 7–9 reflect the number of gating units in these models.

Eqs. 7–9 can be rearranged by multiplying each side of the equation by the mean burst duration in the absence of ATP (which is obtained by setting [ATP] to zero):

\[
\frac{\tau_{B,HH}(0)}{\tau_{B,HH}([ATP])} = \frac{1 + m_{HH} \kappa_{O,HH} [ATP]}{1 + K_{O,HH} [ATP]} \tag{10}
\]

\[
\frac{\tau_{B,MWC}(0)}{\tau_{B,MWC}([ATP])] = \left( \frac{1 + m_{MWC} \kappa_{O,MWC} [ATP]}{1 + K_{O,MWC} [ATP]} \right) \tag{11}
\]

\[
\frac{\tau_{B,D}(0)}{\tau_{B,D}([ATP])] = \left( \frac{1 + m_{D} \kappa_{O,D} [ATP]}{1 + K_{O,D} [ATP]} \right)^2 \tag{12}
\]

In this form, the burst durations are expressed solely as a function of the ATP concentration and the ATP-dependent parameters \( K_0 \) and \( m \). Eqs. 10–12 thus allow comparison of the mean burst duration for channels with different open state energies (i.e., they remove the dependence on the rate constant \( \epsilon \) in Scheme 2). Since individual K\(_{ATP}\) channels (even of the same species) possess quite variable values of \( \tau_B(0) \), plotting the ATP dependence of the mean burst durations averaged from several single-channel patches using Eqs. 10–12 instead of Eqs. 7–9 should substantially reduce error in the determination of \( K_0 \) and \( m \). Second, taking the intrinsic mean burst duration (i.e., that in the absence of ATP) as the average of that measured before and after ATP application should compensate for effects of K\(_{ATP}\) channel rundown.

For simplicity, we define the ratio of the mean burst duration in the absence of ATP to that in the presence of ATP, i.e., \( \tau_B(0)/\tau_B([ATP]) \) as the burst ratio (BR).

The Properties of Connected Tetramers for the Different Models

We next consider how the ATP dependence of BR varies with the gating models shown in Fig. 2 for the different tetrameric constructs. Eqs. 10–12 indicate that there are two ways in which a mutation may affect the ATP dependence of the burst ratio.

First, it may affect the affinity of ATP binding. Regardless of the gating mechanism, a reduction in ATP binding to the open state (\( K_o \)) would result in a decrease in the slope of the relationship between BR and ATP. A simultaneous reduction in \( K_0 \) and \( K_\epsilon \) would also shift the macroscopic ATP dose–response curves to higher ATP concentrations. In the limiting case (where \( K_0 = 0 \), \( K_\epsilon = 0 \)), the channel will not bind ATP at all and gating will be unaffected by the ligand.

Second, a mutation may also affect the parameter \( m \), which describes the increase in the rate of closure due to ATP binding to the open state. Regardless of the gating mechanism, a reduction in \( m \) would reduce the maximal extent of the decrease in the mean burst duration caused by ATP (Eqs. 10–12). If \( K_0 \) and \( n \) in Scheme 2 are unaffected, it follows from the principle of microscopic reversibility that a decrease in \( m \) would simultaneously result in a decrease in \( K_\epsilon \) and in the ratio \( K_o/K_\epsilon \) (since \( K_o = K_o^{**} m/n \)). A decrease in \( m \) will then have two effects on the ATP sensitivity of macroscopic K\(_{ATP}\) currents; it will shift the ATP dose–response curves to higher ATP concentrations (due to the decrease in \( K_\epsilon \)), and it will also increase the fraction of unblocked current at high ATP concentrations (via the increase in the \( K_o/K_\epsilon \) ratio). In principle, a change in \( m \) can be accompanied by a simultaneous change in \( n \), which would either reduce (decrease in \( n \)) or enhance (increase in \( n \)) these effects. In the special case when \( m = n \) and thus \( t = 1 \), the channel will still bind ATP but its gating will be completely unaffected by ligand binding.

Let us assume that the K185E mutation either (a) abolishes ATP binding to the open state (\( K_o = 0 \)) or (b) abolishes the effect of ATP binding to the open state on channel closure (\( m = 1 \)). For all three models, it is possible to show that the ATP dependence of the mean burst duration will be identical whether it is calculated assuming \( K_o = 0 \), or \( m = 1 \). The predictions for the different tetrameric constructs are shown in Fig. 3 (A–C).

It is clear that each model produces a distinct set of relationships between BR and ATP. In the case of the Hodgkin-Huxley model, the BR amplitude is simply proportional to the number of functional ATP-binding sites in the tetramer (Fig. 3 A). In contrast, for the MWC model, the BR amplitude decreases dramatically with the number of wild-type ATP-binding sites in the gating unit (Fig. 3 B). This is because the additive effect of ATP on the energy profile between open and interburst closed state(s) is translated into a multiplicative effect on burst duration. In the case of the dimer model, the BR amplitude is dramatically reduced when there is only one wild-type ATP-binding site per channel and the properties of channels with two functional ATP-binding sites depend on whether they lie within the same or different gating unit (Fig. 3 C).
and 5 mM. Fig. 4 shows representative single-channel records for each tetramer in the absence (top traces) and presence of 1 mM ATP (bottom traces). It is clear that as the number of wild-type ATP-binding sites in the tetramer is reduced, the ATP sensitivity of these channels is gradually impaired. This indicates that binding of a single ATP does not have a dominant effect.

Fig. 5 A gives the mean relationship between BR and ATP concentration for the different tetramers. Comparison of Fig. 5 A with Fig. 3 immediately suggests that the ATP dependence of BR resembles that predicted by the MWC mechanism. The properties of the MWC model can be seen more easily when the data are plotted on a log scale (Fig. 5 B). It then becomes clear that the burst duration of channels containing four mutant subunits (4M) still exhibits a mild ATP dependence. Interestingly, the K185E mutation does not appear to affect the affinity of ATP binding to the open state ($K_0$) as the ATP dependence of BR saturates at a similar ATP concentration to that of the wild-type channel for all channel species. Instead, it is clear that the mutation markedly alters the maximal amplitude of the relationship. Fitting the MWC model (Eq. 11) to the 4M data gave $K_{O,MWC} = 3.3 \pm 1.0 \text{ mM}$ and $m_{MWC} = 1.13 \pm 0.01$.

In all models discussed so far we assumed independence of ATP binding and ATP-induced $K_{ATP}$ channel closure. This may not be the case. One of the simplest possibilities that does not fulfill these two conditions is shown in Fig. 3 D. Because the Hill coefficient for $K_{ATP}$ channel inhibition by ATP is close to 1, it is reasonable to assume that the first ATP bound to the channel may have a dominant effect on $K_{ATP}$ channel closure. This can be modeled by assuming that only one ATP molecule can bind to the channel in the open conformation (top four states of the MWC model in Fig. 2). Using Eq. 6, the burst ratio in this case is given by:

$$\frac{\tau_B(0)}{\tau_B([ATP])} = \frac{1 + j \cdot m \cdot K_0 \cdot [ATP]}{1 + j \cdot K_0 \cdot [ATP]}$$

where $j = 1...4$ is the number of functional ATP binding sites. In this case, a reduction in the number of wild-type ATP binding sites will have only a mild effect on the amplitude of BR (Fig. 3 D).

**ATP-dependent Closure of $K_{ATP}$ Channels Formed from Connected Tetramers Obey the MWC Model**

We next performed single-channel recordings for each of the concatenated tetramers shown in Fig. 1, to determine which ATP–BR relationship is found experimentally. Kir6.2 tetramers were coexpressed with SUR1 and all experiments were performed in Mg-free solution. We recorded single-channel activity from inside-out patches at three different ATP concentrations: 0.2, 1, and 5 mM. Fig. 4 shows representative single-channel records for each tetramer in the absence (top traces) and presence of 1 mM ATP (bottom traces). It is clear that as the number of wild-type ATP-binding sites in the tetramer is reduced, the ATP sensitivity of these channels is gradually impaired. This indicates that binding of a single ATP does not have a dominant effect.

Fig. 5 A gives the mean relationship between BR and ATP concentration for the different tetramers. Comparison of Fig. 5 A with Fig. 3 immediately suggests that the ATP dependence of BR resembles that predicted by the MWC mechanism. The properties of the MWC model can be seen more easily when the data are plotted on a log scale (Fig. 5 B). It then becomes clear that the burst duration of channels containing four mutant subunits (4M) still exhibits a mild ATP dependence. Interestingly, the K185E mutation does not appear to affect the affinity of ATP binding to the open state ($K_0$) as the ATP dependence of BR saturates at a similar ATP concentration to that of the wild-type channel for all channel species. Instead, it is clear that the mutation markedly alters the maximal amplitude of the relationship. Fitting the MWC model (Eq. 11) to the 4M data gave $K_{O,MWC} = 3.3 \pm 1.0 \text{ mM}$ and $m_{MWC} = 1.13 \pm 0.01$.

From the log plot of the data it is also evident that the BR–ATP relationships for all tetramers are distributed at equal intervals and saturate in the same range of ATP concentrations. Thus, in accordance with the MWC model, the effects of ATP binding to both wild-type and mutated...
for the wild-type channel were derived from the global fit to all data obtained from channels containing at least one WT subunit. These were $K_{O,WT} = 3.1 \pm 0.2 \text{ mM}$ and $m_{WT} = 2.8 \pm 0.1$. These values were similar to those obtained by fitting the wild-type data alone (see Fig. 5 A). Thus, the K185E mutation appears to affect the burst duration predominantly by its action on the mechanism by which ATP binding to the open state is transduced into channel closure ($m_M \approx 1$).

Macroscopic Properties of $K_{ATP}$ Channels Formed from Connected Kir6.2 Tetramers

We next determined the ATP sensitivity of all tetrameric constructs (Fig. 6 A). For these experiments we used macroscopic currents, as the ATP sensitivity can be measured more accurately and represents the mean of many channels. At this point, it is important to remember that ATP-binding sites appear to be additive. This allows all the data to be fit simultaneously with a single set of equations:

$$\frac{\tau_{A,0}}{\tau_{A,([\text{ATP}])}} = \left(1 + m_{WT} \cdot K_{O,WT} \cdot [\text{ATP}]\right)^{i} \cdot \left(1 + m_{M} \cdot K_{O,M} \cdot [\text{ATP}]\right)^{4-i},$$

where $K_{O,WT}$ and $K_{O,M}$ are the binding constants for ATP to the open state for wild-type and mutant subunits, respectively; $m_{WT}$ and $m_{M}$ are the relative increases in the rate of the slow gate closure induced by ATP for wild-type and mutant subunits, respectively; and $i$ is the number of wild-type subunits in the tetramer ($i = 1–4$). All the relationships could be well fit with the same set of parameters. The parameters for the 4M channel were assumed to be constant ($K_{O,M} = 3.3 \text{ mM}^{-1}$; $m_{M} = 1.13$) and were obtained from the fit to the 4M data.
through 4M channels was also increased indicates that
the K185E mutation also reduces the affinity of ATP
binding to the long closed states (K_C).

In comparison with other concatenated tetramers, connected 4M channels exhibited more pronounced
rundown; consequently, the values of IC_50 and p for ATP
inhibition of these species decreased noticeably with
time after patch excision. Thus, the estimates of
IC_50 and p from single-channel experiments, conducted over
a long period of time (up to 20 min) were smaller than
those from macroscopic experiments, obtained shortly
after patch excision (see Fig. S11, available at http://
www.jgp.org/cgi/content/full/jgp.200709874/DC1).

The extent of ATP inhibition of the macroscopic currents
will be determined not only by the effect of ATP on the
open state (discussed above), but also by interaction of
ATP with the long closed states of the channel. Thus
this approach enables us to obtain (indirect) informa-
tion on the long closed states.

Figure 5. ATP dependence of burst ratio. Relationship between
burst ratio and ATP concentration measured experimentally for
connected K_ATP channel tetramers containing different numbers
of wild-type and mutant subunits (as indicated to the right of each
trace). Each data point is the average of three to five experiments.
(A) Linear scale. The lines were generated by fitting Eq. 14 to the
data for each tetramer separately. The best fit of Eq. 11 to the
data for 4M channels gave values of K_{O,M} = 3.3 mM\(^{-1}\) and m_M =
1.13, and these were used for the mutant subunits in all other fits.
The best fit of Eq. 14 to K_ATP channels containing one to four WT
subunits was obtained with the following values of K_{O,WT} and m_WT:
K_{O,WT} = 2.7 (1WT:3M); m_WT = 2.7 (2WT:2M cis); K_{O,WT} = 2.9 mM\(^{-1}\); m_WT =
2.7 (2WT:2M trans); K_{O,WT} = 3.1 mM\(^{-1}\) for all other fits.

Figure 6. Macroscopic properties of connected tetramers. (A)
Macroscopic dose–response curves for ATP inhibition of con-
nected K_ATP channels containing different numbers of WT and
K185E (M) subunits (< 4WT; □, 3WT:1M; ●, 2WT:2M cis; ▲, 2WT:2M trans; ▼, 1WT:3M; ♦, 4M). n = 5 for all data. The lines
are best fit of the data with Eq. 5 with IC_50 = 2.6 mM, h = 1.18, P =
0.48 (4M); IC_50 = 690 μM, h = 0.84, P = 0.08 (1WT:3M); IC_50 = 14
μM, h = 1.36, P = 0 (4WT); IC_50 = 31 μM, h = 1.27, P = 0 (3WT:1M); 
IC_50 = 97 μM, h = 0.94, P = 0 (2WT:2M cis); IC_50 = 122 μM, h = 0.98,
P = 0 (2WT:2M trans). (B) Pedestal amplitude as a function of the
number of wild-type subunits in the connected tetramer. Open
circles, 2WT:2M trans; filled circles, all other species. The pedes-
tal was estimated from data in A as (a) the fraction of unblocked
current at 30mM ATP or (b) from fitting Eq. 5 to the data (4M,
1WT:3M). Solid line, spline function fit through the data; dot-
ted line, prediction of the MWC model (Eq. 15) with P_o = 0.75,
F = 0.21, E_o = 0.12 (Table 1) and values K_{C,WT}/K_{O,WT} = 51 and
K_{C,M}/K_{O,M} = 1.9 obtained from the fit of homomeric wild-type and
mutant tetramers.
TABLE I

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Obtained from</th>
<th>Connected tetramers</th>
<th>Unconnected tetramers</th>
</tr>
</thead>
<tbody>
<tr>
<td>$IC_{50}$</td>
<td>macroscopic data</td>
<td>$14.5 \pm 2.5 \mu M$</td>
<td>$8.4 \pm 2.9 \mu M$</td>
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<tr>
<td>$h$</td>
<td>macroscopic data</td>
<td>$1.42 \pm 0.07$</td>
<td>$1.22 \pm 0.16$</td>
</tr>
<tr>
<td>$P_O$</td>
<td>single-channel data</td>
<td>$0.75 \pm 0.04$</td>
<td>$0.33 \pm 0.10$</td>
</tr>
<tr>
<td>$\tau_{s}(0)$</td>
<td>single-channel data</td>
<td>$127 \pm 18 \text{ms}$</td>
<td>$20.7 \pm 6.0 \text{ms}$</td>
</tr>
<tr>
<td>$a$</td>
<td>$\tau_a(0) \cdot \tau_s(0)$</td>
<td>$0.59 \pm 0.02 \text{ms}^{-1}$</td>
<td>$0.54 \pm 0.07 \text{ms}^{-1}$</td>
</tr>
<tr>
<td>$b$</td>
<td>$\tau_b(0)$</td>
<td>$2.8 \pm 0.1 \text{ms}^{-1}$</td>
<td>$2.7 \pm 0.3 \text{ms}^{-1}$</td>
</tr>
<tr>
<td>$c$</td>
<td>$\tau_c(0) \cdot \tau_s(0)$</td>
<td>$0.009 \pm 0.001 \text{ms}^{-1}$</td>
<td>$0.06 \pm 0.02 \text{ms}^{-1}$</td>
</tr>
<tr>
<td>$F$</td>
<td>$a$ and $b$</td>
<td>$0.21 \pm 0.03$</td>
<td>$0.29 \pm 0.05$</td>
</tr>
<tr>
<td>$E_O$</td>
<td></td>
<td>$0.12 \pm 0.02$</td>
<td>$1.83 \pm 1.01$</td>
</tr>
<tr>
<td>$K_O,WT$</td>
<td>$\tau_s(0)/\tau_s([\text{ATP}])$</td>
<td>$3.1 \pm 0.2 \text{mM}^{-1}$</td>
<td>$3.2 \pm 0.2 \text{mM}^{-1}$</td>
</tr>
<tr>
<td>$K_O,WT$</td>
<td>$\tau_s([\text{ATP}])$</td>
<td>$3.8 \pm 0.9 \text{mM}^{-1}$</td>
<td>$3.2 \pm 0.2 \text{mM}^{-1}$</td>
</tr>
<tr>
<td>$m_{WT}$</td>
<td>$\tau_s(0)/\tau_s([\text{ATP}])$</td>
<td>$2.8 \pm 0.1$</td>
<td>$2.7 \pm 0.1$</td>
</tr>
<tr>
<td>$m_{WT}$</td>
<td>$\tau_s([\text{ATP}])$</td>
<td>$2.4 \pm 0.1$</td>
<td>$2.5 \pm 0.1$</td>
</tr>
</tbody>
</table>

$n = 5$ for macroscopic data; $n = 10$ for single-channel data. Values of $K_{O,WT}$ and $m_{WT}$ were obtained by fitting the MWC model (Eq. 11 for burst ratios and Eq. S4 for open times) to the mean values obtained from three to five experiments. All other parameters were calculated from 10 experiments.

The fraction of unblockable current was decreased, but not abolished, for channels containing one wild-type ATP-binding site. The fact that ATP inhibition of cis and trans 2WT:2M species by ATP was almost identical suggests that ATP-dependent gating of connected tetramers does not involve dimeric transitions.

The MWC model assumes transitions only occur between two classes of states (Monod et al., 1965). If ATP binding to single subunit (in the tetramer) is independent of binding to the other subunits for all interburst closed states, then it is possible to lump the interburst closed states together (i.e., as $C_s$). The gating between the open and the lumped closed state ($C_s$) can then be described with MWC model. This model can then be used to describe the macroscopic ATP dose–response curve of the K$_{ATP}$ channel, as in Fig. 2B.

We tested the possibility that the MWC model can be used to describe the measured macroscopic ATP dose–response curve. We fitted the values of the pedestal, $p_i$, which we define as the fraction of unblocked current at saturating ATP concentration for channels with $i$ WT subunits. Fig. 6B plots the pedestal, for the data shown in Fig. 6A, as a function of the number of wild-type subunits. For the MWC model, $p_i$ is given by

$$p_i = \frac{1}{P_O \cdot (1 + F) + P_O \cdot E_O \cdot \left(\frac{K_{C,WT}}{K_{O,WT}}\right)^i \cdot \left(\frac{K_{C,M}}{K_{O,M}}\right)^i} \cdot (1 + i),$$

where $K_{C,WT}$ and $K_{C,M}$ are the binding constants for ATP to the lumped interburst closed state ($C_s$) for wild-type and mutant subunits, respectively; $K_{O,WT}$ and $K_{O,M}$ are the binding constants for ATP to the open state for wild-type and mutant subunits, respectively; $E_O = c/d$ is the equilibrium gating constant for transitions between the open and the lumped closed state (see Schemes 1 and 2), $P_O$ is the intrinsic open probability and $i$ is the number of wild-type subunits in the tetramer.

All tetrameric constructs had similar initial values of intrinsic $P_O$: $0.75 \pm 0.04$, $n = 10$ (4WT); $0.75 \pm 0.02$, $n = 11$ (3WT1M); $0.75 \pm 0.02$, $n = 11$ (cis); $0.74 \pm 0.04$, $n = 10$ (trans); $0.74 \pm 0.03$, $n = 8$ (3M1WT); $0.71 \pm 0.05$, $n = 7$ (4M). Using the values of $P_O$, $F$, and $E_O$ given in Table I, it is possible to calculate the ratios $K_{C,WT}/K_{O,WT}$ and $K_{C,M}/K_{O,M}$ from the corresponding values of $p_i$ and $p_0$ using Eq. 15. These ratios can be used to obtain values of $p$ for the MWC model (dotted line in Fig. 6B). As Fig. 6B shows, the data deviate from the model, suggesting that the assumption that ATP binding to one subunit is independent of that to the other subunits is not correct for at least some of the interburst closed states. Thus the data suggest that while the MWC model can be applied to the transitions between the open state and one or more of the interburst closed states, it cannot be applied to all of the transitions between the different interburst closed states.

It is of course possible that this effect is specific to the K185E mutation and is not observed for other mutations in the ATP-binding site. The deviations from the MWC model observed at high ATP concentrations could result from an effect of the K185E mutation on intersubunit interactions between ATP-binding sites when the channel is closed. We found no obvious effect on the intrinsic open probability of connected homomorphic WT channels ($P_O = 0.75 \pm 0.04$, $n = 10$) and connected homomorphic K185E tetramers ($P_O = 0.71 \pm 0.05$, $n = 7$). However, this does not mean that the mutation cannot somehow alter the distribution of interburst closed states. Unfortunately, this is very difficult to measure accurately.
Comparison of Properties of $K_{ATP}$ Channels Formed from Connected and Separate Kir6.2 Subunits

Finally, in order to assess the physiological relevance of the results we obtained on connected tetramers, we examined the single-channel properties of unconnected tetramers. Full details are given in the online supplementary material (available at http://www.jgp.org/cgi/content/full/jgp.200709874/DC1); here we summarize only the major results.

Effects on Open States

The values of $K_0$ and $m$ obtained from the best fit of the BR data for separated 4WT channels with the MWC model (Eq. 11) were $K_{O,WT} = 3.25 \text{ mM}^{-1}$ ($K_d = 308 \mu \text{M}$) and $m_{WT} = 2.7$ (Table I). These values are very similar to those for the connected 4WT channel ($K_d = 320 \mu \text{M}$, $m_{WT} = 2.8$). Thus we conclude that the linkers introduced between Kir6.2 subunits in connected tetramers do not substantially alter ATP binding to the open state ($K_0$) or the transduction parameter $m$.

Similar values for $K_0$ and $m$ were also obtained from fitting the ATP dependence of the mean open times obtained for both connected and unconnected 4WT $K_{ATP}$ channels (Table I; Fig. S13 C). These results provide further support for the idea that ATP reduces the burst duration via interaction with the open state of the channel (Li et al., 2002). They also confirm that ATP exerts only a mild effect on the intraburst transitions (thus validating the use of Scheme 2) and indicate that errors associated with the methods employed for measuring burst durations and open times were small.

To examine the properties of unconnected $K_{ATP}$ channels containing various numbers of mutant subunits, we analyzed single-channel records from patches pulled from oocytes injected with different ratios of wild-type and mutant Kir6.2 mRNAs (Fig. S12 A). In single-channel patches, it was possible to identify channels with kinetic signatures that resembled those observed for connected tetramers with different numbers of WT and M subunits. The BR-ATP relationship for these channels was similar to that of the corresponding connected tetramers (Fig. S12 B). We therefore conclude that in unconnected mixed WT:M tetramers, as in their connected counterparts, ATP binding to Kir6.2 is also likely to be independent.

The intrinsic $P_0$ of connected 4WT $K_{ATP}$ channels was $\approx 2.3$-fold higher than that of unconnected 4WT channels (Table I). The mean burst duration was also increased (approximately sixfold, Table I). Calculation of the intrinsic rate constants $a$, $b$, and $c$ from data obtained in the absence of ATP indicated that these effects are caused by a decrease in the rate constant $c$ in the connected tetramer (Table I). Such a shift in the gating equilibrium toward the open state is expected to indirectly reduce the ATP sensitivity of the $K_{ATP}$ channel (Trapp et al., 1998). As anticipated, connected 4WT tetramers had a slightly reduced ATP sensitivity: the $IC_{50}$ increased from 8.4 to 14.5 $\mu \text{M}$ (Table I). This small increase in $IC_{50}$ can be fully accounted for by the sixfold increase in the mean burst duration (Trapp et al., 1998).

Taken together, these results indicate that connected tetramers provide a good model for studying the interaction of ATP with the open state of the $K_{ATP}$ channel. Although the increase in intrinsic $P_0$ altered the ATP sensitivity, it also substantially stabilized channel activity in excised patches, making it particularly suitable for analysis of single-channel kinetics.

DISCUSSION

In this paper, we show that the ATP dependence of the mean burst duration of connected tetramers can be used to determine the mechanism by which ATP induces closing of the $K_{ATP}$ channel. Although experimentally laborious, this method can be easily extended for other types of ligand-gated multimeric ion channels.

We considered the possibility that the subunits within a concatenated tetramer do not assemble into the same tetrameric channel, as previously suggested for connected dimers of Shaker $K^+$ channels (McCormack et al., 1992). However, this did not seem to be the case. Each of the different tetrameric channels we constructed had very different single-channel properties. Expression of a single tetrameric species gave rise to single channels with similar properties; we never observed channels with properties corresponding to more than one type of channel. It is possible that the presence of SUR1, which surrounds the Kir6.2 tetramer (Mikhailov et al., 2005), may act as a chaperone, or simply as a physical obstruction,
and prevent aberrant associations between subunits from two different concatenated tetramers.

Effects of ATP on the Open State

We show (for the first time) that the decrease in the burst and open time duration of the $K_{\text{ATP}}$ channel produced by ATP saturates at high ATP concentration. This is indicative of the existence of ATP bound-open states in the wild-type channel. Analysis of burst and open times can be thus expanded to include effects of all four ATP molecules on channel closure. In contrast, previous studies considered the effect of only one ATP molecule on channel closure because they assumed a linear relationship between $1/\tau_0$ and $[\text{ATP}]$ (Davies et al., 1992; Li et al., 2002).

Our results demonstrate that the ATP-dependent decrease in mean open and burst durations can be described by the MWC mechanism. Together with the data obtained by Drain et al. (2004), it can thus be concluded that Kir6.2 subunits (either liganded or unliganded) exert an additive effect on the energy of the open state of the channel. Furthermore, our results show that when the channel is open, ATP binds to a single ATP-binding site with a $K_d$ of $\sim 300$ μM (as the channel has four ATP-binding sites, the overall $K_d$ for binding to the open state is $\sim 75$ μM). This means that in the intact cell, where ATP ranges from 1 to 10 mM, an open channel will have ATP bound to Kir6.2 for most of the time.

The MWC model is inherently easier to reconcile with the structure of the $K_{\text{ATP}}$ channel than are the other two models tested. Let us consider that each gating unit represents the cytosolic domain of a single Kir6.2 subunit. In the HH model, the cytosolic domain of an individual Kir6.2 subunit undergoes a conformational change on ATP binding that does not influence other subunits. In the MWC model, the four cytosolic domains function as a single unit, and ATP binding to a single subunit produces a global conformational change in all four cytosolic domains that leads to pore closure. This seems reasonable, given that residues in two adjacent subunits contribute to a single ATP-binding site in a molecular model of Kir6.2 (Antcliff et al., 2005).

Since we restricted our analysis to the mean burst durations, it is possible that more complex concerted models than the MWC model will be required to describe the ATP-dependent closing reaction fully. However, our data suggest that any such model will also have to be concerted. Furthermore, in order to produce the exponential dependence of burst duration on the number of wild-type subunits (Figs. 2 and 5), it should also satisfy the criterion that ATP binding at one subunit is independent of other subunits.

Effects of K185E Mutation on ATP Binding

Residue K185 plays a crucial role in ATP binding to Kir6.2. Mutation of K185 has a marked effect on ATP block of channel activity (Reimann et al., 1999; John et al., 2003; Ribalet et al., 2003; Wang et al., 2007), and binding of labeled ATP to Kir6.2 is reduced when K185 is mutated (Tanabe et al., 2000). Molecular dynamic simulations of ATP binding to a homology model of Kir6.2 suggest that the strongest interaction of ATP with Kir6.2 is via the side chain of K185 (Haider et al., 2005b).

Our results show that the K185E mutation does not alter the affinity of ATP binding to the open state but instead impairs the conformational change induced on ATP binding that leads to channel closure. The affinity of ATP binding to the closed state of the channel is also impaired. Taken together, the data are consistent with a model in which residue 185 may not be accessible to ATP in the open state, but where the closure of the channel leads to a conformational change that facilitates entry of ATP into its binding site, thereby providing access to residue 185. The lack of effect of replacing lysine with glutamate on ATP binding to the open state also raises the possibility that residue 185 does not interact directly with ATP as implied by molecular modeling (Antcliff et al., 2005), or that it only interacts in the closed state.

Conclusions

Our results demonstrate that it is important to consider the interaction of ATP with the open state of the channel for both a qualitative and a quantitative understanding of ATP inhibition. We have previously used this interaction to quantify a gating defect caused by the C166S mutation (Trapp et al. 1998). In this study, we show how the ATP-dependent closing rate can be also used to measure ATP binding and to quantify the mechanism by which ATP binding is transduced into channel closure. The latter is of particular interest as this mechanism is poorly understood. The fact that the ATP dependence of the mean open and burst durations can be described by a simple MWC model is useful for the analysis of the molecular mechanism of $K_{\text{ATP}}$ channel mutations, such as those that cause neonatal diabetes (Ashcroft, 2005; Hattersley and Ashcroft, 2005). In the future, it will be necessary to develop more complex kinetic models in order to describe the gating of $K_{\text{ATP}}$ channels more completely.

APPENDIX

Modeling of the ATP Dependence of the BR of Unconnected Wild-Type $K_{\text{ATP}}$ Channels for the Different Types of Models (Fig. 3)

Fig. 2 A and Fig. 3 A. The Hodgkin-Huxley model (4GU$_1$). The lines were drawn to

$$\frac{\tau_{B,HH}(0)}{\tau_{B,HH}([\text{ATP}])} = 1 + \frac{j(m_{HH} - 1)K_{O,HH} [\text{ATP}]}{4(1 + K_{O,HH} [\text{ATP}])},$$

where $j = 1...4$ is the number of wild-type subunits in the tetramer, $K_{O,HH} = 0.88$ mM$^{-1}$, and $m_{HH} = 60$. 

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Fig. 2 B and Fig. 3 B. The Monod-Wyman-Changeux model (GU). The lines were drawn to

$$\frac{\tau_{B,\text{MWC}}(0)}{\tau_{B,\text{MWC}}([ATP])} = \left(1 + \frac{m_{\text{MWC}} \cdot K_{O,\text{MWC}} \cdot [ATP]}{1 + K_{O,\text{MWC}} \cdot [ATP]}\right)^j,$$

(17)

where $j = 1..4$ is the number of wild-type subunits in the tetramer, $K_{O,\text{MWC}} = 3.2$ mM$^{-1}$, and $m_{\text{MWC}} = 2.7$.

Fig. 2 C and Fig. 3 C. The dimer model (2GU$_2$). The lines were drawn according to the following equations with $K_{O,D} = 2.1$ mM$^{-1}$ and $m_0 = 7.3$:

$$\frac{\tau_{B,D}(0)}{\tau_{B,D}([ATP])} = \left(1 + m_0 \cdot K_{O,D} \cdot [ATP]\right)^2,$$

(18)

$$\frac{\tau_{B,D}(0)}{\tau_{B,D}([ATP])} = \left(1 + \frac{1 + m_0 \cdot K_{O,D} \cdot [ATP]}{2}\right)^j,$$

(19)

$$\frac{\tau_{B,D}(0)}{\tau_{B,D}([ATP])} = \left(1 + \frac{1 + m_0 \cdot K_{O,D} \cdot [ATP]}{2}\right)^j,$$

(20)

$$\frac{\tau_{B,D}(0)}{\tau_{B,D}([ATP])} = \left(1 + \frac{1 + m_0 \cdot K_{O,D} \cdot [ATP]}{2}\right)^j,$$

(21)

$$\frac{\tau_{B,D}(0)}{\tau_{B,D}([ATP])} = \left(1 + \frac{1 + m_0 \cdot K_{O,D} \cdot [ATP]}{2}\right)^j,$$

(22)

for channels with 4 (Eq. 19), 3 (Eq. 19), 2DD (Eq. 20), 2SD (Eq. 21), and 1 (Eq. 22) functional ATP-binding site(s).

Fig. 2 D. The lines were drawn to

$$\frac{\tau_{B,\text{DOM}}(0)}{\tau_{B,\text{DOM}}([ATP])} = \frac{1 + m_{\text{DOM}} \cdot K_{O,\text{DOM}} \cdot [ATP]}{1 + j \cdot K_{O,\text{DOM}} \cdot [ATP]},$$

(23)

where $j = 1..4$ is the number of functional ATP-binding sites, $K_{O,\text{DOM}} = 0.22$ mM$^{-1}$, and $m_{\text{DOM}} = 60$.

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