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MgATP activates the β cell K_{ATP} channel by interaction with its SUR1 subunit

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ABSTRACT **ATP-sensitive potassium (K_{ATP}) channels in the pancreatic β cell membrane mediate insulin release in response to elevation of plasma glucose levels. They are open at rest but close in response to glucose metabolism, producing a depolarization that stimulates Ca^{2+} influx and exocytosis. Metabolic regulation of K_{ATP} channel activity currently is believed to be mediated by changes in the intracellular concentrations of ATP and MgADP, which inhibit and activate the channel, respectively. The β cell K_{ATP} channel is a complex of four Kir6.2 pore-forming subunits and four SUR1 regulatory subunits: Kir6.2 mediates channel inhibition by ATP, whereas the potentiatory action of MgADP involves the nucleotide-binding domains (NBDs) of SUR1. We show here that MgATP (like MgADP) is able to stimulate K_{ATP} channel activity, but that this effect normally is masked by the potent inhibitory effect of the nucleotide. Mg^{2+} caused an apparent reduction in the inhibitory action of ATP on wild-type K_{ATP} channels, and MgATP actually activated K_{ATP} channels containing a mutation in the Kir6.2 subunit that impairs nucleotide inhibition (R50G). Both of these effects were abolished when mutations were made in the NBDs of SUR1 that are predicted to abolish MgATP binding and/or hydrolysis (D853N, D1505N, K719A, or K1384M). These results suggest that, like MgADP, MgATP stimulates K_{ATP} channel activity by interaction with the NBDs of SUR1. Further support for this idea is that the ATP sensitivity of a truncated form of Kir6.2, which shows functional expression in the absence of SUR1, is unaffected by Mg^{2+}.**

ATP-sensitive K^+ (K_{ATP}) channels couple cell metabolism to electrical activity. They thereby regulate insulin secretion from pancreatic β cells, participate in the response to cardiac and cerebral ischemia, control vascular smooth muscle tone, modulate transmitter release at brain synapses, and mediate K^+ fluxes across epithelial cells (1–3). It is thought that metabolic regulation is achieved, at least in part, by variation in the intracellular concentrations of the adenine nucleotides ATP and ADP. These nucleotides influence K_{ATP} channel activity by interacting with two physically distinct sites on the K_{ATP} channel: interaction with one site leads to channel inhibition whereas interaction with the other site causes an increase in channel activity (4, 5). ADP interacts with both sites, being stimulatory at low concentrations and inhibitory at high concentrations. Although it is well established that ATP interacts with the inhibitory site, it is not known whether it is also effective at the stimulatory site. The properties of the two sites differ in their requirement for Mg^{2+}, the divalent cation being required for the potentiatory but not the inhibitory action of ADP (4, 6, 7).

The β cell K_{ATP} channel consists of two types of subunit (8, 9): an inwardly rectifying K^+ channel subunit (Kir6.2) and a sulfonylurea receptor subunit (SUR1), which assemble in a 4:4 stoichiometry to form an octameric channel (10–12). Both subunits are required to form a fully functional K_{ATP} channel. The Kir6.2 subunit serves as the K_{ATP} channel pore, whereas SUR1 endows Kir6.2 with sensitivity to sulfonylureas and K^+ channel openers (5, 13). There is evidence that the inhibitory effect of ATP (and ADP) results from interaction of the nucleotide with Kir6.2 (5). By contrast, the potentiatory effects of MgADP are mediated through the sulfonylurea receptor subunit (5, 14–16). Sequence and hydropathy analysis suggest that SUR1 has two cytosolic nucleotide-binding domains (NBDs) and multiple transmembrane domains (17, 18). The NBDs of several other members of the ABC transporter family, to which SUR1 belongs, are known to be involved in ATP binding and hydrolysis (19). In the case of the cystic fibrosis transmembrane conductance regulator, CFTR, the energy of ATP hydrolysis is used to drive a conformational change that opens an intrinsic Cl^- channel (20–22). There is evidence that both NBDs of SUR1 are involved in channel activation by MgADP (15, 16).

The fact that MgGTP, like MgADP and MgGDP, stimulates K_{ATP} channel activity (23) raises the possibility that MgATP also may have a stimulatory effect, mediated by the NBDs, which normally is obscured by the inhibitory effect of the nucleotide. This possibility is supported by the fact that the sensitivity of native β cell K_{ATP} channels to ATP inhibition is increased by the removal of Mg^{2+} ions (24, 25). Originally, this finding was interpreted to indicate that the β cell K_{ATP} channel is inhibited by the free base, ATP^{4-}, whose concentration is reduced in solutions containing Mg^{2+}. An alternative explanation, however, is that, like MgADP, MgATP has a small stimulatory effect on K_{ATP} channel activity that causes an apparent reduction in ATP sensitivity in the presence of Mg^{2+}.

In this paper we show that the effect of Mg^{2+} on the ATP sensitivity of the β cell K_{ATP} channel is mediated by the sulfonylurea receptor subunit. We further show that this effect requires interaction of MgATP with the NBDs of SUR1. Our results suggest that, like MgADP, MgATP has dual stimulatory and inhibitory actions on the K_{ATP} channel. Removal of Mg^{2+} enhances the apparent ATP sensitivity by abolishing the stimulatory effect. MgATP is less effective at increasing K_{ATP} channel activity than MgADP. It is possible that the stimulatory effect of MgATP also contributes to the metabolic regulation of K_{ATP} channel activity.

METHODS

Molecular Biology. Mouse Kir6.2 (GenBank D50581; refs. 8 and 9) and rat SUR1 (GenBank L40624; ref. 17) were used in this study. A 36- (or 26)-aa C-terminal deletion of mouse

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Abbreviations: NBD, nucleotide-binding domain; K_{ATP} channel, ATP-sensitive K^+ channel.

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Kir6.2 (Kir6.2ΔC36; Kir6.2ΔC26) was made by introduction of a stop codon at the appropriate residue by using site-directed mutagenesis (5). Site-directed mutagenesis was carried out by subcloning the appropriate fragments into the pALTER vector (Promega). Synthesis of mRNA was carried out by using the mMessage mMachine large-scale *in vitro* transcription kit (Ambion).

Electrophysiology. Female *Xenopus laevis* were anesthetized with MS222 (2 g/liter added to the water). One ovary was removed via a mini-laparotomy, the incision was sutured, and the animal was allowed to recover. Once the wound had completely healed, the second ovary was removed in a similar operation, and the animal then was killed by decapitation while under anesthesia. Immature stage V-VI *Xenopus* oocytes were incubated for 75 min with 1.5 mg/ml of collagenase (Boehringer, type A) and manually defolliculated. In some experiments, oocytes were injected with ≈2 ng of mRNA encoding either wild-type or mutant Kir6.2ΔC36 (or Kir6.2ΔC26). In coexpression experiments, ≈0.04 ng of wild-type or mutant Kir6.2 was coinjected with ≈2 ng of wild-type or mutant SUR1 (giving ≈1:50 ratio). The final injection volume was ≈50 nl/oocyte. Control oocytes were injected with water. Isolated oocytes were maintained in tissue culture and studied 1–4 days after injection (26).

Macroscopic currents were recorded from giant excised inside-out patches at a holding potential of 0 mV and 20–24°C (26). Patch electrodes were pulled from thick-walled borosilicate glass (GC150; Clark Electromedical Instruments, Pangbourne, U.K.) and had resistances of 200–400 kΩ when filled with pipette solution. Currents were evoked by repetitive 3-s voltage ramps from −110 mV to +100 mV (holding potential, 0 mV) and recorded by using an EPC7 patch-clamp amplifier (List Electronics, Darmstadt, Germany). They were filtered at 0.2 kHz, digitized at 0.5 kHz by using a Digidata 1200 Interface, and analyzed by using pClamp software (Axon Instruments, Foster City, CA).

The pipette solution contained 140 mM KCl, 1.2 mM $MgCl_2$, 2.6 mM $CaCl_2$, and 10 mM Hepes (pH 7.4 with KOH), and the internal (bath) solution contained 110 mM KCl, 1.4 mM $MgCl_2$, 30 mM KOH, 10 mM EGTA, 10 mM Hepes (pH 7.2 with KOH), and nucleotides as indicated. The zero magnesium solution contained 110 mM KCl, 2.6 mM $CaCl_2$, 30 mM KOH, 10 mM EDTA, 10 mM Hepes (pH 7.2 with KOH), and nucleotides as indicated. ATP was added as either the Mg^{2+} or K^+ salt. ADP was added as the K^+-salt and 1 mM $MgCl_2$ was added per 1 mM ADP to maintain the free Mg^{2+} concentration constant. Rapid exchange of solutions was achieved by positioning the patch in the mouth of one of a series of adjacent inflow pipes placed in the bath.

Data Analysis. The slope conductance (G) was measured by fitting a straight line to the current-voltage relation between −20 mV and −100 mV: the average of five consecutive ramps was calculated in each solution. Dose-response relations for ATP block of K_{ATP} currents were obtained by alternating test solutions with control (ATP-free) solution. To control for rundown, the control conductance (G_c) was taken as the mean of that obtained in the control solution before and after application of ATP.

ATP dose-response relationships were fitted to the Hill equation $G/G_c\ (\%) = 100/(1 + ([ATP]/K_i)^h)$ where [ATP] is the ATP concentration, K_i is the ATP concentration at which inhibition is half maximal, and h is the slope factor (Hill coefficient). All data are given as mean ± one SEM, and the symbols in the figures indicate the mean and the vertical bars one SEM. (where this is larger than the symbol). Statistical significance was tested by using an unpaired Student's t test: P values of <0.05 were taken to indicate that the data were significantly different.

RESULTS

Large currents were recorded in giant inside-out membrane patches excised from oocytes coinjected with mRNAs encoding Kir6.2 and SUR1. These currents were reversibly inhibited by ATP, both in the absence and presence of Mg^{2+} (Fig. 1*Aa*). However, ATP was more effective in the absence of Mg^{2+}, in agreement with what has been described for native β cell K_{ATP} channels (24–26). Fig. 1*Ba* shows the relationship between ATP concentration and the extent of inhibition of Kir6.2/SUR1 currents, measured in the presence or absence of Mg^{2+}. Mg^{2+} removal significantly enhanced the ATP sensitivity, the K_i decreasing from 28 ± 4 μM (n = 15) in the presence of 1.4 mM Mg^{2+} to 5.8 ± 1.0 μM (n = 7) in Mg^{2+}-free solution (P = 0.0007, t test). The Hill coefficients (h) were 1.1 ± 0.1 and 1.0 ± 0.2, in the presence and absence of Mg^{2+}, respectively.

We next explored whether the SUR1 subunit was required for the enhancement of ATP sensitivity by Mg^{2+}. To do this, we exploited the fact that deletion of the last 36 amino acids

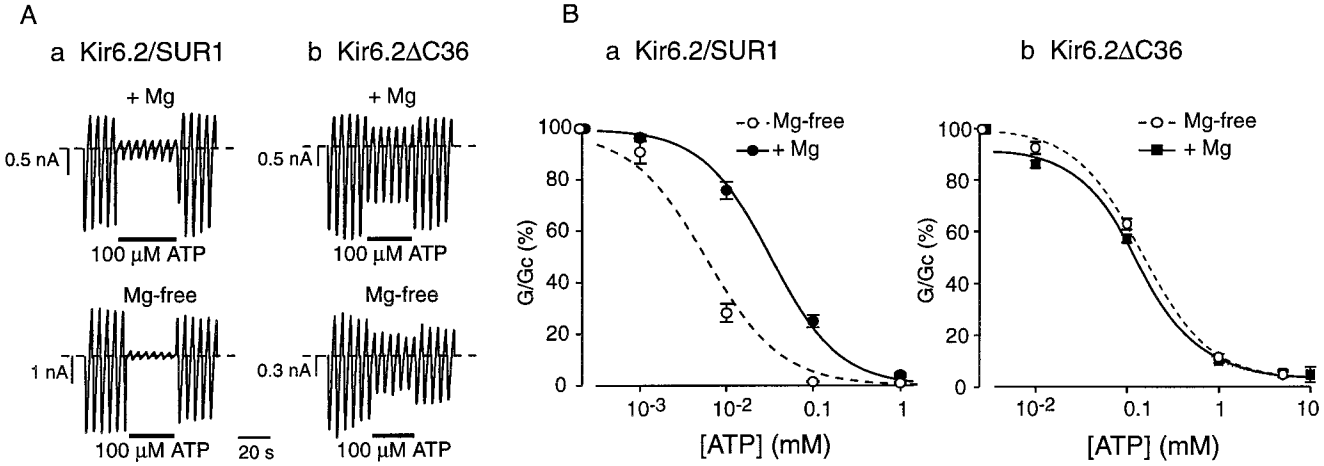

FIG. 1. (*A*) Macroscopic currents recorded from inside-out patches excised from oocytes coinjected with Kir6.2 and SUR1 mRNAs (*a*) or injected with Kir6.2ΔC36 mRNA (*b*) in response to a series of voltage ramps from −110 mV to +100 mV. ATP (100 μM) was added to the internal solution as indicated. (*B*) Mean ATP dose-response relationships for Kir6.2/SUR1 currents (*a*) or Kir6.2ΔC36 currents (*b*) in the presence (●) or absence (○) of Mg^{2+}. Test solutions were alternated with control solutions, and the slope conductance (G) is expressed as a percentage of the mean (G_c) of that obtained in control solution before and after exposure to ATP. Conductance was measured between −20 and −100 mV and is the mean of five voltage ramps. The number of patches was (*a*) ● = 7; ○ = 15 and (*b*) ■ = 11; ○ = 15. The lines are the best fit of the data to the Hill equation by using the mean values for K_i and h given in the text.

of Kir6.2 (Kir6.2ΔC36) enables its independent functional expression (5). Fig. 1 *Ab* and *Bb* shows that the ATP sensitivity of Kir6.2ΔC36 currents was unaffected by Mg^{2+} removal, the K_i being 115 ± 6 μM (n = 11) in the presence of 1.4 mM Mg^{2+} compared with 145 ± 13 μM (n = 5) in Mg^{2+}-free solution (not significant). The Hill coefficients were 1.0 ± 0.1 and 1.3 ± 0.1, respectively. This result therefore suggests that the effect of Mg^{2+} on the ATP sensitivity of the wild-type β cell K_{ATP} channel may be mediated by the sulfonylurea receptor subunit. The reduced ATP sensitivity of Kir6.2ΔC36 currents compared with Kir6.2/SUR1 currents has been reported previously (5).

A possible explanation for the apparent decrease in the ATP sensitivity of Kir6.2/SUR1 currents in the presence of Mg^{2+} is that, like MgADP, MgATP is able to stimulate channel activity. In this case channel inhibition (only) would occur in the absence of Mg^{2+}, whereas in the presence of Mg^{2+} channel activity would be determined by the balance between the inhibitory and stimulatory effects of MgATP. Because MgADP stimulates K_{ATP} channel activity by interaction with the NBDs of SUR1 (15–17), we hypothesized that the interaction of MgATP with the NBDs of SUR1 also might induce channel activation. To explore this possibility further, we used mutations in SUR1 that are predicted to alter MgATP binding and/or hydrolysis. In other ABC transporters, ATP hydrolysis requires two conserved motifs in the NBDs known as the Walker A (W_A) and Walker B (W_B) motifs (19). The W_A motif contains an invariant lysine residue that is believed to coordinate the negatively charged tail of the nucleotide. The critical amino acid in the W_B motif is an aspartate residue, which may be involved in coordination of the Mg^{2+} ion of MgATP. Mutation of these residues is predicted to impair the binding and/or hydrolysis of ATP. It previously has been shown that mutations in either the W_A lysine or W_B aspartate residues of SUR1 abolish the stimulatory effects of MgADP, MgGDP, and MgGTP (14–16, 23, 27). We therefore explored the effects of mutation of the W_B aspartate to asparagine in either the first (D853N) or the second (D1505N) NBD on the sensitivity of the channel to inhibition by ATP.

Giant patches excised from oocytes coinjected with mRNAs encoding Kir6.2 and either D853N-SUR1 or D1505N-SUR1 developed large K^+ currents, which were of comparable amplitude to those observed for wild-type channels, after patch excision. The mean macroscopic conductance was 95 ± 24 nS (n = 5) for D853N-SUR1 channels, 48 ± 16 nS (n = 8) for D1505N-SUR1 channels, and 49 ± 6 nS (n = 18) for wild-type channels. The mutations therefore do not impair channel expression.

Fig. 2 compares the relationship between ATP concentration and channel inhibition for wild-type, D853N-SUR1, and D1505N-SUR1 currents. In the presence of Mg^{2+}, the W_B mutant channels were more sensitive to ATP than Kir6.2-SUR1 channels. The K_i for ATP inhibition of D853N-SUR1 currents was 13.4 ± 0.2 μM (n = 4), and that of D1505N-SUR1 was 16.0 ± 2.6 μM (n = 5), compared with 28 μM for wild-type currents (P = 0.05 by ANOVA). The K_i values measured for ATP inhibition of W_B mutant channels are similar to those found when the W_A lysine is mutated (15). A reduction in the K_i for ATP-inhibition also is observed for the wild-type K_{ATP} channel in the absence of Mg^{2+} (K_i = ≈6 μM). It has been shown previously that mutation of either of the W_B aspartates, or W_A lysines, blocks the ability of MgADP to potentiate K_{ATP} channel activity (14–16, 27). Our results therefore suggest that MgATP, like MgADP, may interact with the NBDs of SUR1 to enhance channel activity. In the case of ATP, this effect is apparent as a reduction in the efficacy of ATP block.

If the stimulatory effect of MgATP on K_{ATP} channel activity normally is masked by the inhibitory effect of the nucleotide, it should be possible to demonstrate MgATP-dependent activation directly by using a mutant channel that is much less

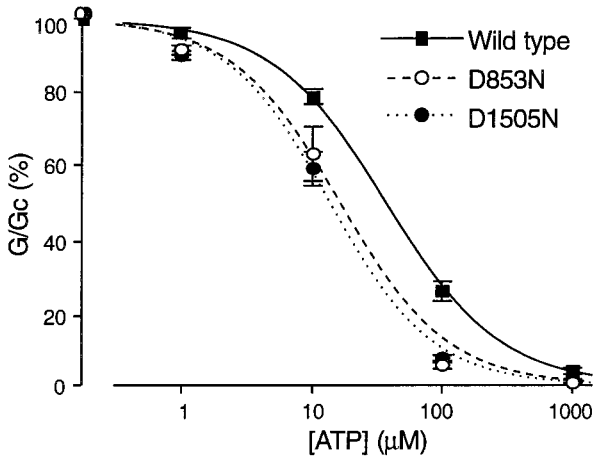

FIG. 2. Mean ATP dose-response relationships for Kir6.2/SUR1 (■, n = 15), Kir6.2/SUR1-D853N (○, n = 4) or Kir6.2/SUR1-D1505N (●, n = 5) currents, measured in the presence of Mg^{2+}. Test solutions were alternated with control solutions, and the slope conductance (G) is expressed as a percentage of the mean (G_c) of that obtained in control solution before and after exposure to ATP. Conductance was measured between −20 and −100 mV and is the mean of five voltage ramps. The lines are the best fit of the data to the Hill equation by using the mean values for K_i and h given in the text.

sensitive to ATP inhibition. Mutation of the arginine residue at position 50 of Kir6.2 to glycine (R50G) reduced the K_i for ATP inhibition from 106 μM for Kir6.2ΔC26 currents to 3.4 mM for Kir6.2ΔC26-R50G currents (28). We therefore engineered this mutation in the full-length form of Kir6.2 (Kir6.2-R50G) and coexpressed it with SUR1. Because Kir6.2 does not express functional channels independently of SUR, this procedure ensures that the K_{ATP} current we record only reflects current flow through channels comprising both Kir6.2 and SUR1 subunits. As predicted, when Kir6.2-R50G was coexpressed with SUR1, MgATP now stimulated channel activity (Fig. 3). The potentiatory effect of MgATP was dose-dependent and significantly less that that of MgADP. These data support the idea that MgATP exerts both excitatory and inhibitory effects on the wild-type K_{ATP} channel. The effects of MgATP and MgADP were not additive, because MgATP was ineffective when added in the presence of MgADP: 100 μM MgATP activated Kir6.2-R50G/SUR1 currents by 138 ± 7% (n = 5) in the absence, and by 102 ± 2% (n = 4) in the presence, of 100 μM MgADP.

Finally, we examined the effect of ATP on currents recorded from giant patches excised from oocytes coexpressing Kir6.2-R50G and an SUR1 subunit in which the W_A lysines in both NBDs had been mutated (K719A/K1384M). These mutations are predicted to abolish or severely impair nucleotide hydrolysis, and they prevent the stimulatory action of MgADP (15). As expected if MgATP stimulation requires the NBDs of SUR1, the currents were inhibited, not activated by MgATP (Fig. 4 *A* and *B*). Mutation of the W_A lysine in a single NBD also abolished the stimulatory effect of MgATP (Fig. 4*C*). This finding suggests that both NBDs are required for channel activation by MgATP, as is the case for MgADP and MgGDP (15, 16, 23). The relative extent of activation and inhibition of the K_{ATP} channel by MgATP, at different ATP concentrations, can be estimated by comparing the ATP dose-response curve for wtKir6.2/SUR1 (Fig. 4*B*, dashed line), where ATP inhibition predominates, with that for Kir6.2-R50G/SUR1 currents (Fig. 4*B*, ■) in which the stimulatory effect of MgATP is dominant.

DISCUSSION

Our results demonstrate that, like MgADP, MgATP exerts a stimulatory effect on the β cell K_{ATP} channel. This stimulation

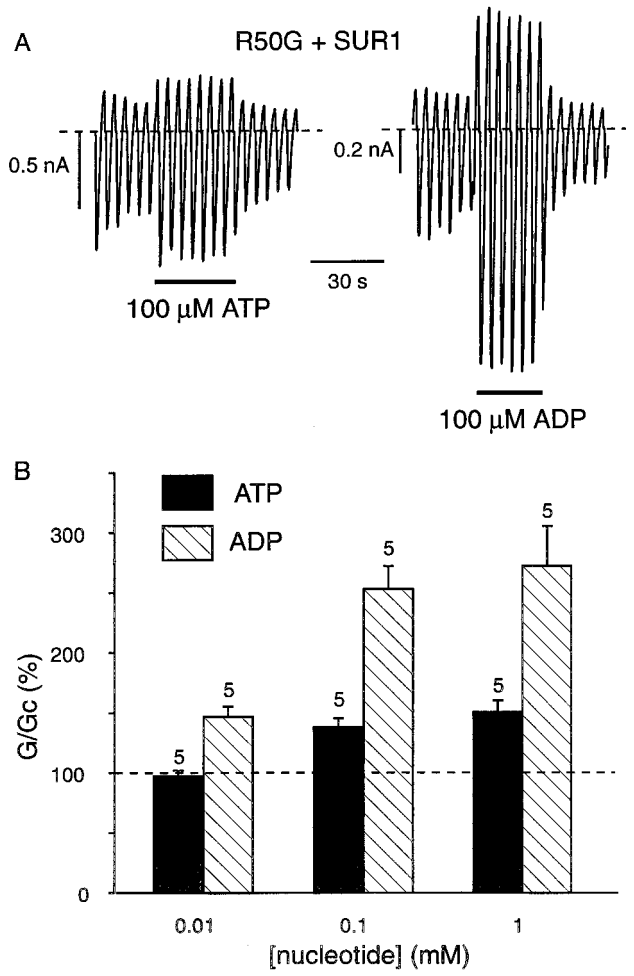

FIG. 3. (*A*) Macroscopic currents recorded from the same inside-out patch from an oocyte coinjected with Kir6.2-R50G and SUR1. Currents were elicited by a series of voltage ramps from −110 mV to +100 mV. ATP (100 μM) and ADP (100 μM) were added to the internal solution as indicated. Mg^{2+} (1.4 mM) was present throughout so ATP and ADP will exist as the Mg^{2+} salts. (*B*) Mean macroscopic slope conductance for Kir6.2-R50G/SUR1 currents, recorded in the presence of MgATP (solid bars) or MgADP (hatched bars), expressed as a percentage of the slope conductance in control solution (no additions). The dashed line indicates the conductance level in control solution. The number of oocytes is given above the bars.

normally is masked by an additional potent inhibitory effect of the nucleotide. In contrast, the inhibitory action of MgADP is apparent only at high nucleotide concentrations, and channel stimulation usually is observed at MgADP concentrations <1 mM. Several lines of evidence support the hypothesis that MgATP activates Kir6.2/SUR1 currents by interacting with the NBDs of SUR1. First, Mg^{2+} is without effect on Kir6.2ΔC36 currents, but causes an apparent reduction in ATP inhibition of Kir6.2/SUR1 currents (Fig. 1). Second, mutation of either the W_B aspartate residues, and/or the W_A lysines, in the NBDs of SUR1 enhanced the sensitivity of Kir6.2/SUR1 currents to inhibition by ATP (Fig. 2; ref. 15). Third, the inhibitory effect of 0.1 mM ATP on Kir6.2/SUR1 channels containing mutations in the NBDs of SUR1 was similar to that of the wild-type channel in the absence, but not the presence, of Mg^{2+} (16). Finally, MgATP activated K_{ATP} channels containing mutations in the Kir6.2 subunit, which impair ATP sensitivity, and this effect was abolished when additional mutations were made in the W_A lysine residues of SUR1 (Figs. 3 and 4).

Taken together, these results suggest that ATP, like ADP, has a dual regulatory effect on the K_{ATP} channel. It inhibits the

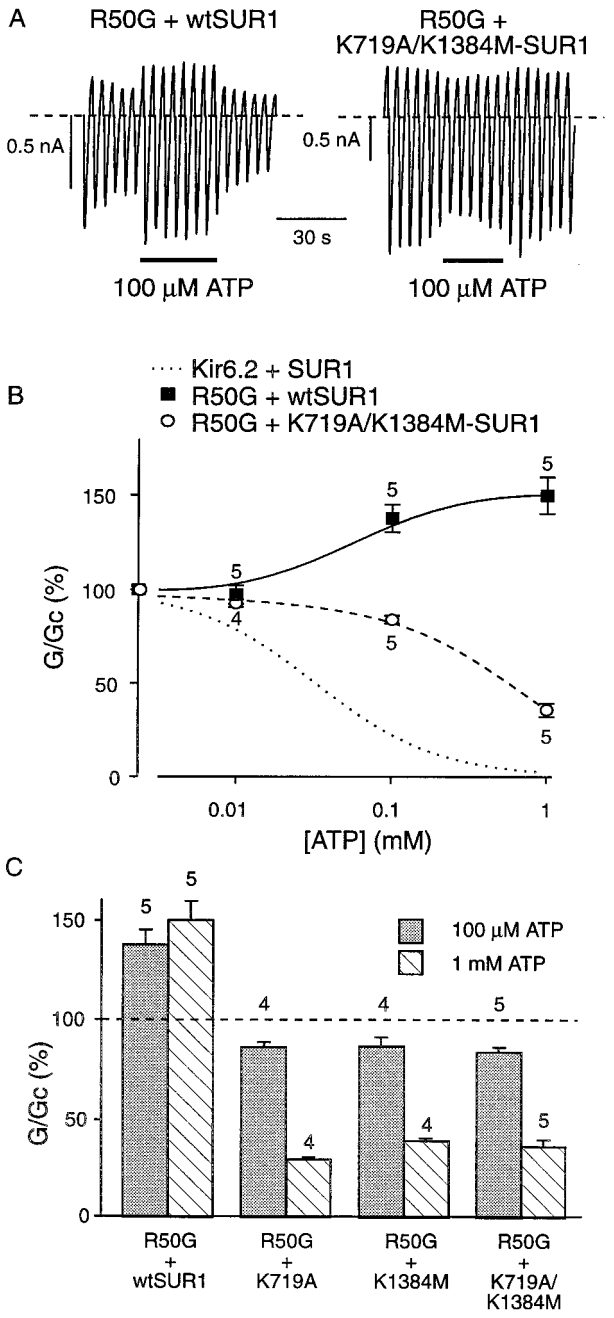

FIG. 4. (*A*) Macroscopic currents recorded from inside-out patches excised from oocytes coinjected with Kir6.2-R50G and either SUR1 or K719A/K1384M-SUR1 mRNAs. Currents were elicited by a series of voltage ramps from −110 mV to +100 mV. ATP (100 μM) was added to the internal solution as indicated. Mg^{2+} (1.4 mM) was present throughout so ATP will exist as the Mg^{2+} salt. (*B*) Mean ATP dose-response relationships for Kir6.2-R50G/SUR1 or Kir6.2-R50G/SUR1-K719A/K1384M currents, measured in the presence of Mg^{2+}. The slope conductance (G) is expressed as a percentage of the mean (G_c) of that obtained in control (ATP-free) solution. The lines are drawn through the points by eye, and the number of patches is indicated next to each data point. The dotted line indicates the effect of ATP on Kir6.2/SUR1 currents and is the same data as in Fig. 1*B*. (*C*) Mean macroscopic slope conductance recorded in the presence of 100 μM or 1 mM MgATP, expressed as a percentage of the slope conductance in the absence of ATP, for channels comprising Kir6.2-R50G and SUR1 containing the mutations indicated. The dashed line indicates the conductance level in control solution. The number of oocytes is given above the bars.

channel by binding to a site probably located on Kir6.2, in a reaction that is independent of Mg^{2+}. In addition, MgATP

stimulates channel activity by interaction with the NBDs of SUR1. This effect requires Mg^{2+}, but it is not clear whether the cation simply facilitates nucleotide binding or if it is required for hydrolysis of MgATP. Our results also suggest that MgATP and MgADP stimulate channel activity through the same pathway, because once Kir6.2-R50G/SUR1 channels had been maximally activated by MgADP they could not be further stimulated by MgATP.

Both NBDs are needed for channel activation by MgATP, because mutation of a single NBD was sufficient to cause a reduction in the ATP sensitivity of Kir6.2/SUR1 currents and to prevent MgATP-dependent activation of Kir6.2-R50G/SUR1 currents. Likewise, the potentiatory effects of MgADP require both NBDs (15, 16). The K_i for ATP inhibition of the W_B mutant channels was not as small as that observed for the wild-type K_{ATP} channel in the absence of Mg^{2+} ($P = 0.001$ by ANOVA). One explanation for this difference may be that the mutations substantially reduce, but do not completely abolish, MgATP binding (or hydrolysis) at the NBDs of SUR1.

Nucleotide binding to SUR1 has been demonstrated directly by Ueda and colleagues (29). They further showed that mutation of either the W_A lysine or the W_B aspartate in NBD1 impaired the binding of 8-azido-ATP, whereas the equivalent mutations in NBD2 did not. In contrast, our results suggest that both NBDs are required for channel activation by MgATP and that mutations in either NBD reduce the potentiatory effect of the nucleotide. It remains possible, however, that MgATP (rather than 8-azido-ATP) binds to NBD2 as well as to NBD1.

Our data also indicate that the ability of MgADP to activate the K_{ATP} channel is greater than that of MgATP. Similarly, MgGDP has been shown to be more effective than MgGTP (23). This suggests either that nucleotide diphosphates bind with higher affinity or that their binding is more effectively translated into changes in channel activity. The former idea is less likely because the greatest increase in activation occurs over the same concentration range (0.1–1 mM) for both MgATP and MgADP (Fig. 3*B*). One possible explanation of our findings is that the diphosphate is the effective ligand, and that MgATP must be hydrolyzed by the NBDs of SUR1 to MgADP before it is able to enhance channel activity. Because the NBDs would be occupied by MgADP for a smaller proportion of time in ATP solutions than in ADP solutions, this would result in an apparent lower efficacy of MgATP. One way to test this hypothesis would be to examine the effect of preventing ATP hydrolysis, which would be expected to abolish MgATP, but not MgADP, activation of the channel. This experiment is not straightforward, however, as operations designed to prevent nucleotide hydrolysis—such as removal of external Mg^{2+}, or the use of nonhydrolyzable ATP analogues—also may influence nucleotide binding. We also cannot be certain that mutation of the W_A lysines of SUR1, which are predicted to prevent ATP hydrolysis, do not also impair MgATP binding. Furthermore, it remains possible that MgADP may itself be hydrolyzed by SUR1.

Cardiac K_{ATP} channels differ from those of the β cell in that SUR2A, rather than SUR1, serves as the sulfonylurea receptor subunit (13, 30). It is therefore of interest that the apparent ATP sensitivity of K_{ATP} channels in cardiac myocytes is enhanced, rather than reduced, in the presence of Mg^{2+} (31, 32). Because Kir6.2 is believed to form the pore of both β cell and cardiac K_{ATP} channels (8, 9, 13), this provides further support for our hypothesis that the effect of Mg^{2+} on the ATP sensitivity of the β cell K_{ATP} channel is conferred by the sulfonylurea receptor subunit.

MgATP activation of the β cell K_{ATP} channel may be of physiological significance. Although MgADP is a more effective stimulator of the K_{ATP} channel than MgATP, it is also present at a much lower intracellular concentration. The average values for total ATP and ADP in β cells are estimated as $\approx$5 mM and $\approx$2 mM, respectively (33), but it is believed that most ADP is bound to cytosolic proteins and in β cells the free MgADP concentration may be <100 μM (34). In contrast, cytosolic levels of MgATP are much higher. This suggests that in the intact β cell MgATP may compete with MgADP for the nucleotide-binding sites on SUR1. The cytosolic concentrations of MgADP and MgATP also will depend on the intracellular Mg^{2+} concentration and a rise in Mg^{2+} therefore may enhance channel activation by both adenine nucleotides.

In conclusion, MgATP (like MgGTP, MgGDP, and MgADP) stimulates K_{ATP} channel activity by interaction with the NBDs of SUR1. This effect has not been recognized in previous studies because ATP, unlike GTP, also has a potent inhibitory effect. Our results provide additional support for the idea that the potentiatory and inhibitory sites have distinct nucleotide sensitivities and are located on different K_{ATP} channel subunits.

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