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# $K_{ATP}$ channel inhibition by ATP requires distinct functional domains of the cytoplasmic C terminus of the pore-forming subunit

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**ABSTRACT** **ATP-sensitive potassium ("$K_{ATP}$") channels are rapidly inhibited by intracellular ATP. This inhibition plays a crucial role in the coupling of electrical activity to energy metabolism in a variety of cells. The $K_{ATP}$ channel is formed from four each of a sulfonylurea receptor (SUR) regulatory subunit and an inwardly rectifying potassium ($K_{ir}6.2$) pore-forming subunit. We used systematic chimeric and point mutagenesis, combined with patch-clamp recording, to investigate the molecular basis of ATP-dependent inhibition gating of mouse pancreatic $\beta$ cell $K_{ATP}$ channels expressed in *Xenopus* oocytes. We identified distinct functional domains of the presumed cytoplasmic C-terminal segment of the $K_{ir}6.2$ subunit that play an important role in this inhibition. Our results suggest that one domain is associated with inhibitory ATP binding and another with gate closure.**

The ATP-sensitive potassium ($K_{ATP}$) channel couples membrane electrical activity to energy metabolism in a variety of cells and is important in several physiological systems. In pancreatic $\beta$ cells, for example, it is essential for coupling the rate of insulin release to blood glucose levels. Although the channel's name reflects its characteristic inhibition by intracellular ATP, little is known about the molecular nature of this property.

To understand the molecular mechanisms underlying ATP-dependent inhibition gating in the $K_{ATP}$ channel, one must identify those parts of the channel complex that form the ATP-binding site, the inhibition gate, and the "linkage" domains handling signal flow between them. The $K_{ATP}$ channel is assembled from four each of two subunit types, a regulatory sulfonylurea receptor (SUR) or SUR1 and a potassium pore-forming subunit or $K_{ir}6.2$ (1–4). SUR1 is a member of the ATP-binding cassette (ABC) family of proteins featuring two cytoplasmic nucleotide-binding folds, viewed initially as likely mediators of inhibition by ATP (5, 6). However, a mutant $K_{ir}6.2$ in which the C-terminal 26 residues are deleted remarkably gives rise to potassium channels that retain much ATP sensitivity in the absence of SUR1 (7). The truncation does, however, diminish ATP sensitivity of the $K_{ATP}$ channel 10-fold, as does SUR2 when coexpressed with wild-type $K_{ir}6.2$ (6). These results have led to opposing models in which ATP acts either on $K_{ir}6.2$ or on SUR to inhibit the $K_{ATP}$ channel.

We tested whether major components of the ATP-dependent inhibition gating mechanism reside in the pore-forming subunit. By systematically mutating $K_{ir}6.2$, we localized molecular components of ATP-dependent inhibition gating to distinct regions of its cytoplasmic C-terminal segment. Our results confirm and extend the findings of Tucker *et al.* (7) that the primary site of action of inhibitory ATP lies on $K_{ir}6.2$. We go on to show that one of the regions we identified is likely associated with inhibitory ATP binding, whereas a second region appears to be associated with inhibition gate closure.

## MATERIALS AND METHODS

**Molecular Biology.** *Cloning of mouse SUR1 and $K_{ir}6.2$ from $\beta$HC9 cells.* Mouse pancreatic $\beta$HC9 cells (8, 9) grown in DMEM with 10% fetal calf serum and 30 mM glucose were a kind gift from the Diabetes Center at the University of Pennsylvania. Total RNA was isolated with a Fast RNA Green Kit (Bio 101) as specified by the supplier. Briefly, $10^7$ cells were placed with extraction buffer into a tube and homogenized in a FastPrep machine (FP120, Savant). This step was followed by several steps of phenol/chloroform extraction and 2-propanol precipitation. For mouse SUR1, total RNA was used for reverse transcription–PCR by using oligonucleotides based on the hamster SUR1 (5) as gene-specific primers. A full-length clone that expressed functional SUR1 was sequenced. For mouse $K_{ir}6.2$, the same approach was used with gene-specific primers initially based on $K_{ir}6.1$ (10) and later based on $K_{ir}6.2$ from the MIN6 cell line (1).

*Site-directed mutagenesis.* Preparation of cRNA and site-directed mutagenesis were as described (11). All mutations were confirmed by DNA sequencing.

**Electrophysiology.** Preparation and injection of *Xenopus* oocytes, patch pipet fabrication, and recording techniques were as described (11). Macroscopic and single-channel currents were recorded at −80 mV with the inside-out patch configuration unless indicated otherwise. Pipet solution (mM): 150 KCl, 10 NaCl, 1 $CaCl_2$, 10 EGTA, and 10 Hepes, pH 7.4 ± 0.05. Bath solution: same as pipet solution but with 1.3 MgATP. Constant perfusion of the cytoplasmic face of patches was performed by using either a six-sewer pipe syringe-pressurized system (custom made) or the DAD-12 superfusion system (Adams–List, Westbury, NY). Recordings were always begun within 1 min after excision with the patch pipet partially inserted into one of the sewer pipes. ATP was added as the magnesium salt to minimize rundown, and no other modulatory ligands were added until after the ATP dose-response data were obtained. Experiments showing "rundown" (13), observed as a sudden and significant decrease in channel open probability, $P_O$, were terminated and not used. Patch clamp currents were amplified with an Axopatch 200A (Axon Instruments, Foster City, CA) or EPC-9 (HEKA Electronics, Lambrecht/Pfalz, Germany), low-pass filtered with an 8-pole Bessel filter (Frequency Devices, Haverhill, MA) at a corner frequency of 2 or 4 kHz, and sampled at 20 kHz by using HEKA PULSE version 8.0 (HEKA Electronics). Leak currents (as

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Abbreviations: $P_O$, channel open probability; SUR, sulfonylurea receptor; ABC, ATP-binding cassette.

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determined after complete rundown or inhibition with saturating tolbutamide or ATP) were typically 10 pA, always ≤5% of the macroscopic $K_{ATP}$ currents, and were not subtracted from the data. Unless indicated otherwise, these recording conditions were used.

**Data Analysis.** Analysis and display were done by using TAC version 3.0.3 (Bruxton, Seattle, WA), IGOR PRO version 3.01 (WaveMetrics, Lake Oswego, OR), and PAGEMAKER version 6.5 (Adobe Systems, San Jose, CA). Dose-response measurements were fit to the Hill equation, $I/I_{max} = 1/\{1 + ([ATP]/K_i)^{\alpha_H}\}$, where [ATP] is the concentration of ATP and $I/I_{max}$ is the fractional current at the indicated [ATP] relative to that in the same solution in the absence of added ATP. The inhibition constant, $K_i$, is the [ATP] at which inhibition is one-half maximal, and $\alpha_H$ is the slope factor or Hill coefficient. $I_{max}$ measurements taken before and after I measurements were averaged. Data are presented as mean ± SEM. For Hill plots of data from mutant $K_{ATP}$ channels with very large $K_i$ values, we constrained $\alpha_H$ to 1.0. This is reasonable given that when $\alpha_H$ was a free variable, $\alpha_H = 1.0 \pm 0.1$ for the wild-type channel and $\alpha_H = 1.0 \pm 0.3$ for all our less severely affected $K_i$ mutant channels. Single-channel current event duration analysis was done by using TAC-FIT version 3.0.3 (Bruxton), which uses the transformations of Sigworth and Sine (12) to construct and fit duration histograms.

## RESULTS

**Mouse SUR1 and Mouse $K_{ir}6.2$ Coexpressed in *Xenopus* Oocytes Produces Potassium Currents Inhibited by ATP.** To obtain functional $K_{ATP}$ channels formed from species-homologous subunits, we cloned both SUR1 and $K_{ir}6.2$ from the insulin-secreting βHC9 cell line (8, 9) and coinjected the subunit cRNAs into *Xenopus* oocytes. Expression of either subunit alone failed to increase membrane potassium conductance. Fig. 1*A* shows a representative example of currents from the cloned $K_{ATP}$ channels in inside-out patches in symmetrical 150 mM potassium solutions at −80 mV. The current almost was fully inhibited by internal [ATP] at concentrations above 500 μM and was approximately one-half maximal at 10 μM ATP. At low [ATP], here 0.1 μM, the $K_{ATP}$ current decreased significantly over tens of seconds, in a complex process called "rundown" (13). The apparent dissociation constant for inhibition by ATP ($K_i$) was 12.3 ± 3.5 μM with a Hill coefficient

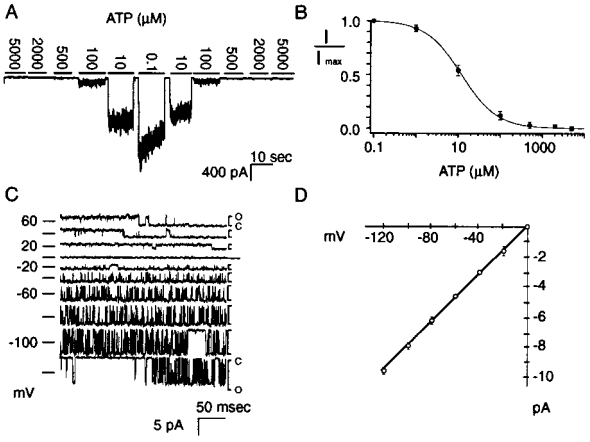


FIG. 1. Mouse SUR1 and mouse $K_{ir}6.2$ coexpressed in *Xenopus* oocytes generate $K_{ATP}$ channels. (*A*) Macroscopic currents from a representative inside-out patch in symmetrical 150 mM KCl at −80 mV. ATP at the indicated concentrations was superfused onto the cytoplasmic face of the patch. (*B*) Hill fit to data from eight oocytes. (*C*) Single channel currents at voltages from −120 to 60 mV in symmetrical 150 mM KCl and no ATP. (*D*) Linear fit to single-channel current amplitude as a function of voltage.

($\alpha_H$) of 1.03 ± 0.10 ($n = 8$ oocytes; Fig. 1*B*). Single channel experiments (Fig. 1*C*) showed multiple openings in rapid succession separated by long-lived closed times, and these bursting gating kinetics were more conspicuous at negative voltages. In the absence of ATP, there was a single voltage-independent short "intraburst" closed time, whereas open times became shorter in the negative voltage range. Between −40 and −100 mV the open state dwell times at a given voltage were well fit by single exponentials. At −80 mV, the mean open time was 1.85 ± 0.23 ms and the mean intraburst closed time was 0.42 ± 0.22 ms ($n = 10$). In the absence of ATP, longer duration ("interburst") closed events were relatively rare. ATP did not obviously affect the open and intraburst closed durations. Rather, it reduced the channel open probability, $P_O$, by shortening the burst durations with single exponential kinetics and lengthening the interburst (inhibition) dwell times in a concentration-dependent manner. A simple model for these ATP-dependent gating kinetics is one in which binding of a single ATP molecule inhibits the $K_{ATP}$ channel, and binding of additional ATP molecules further stabilizes the inhibited conformation (14, 15). Analysis of the single-channel current–voltage relationship in symmetrical 150 mM potassium solutions and over the negative voltage range yielded a slope conductance of 79 ± 3 pS ($n = 10$). These properties of the cloned mouse $K_{ATP}$ channel expressed in oocytes are similar to those of the pancreatic β cell $K_{ATP}$ channel in its native environment (16–18). The 390-aa residue sequence of $K_{ir}6.2$ cloned from βHC9 cells is identical to that of the original $K_{ir}6.2$ cloned from MIN6 cells at the protein level and nearly identical at the DNA level (1). The 1,582 residue sequence of the mouse SUR1 is 95.3% identical to that of hamster, 98.8% identical to that of rat, and 95.6% identical to that of human (5).

**Cytoplasmic N-terminal Domain of $K_{ir}6.2$ Is Unlikely To Be a Major Determinant of ATP Sensitivity of the $K_{ATP}$ Channel.** We tested for a role of the presumed cytoplasmic domains of $K_{ir}6.2$ in ATP sensitivity of the $K_{ATP}$ channel because cytoplasmic but not external ATP inhibits the channel (17, 18) and cytoplasmic but not external mild trypsin treatment diminishes ATP sensitivity (19–21). We mutated $K_{ir}6.2$ rather than SUR1 for several reasons. First, inhibition of the $K_{ATP}$ channel does not require hydrolyzable ATP whereas regulation of the cystic fibrosis transmembrane conductance regulator (CFTR), another ABC-channel protein, does (see below). Second, Tucker *et al.* had demonstrated that deletion of the C-terminal 26 residues of $K_{ir}6.2$ yields channels that retain substantial ATP sensitivity in the absence of SUR1 (7). Finally, mutations of several residues in both nucleotide-binding folds of SUR, designed to impair or abolish nucleotide binding or hydrolysis, had failed to affect inhibition of $K_{ATP}$ channels by ATP (22–24). These earlier findings suggest that molecular components for ATP inhibition gating are in the cytoplasmic domains of $K_{ir}6.2$. We therefore systematically swapped the cytoplasmic N- and C-terminal domains of $K_{ir}6.2$ with their counterparts from $K_{ir}1.1$, which forms potassium channels that are not inhibited by ATP (25). Chimeric constructs were made of $K_{ir}6.2$ and $K_{ir}1.1$, with junctions within the transmembrane segments M1 or M2 (Fig. 2). Replacing the N-terminal 73 residues of $K_{ir}6.2$ with the corresponding residues of $K_{ir}1.1$ and coexpressing with SUR1 failed to alter ATP sensitivity substantially: this "1.1–6.2–6.2" chimeric channel had a $K_i$ of 30 ± 5 μM ($n = 4$), a modest 2.5-fold reduction in ATP sensitivity compared with $K_{ir}6.2$. The reciprocal 6.2–1.1–1.1 chimera, in which the N-terminal 75 residues of $K_{ir}6.2$ replaced the corresponding residues of $K_{ir}1.1$, when coexpressed with SUR1 was indistinguishable from $K_{ir}1.1$, exhibiting no ATP sensitivity ($n = 5$). These results suggest that the N-terminal cytoplasmic domain of $K_{ir}6.2$ is not a major determinant of the ATP-dependent inhibition gating of the $K_{ATP}$ channel.

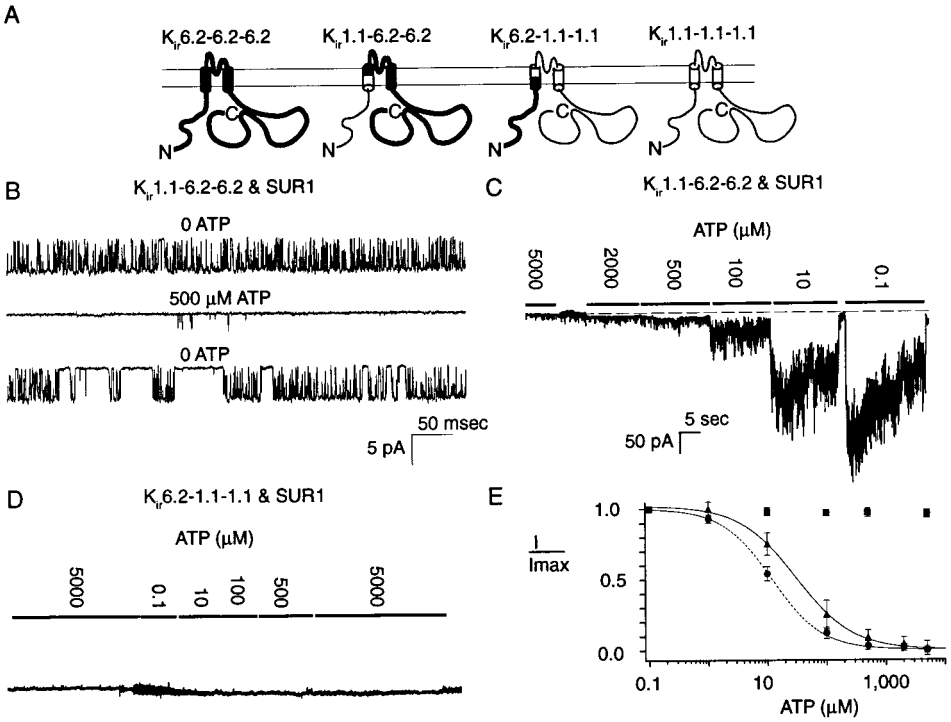


FIG. 2. The N-terminal cytoplasmic segment of $K_{ir}6.2$ is unlikely to be a major determinant of the ATP-dependent inhibition gating. Each construct was coexpressed with SUR1 and studied at −80 mV. (*A*) Parental and chimeric $K_{ir}$ constructs in which the N-terminal cytoplasmic segments are swapped. (*B*) Single channel records showing strong ATP sensitivity of channels formed from $K_{ir}1.1$–6.2–6.2 and SUR1. (*C*) Corresponding macroscopic currents. (*D*) Macroscopic currents (≈730 pA) through channels formed from $K_{ir}6.2$–1.1–1.1 show no ATP sensitivity. (*E*) Hill plots of ATP sensitivity of the chimeric constructs: ●, wild-type $K_{ir}6.2$ channels, $K_i = 12.3 \pm 3.5$ μM and $\alpha_H = 1.03 \pm 0.10$ ($n = 8$; taken from Fig. 1*B*); ▲, channels formed from $K_{ir}1.1$–6.2–6.2, $K_i = 30.0 \pm 5.4$ μM and $\alpha_H = 1.0 \pm 0.2$ ($n = 4$); and ■, $K_{ir}6.2$–1.1–1.1 (insensitive to ATP; $n = 5$).

**Distinct Regions of the Cytoplasmic C-Terminal Domain of $K_{ir}6.2$ Are Required For Wild-Type ATP Sensitivity.** Next, we explored the presumed cytoplasmic C-terminal domain of $K_{ir}6.2$. Twelve chimeric constructs, in which all or large parts of the domain were exchanged for corresponding parts from other $K_{ir}$ subunits, failed to yield functional potassium channels when coexpressed with SUR1. However, constructs in which only one or a few C-terminal domain residues of $K_{ir}6.2$ were replaced by the corresponding residues of $K_{ir}1.1$, $K_{ir}2.1$, $K_{ir}4.1$, or $K_{ir}6.1$ were functional. Large decreases in ATP sensitivity were observed when the four-residue sequence $^{334}$GNTI$^{337}$ of $K_{ir}6.2$ was replaced by that of $K_{ir}4.1$ (26, 27) or $K_{ir}2.1$ (28) (Fig. 3). The "$K_{ir}6.2$::4.1[334–337]" chimera coexpressed with mouse SUR1 always gave rise to potassium channel activity in the cell-attached configuration that, after patch excision into the inside-out configuration, was virtually refractory to [ATP] as high as 2,000 μM. We also studied this construct at the macroscopic current level. Because it is largely resistant to ATP, in three experiments we simply alternated application of 0.1 and a maximal 5,000 μM ATP to the internal face of the channels (Fig. 3*A*). We found $K_i > 10{,}000$ μM ($n = 8$; $\alpha_H$ was constrained to 1.0, see *Materials and Methods*), a >1,000-fold loss of ATP sensitivity (Fig. 3*B*). Further analysis showed that the G334D substitution accounted for nearly the entire 1,000-fold effect, N335Q had only a 2-fold effect, and T336V and I337V were without effect. Of the other chimeras of this region, the $K_{ir}6.2$::2.1[334–337] channel had a $K_i$ of 520 ± 20 μM ($n = 4$; $\alpha_H = 1.1 \pm 0.1$), a >40-fold decrease in ATP sensitivity, whereas the remaining chimeras were similar to $K_{ir}6.2$ ($n = 4$), exhibiting strong ATP sensitivity. These results demonstrate that the 334–337 region of $K_{ir}6.2$ is required for wild-type ATP sensitivity.

A second region of $K_{ir}6.2$ that is required for wild-type ATP sensitivity of the $K_{ATP}$ channel also was identified. When coexpressed with SUR1, $K_{ir}6.2$::T171A exhibited a $K_i$ of 500 ±

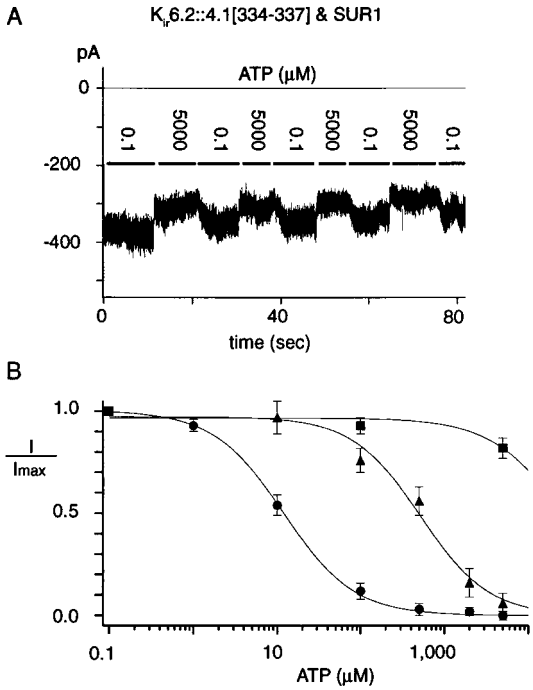


FIG. 3. Residues from the 334–337 region of $K_{ir}6.2$ are required for wild-type ATP sensitivity. (*A*) Representative macroscopic current at −80 mV from a patch expressing channels formed from $K_{ir}6.2$::4.1[334–337] and SUR1 in response to alternating 0.1 and 5,000 μM ATP. The thin horizontal line indicates zero current. (*B*) ATP sensitivity of the 334–337 region chimeras: ▲, $K_{ir}6.2$::2.1[334–337] channels and ■, $K_{ir}6.2$::4.1[334–337] channels, compared with ●, wild-type $K_{ir}6.2$ channels, taken from Fig. 1*B*.

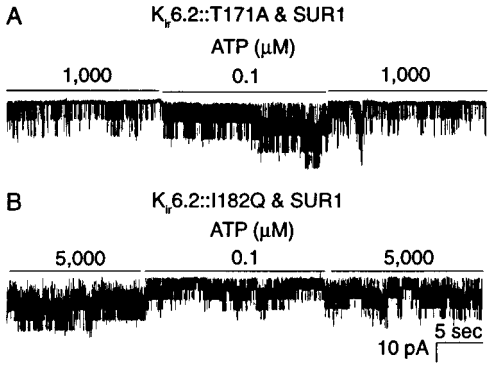


FIG. 4. $K_{ir}$6.2::T171A and $K_{ir}$6.2::I182Q have low ATP sensitivity. (*A*) Representative single-channel currents at −80 mV from channels formed by coexpression of $K_{ir}$6.2::T171A and SUR1. The mutant channels exhibit moderate decrease in ATP sensitivity, exemplified by lowered but still substantial activity in the presence of 1,000 μM ATP. (*B*) Single channel currents at −80 mV from channels formed by $K_{ir}$6.2::I182Q and SUR1. The decrease in channel activity in response to low [ATP] is typical for this mutant. These channels are not detectably inhibited by ATP and appear to run down rapidly when [ATP] is below 10 μM.

41 μM ($n$ = 4) and $K_{ir}$6.2::I182Q a $K_i$ >10,000 μM ($n$ = 12; Fig. 4). We tested 12 additional mutants of $K_{ir}$6.2, which when coexpressed with SUR1 gave rise to functional channels. None of the 12 mutations, which were spaced across the region from K207 to S327 (K207Q, K222Q, H234D, G243D, G243V, G245V, N247D, E241G, V244S, A253S, T293D, and S327K), had a substantial effect on ATP sensitivity. Taken together, these findings indicate that at least two regions of $K_{ir}$6.2, residues 171–182 and residues 334–337, are required for wild-type ATP sensitivity of the $K_{ATP}$ channel. Next, we provide evidence that a residue of the former region acts as if it is involved in inhibition gate closure and a residue of the latter in inhibitory ATP binding.

**Regions of the Channel Required for ATP-Dependent Inhibition Gate Closure and for ATP Binding.** The truncated subunit $K_{ir}$6.2ΔC26 ($K_{ir}$6.2Δ365–390), expressed in the absence of SUR, generates potassium channels retaining much ATP sensitivity (7). We observed, however, important differences from wild-type channels (Fig. 5). The truncated channel (Fig. 5*A*) exhibited an obviously lowered $P_O$: Immediately following patch excision into ATP-free solution, $P_O$ was 0.12 ± 0.02 ($n$ = 5) compared with 0.70 ± 0.14 ($n$ = 5) for wild-type. The truncated channels also exhibited characteristic "spiking" single-channel gating kinetics in the absence of ATP. The spiking kinetics, which differ dramatically from the long bursts typical of wild-type channels, results from a considerable shortening of burst durations (i.e., an increased rate of entry into long-lived interburst closed states). To account for these kinetics we hypothesize that the truncated channel, in the absence of ATP, spontaneously undergoes transitions into long-lived closed conformations, very similar to the ATP-inhibited conformations of the wild-type channel. We recall a remarkable property of ATP-inhibited conformations of the wild-type $K_{ATP}$ channel is that their dwell times are [ATP] dependent (14, 15). We found that this still holds for the truncated channel: In the absence of ATP the long-lived closed times largely followed a single exponential distribution with a 14.2 ± 1.3 ms mean dwell time ($n$ = 4; Fig. 5); in 500 μM ATP the mean dwell times increased to 26.3 ± 3.5 ms ($n$ = 4) and in 1,000 μM ATP to 82.6 ± 9.5 ms ($n$ = 4). Mean dwell times of the single open state and of the short-lived intraburst closed state remained, as in wild-type, largely unaffected by ATP.

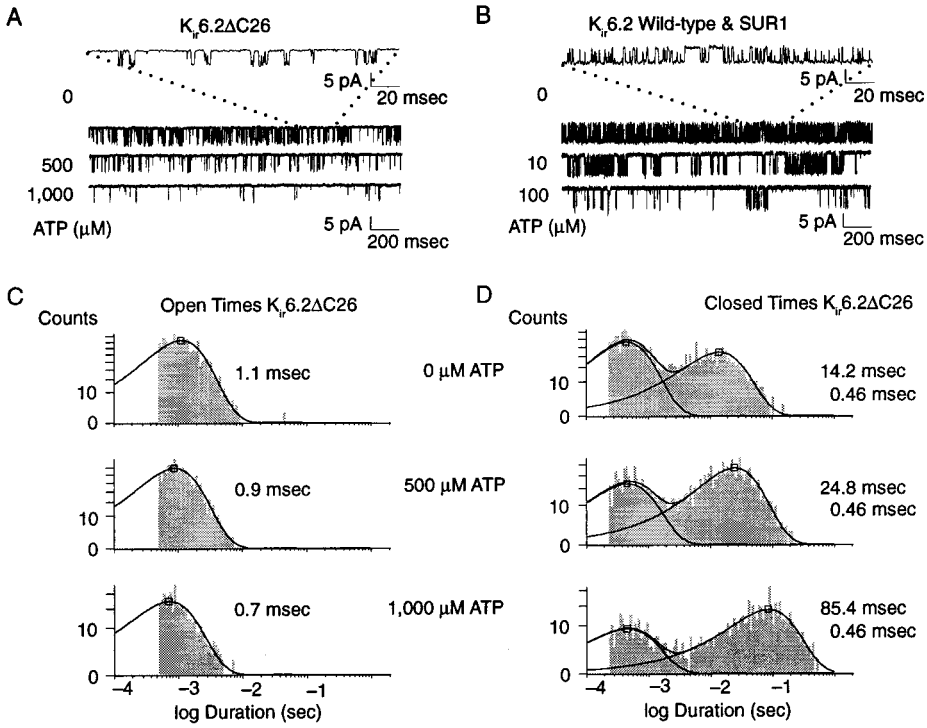


FIG. 5. Long-lived closed times of truncated $K_{ir}$6.2ΔC26 largely fit into a single kinetic component and increase with [ATP]. (*A*) Truncated channel in the absence of SUR1. Top two traces in the absence of added ATP. Notice the very short bursts of openings and frequent long-lived closed times even in the absence of ATP. Bottom two traces at the [ATP] indicated. (*B*) Wild-type $K_{ATP}$ channels. Notice the long bursts of openings and relatively rare long-lived closed times. (*C*) Open-time histograms of the truncated $K_{ir}$6.2ΔC26 channel in the absence of SUR1 at the indicated [ATP], representative of four similar experiments. (*D*) Closed time histograms of the truncated $K_{ir}$6.2ΔC26 channel in the absence of SUR1 at the indicated [ATP], representative of four similar experiments. The event duration histogram construction and fitting (12) results in a function of peaked exponential terms. The time constant of each term is specified by the $x$ coordinate of the peak, indicated in the histograms by an open box. All currents were recorded at −80 mV.

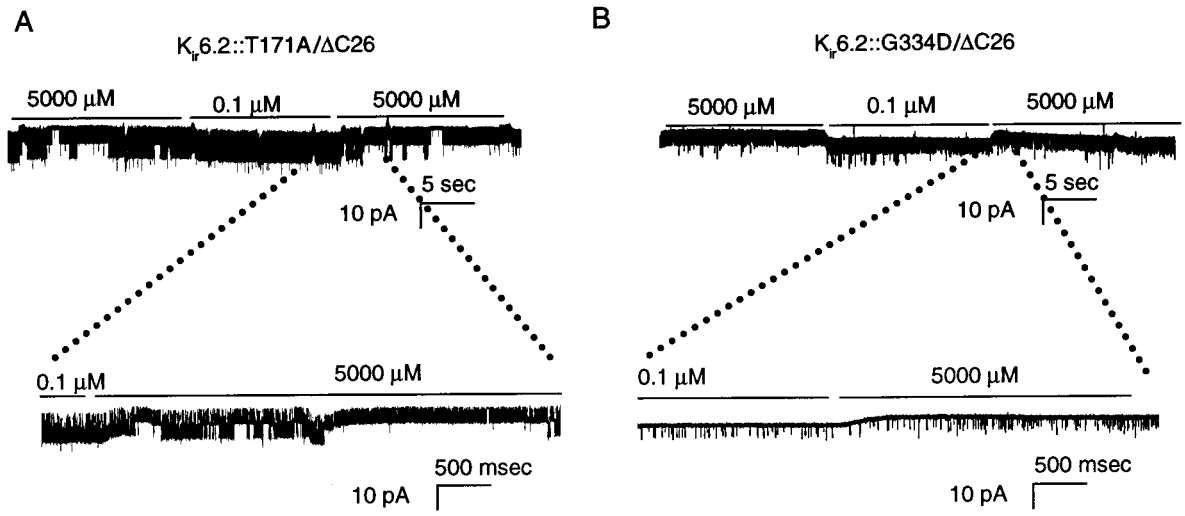


FIG. 6. On the ΔC26 background, T171A mutation restores bursting single-channel kinetics, whereas G334D mutation preserves spiking single-channel kinetics. (*A*) $K_{ir}$6.2::T171A/ΔC26 in the absence of SUR1, slow time scale, with a segment of the recording expanded on a fast time scale as indicated. On average, the 5,000 μM ATP inhibits one of two channels. Notice that the T171A mutation reverts the spiking single-channel gating kinetics of the ΔC26 parent channel to the long bursts of rapid successive openings similar to the wild-type $K_{ATP}$ channel (see Fig. 5*B*). (*B*) $K_{ir}$6.2::G334D/ΔC26 in the absence of SUR1, slow time scale, with a segment of the recording expanded on a fast time scale as indicated. Notice that the channels retain the spiking single-channel gating kinetics of the ΔC26 parent channel. (See Fig. 5*A*). All currents were recorded at −80 mV.

According to our hypothesis, a mutation that disrupts the channel's ATP sensitivity by preventing gate closure (an action subsequent to ATP binding) also could be expected to prevent the spontaneous entry of truncated channels into long-lived closed states. In this case, such a mutation should restore wild-type bursting kinetics in truncated channels in the absence of ATP. Conversely, a mutation that affects only ATP binding need not be expected to prevent spontaneous entry of the truncated channel into the inhibited state in the absence of ATP. In this case, such a mutation should preserve the spiking kinetics of truncated channels. To test these predictions, we combined mutations in either the 171–182 region or the 334–337 region with the ΔC26 truncation and expressed these double-mutant channels in the absence of SUR1.

We found that T171A dramatically converts the spiking kinetics of ΔC26 back to long bursting kinetics (Fig. 6). In the absence of SUR and with little or no ATP present, the T171A/ΔC26 double mutant channel shows the long bursting kinetics typical of the wild-type $K_{ATP}$ channel observed under the same experimental conditions ($n = 10/10$). Still in the absence of SUR, the double mutant channel remained refractory to ATP inhibition ($n = 5$), demonstrating that the loss of ATP sensitivity caused by T171A is not somehow mediated by SUR1. We therefore propose that the T171A mutation affects gating transitions downstream from those associated with ATP binding. A plausible interpretation is that T171A disrupts conformational transitions required for inhibition gate closure. On the other hand, the G334D ($n = 4/4$) and 4.1[334–337] ($n = 4/4$) mutations in the truncated channel failed to restore the long bursting kinetics in the absence of ATP. The G334D/ΔC26 and 4.1[334–337]/ΔC26 double mutant channels, however, remained refractory to ATP inhibition. These results support our interpretation that the 334–335 region is required for steps preceding gate closure, presumably those associated with ATP binding *per se* (see below).

## DISCUSSION

Our results suggest a molecular mechanism in which C-terminal residues of $K_{ir}$6.2 are associated with distinct functional domains of the ATP-dependent inhibition gating mechanism. For example, the $K_{ir}$6.2 sequence $^{333}$FGNTIK$^{338}$, which we identify here as a likely component of the ATP-binding site, includes an ATP-binding motif $FX_4K$ that is highly conserved in the intracellular ATP-binding loop of ion-motive ATPases (29). Although both are sensitive to μM ATP, neither $K_{ir}$6.2 nor ion-motive ATPases contain Walker-type ATP-binding motifs. Our proposal that the 333–338 region of $K_{ir}$6.2 is part of the inhibitory ATP-binding site also is consistent with the failure of G334D- and 4.1[334–337]-mutated $K_{ir}$6.2ΔC26 channels to revert from spiking single-channel kinetics to long bursting kinetics similar to wild-type.

The proposal that the region including T171 of $K_{ir}$6.2 participates in inhibition gating is supported by the ability of the T171A mutation to convert the spiking single-channel kinetics of truncated $K_{ir}$6.2ΔC26 channels back to wild-type bursting kinetics. Our mutagenesis in this region, including detailed analysis of T171A on the ΔC26 background, now suggests it is required for inhibition gate closure. Shyung *et al.* (24) have shown moderate effects of substitutions at position 160 in the M2 region on ATP sensitivity. Residues 160–171, which span part of the second transmembrane domain M2 and the initial cytoplasmic C-terminal segment, are therefore likely to participate in gating. Interestingly, mutant analysis of the homologous position in the *Shaker* potassium channel implicates its involvement in voltage-dependent activation–deactivation gating (30). We expect further studies to reveal additional shared properties between deactivation gating in *Shaker* and inhibition gating in $K_{ATP}$ channels.

Our finding confirms and extends the report of Tucker *et al.* (7) that truncation of $K_{ir}$6.2 yields channels that remain ATP sensitive in the absence of SUR. Tucker *et al.* also reported that mutation of a single C-terminal residue K185Q, on the $K_{ir}$6.2ΔC26 background, decreases by 40-fold sensitivity to inhibition by ATP. We also found that mutation I182Q, on the wild-type $K_{ir}$6.2 background, decreases by >1,000-fold inhibition gating by ATP. The proximity of I182 to K185 may suggest a common role for these two residues in ATP-dependent inhibition gating. It will be interesting to determine the single-channel gating behavior of these and other mutations on the ΔC26 background in the absence of SUR and ATP.

Our findings fit well with the observation that numerous mutations in either or both nucleotide-binding folds of SUR fail to alter ATP-dependent inhibition gating of the $K_{ATP}$

channel (22–24). Mutations in the nucleotide-binding folds of the SUR subunit can disrupt stimulation by MgADP and sensitivity to potassium channel openers, with little or typically no effect on ATP inhibition. The nucleotide-binding folds of ABC proteins have been generally associated with ATP hydrolysis-dependent functions. For example, in CFTR, ATP hydrolysis at one nucleotide-binding fold is required for opening of the chloride conduction pathway, whereas hydrolysis at the other is required for closure (31–35). When both ATP and its nonhydrolyzable analog adenosine-5′-($\beta$,$\gamma$-imido)triphosphate (AMP-PNP) are present, the latter can bind to an open channel's nucleotide-binding fold and prevent closure, producing CFTR channels with extraordinarily extended burst times. In the case of the $K_{ATP}$ channel, however, AMP-PNP and ATP each inhibit conductance with comparable efficacy, suggesting that no hydrolysis is required (17, 18, 36). Major molecular components required for ATP inhibition of the $K_{ATP}$ channel rather reside in distinct functional domains of the cytoplasmic C-terminal segment of $K_{ir}6.2$ that we identify in the present study.

Any molecular model for ATP-dependent inhibition gating must account for signal flow from ATP binding *per se* to closure of a gate during inhibition and from ATP unbinding to opening of the gate during recovery. In the present study, we show that residues 333–338 look and behave as if they are part of an ATP-binding domain. In view of the multimeric structure proposed for the $K_{ATP}$ channel (2–4), our results suggest that each $K_{ir}6.2$ subunit might contribute a functional ATP-binding site to the $K_{ATP}$ channel. Such multiple sites could readily explain the ATP concentration dependence of the long-lived closed times of both the native $K_{ATP}$ channel (14, 15) and the truncated $K_{ir}6.2\Delta C26$ channel (shown here).

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