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The Nucleotide-Binding Domains of Sulfonylurea Receptor 2A and 2B Play Different Functional Roles in Nicorandil-Induced Activation of ATP-Sensitive K^+ Channels

Mitsuhiko Yamada and Yoshihisa Kurachi

Department of Pharmacology II, Graduate School of Medicine, Osaka University, Osaka, Japan

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ABSTRACT

Nicorandil activates ATP-sensitive K^+ channels composed of Kir6.2 and either sulfonylurea receptor (SUR) 2A or 2B. Although SUR2A and SUR2B differ only in their C-terminal 42 amino acids (C42) and possess identical drug receptors and nucleotide-binding domains (NBDs), nicorandil more potently activates SUR2B/Kir6.2 than SUR2A/Kir6.2 channels. Here, we analyzed the roles of NBDs in these channels' response to nicorandil with the inside-out configuration of the patch-clamp method. Binding and hydrolysis of nucleotides by NBDs were impaired by mutations in the Walker A motif of NBD1 (K708A) and NBD2 (K1349A) and in the Walker B motif of NBD2 (D1470N). Experiments were done with internal ATP (1 mM). In SUR2A/Kir6.2 channels, the K708A mutation abolished, and the K1349A but not D1470N mutation reduced the sensitivity to nicorandil. ADP (100 μM) significantly increased the wild-type channels' sensitivity to nicorandil, which was abolished by the K1349A or D1470N mutation. Thus, the SUR2A/Kir6.2 channels' response to nicorandil critically depends on ATP-NBD1 interaction and is facilitated by interactions of ATP or ADP with NBD2. In SUR2B/Kir6.2 channels, either the K708A or K1349A mutation partially suppressed the response to nicorandil, and double mutations abolished it. The D1470N mutation also significantly impaired the response. ADP did not sensitize the channels. Thus, NBD2 hydrolyzes ATP, and NBD1 and NBD2 equally contribute to the response by interacting with ATP and ADP, accounting for the higher nicorandil sensitivity of SUR2B/Kir6.2 than SUR2A/Kir6.2 channels in the presence of ATP alone. Thus, C42 modulates the interaction of both NBDs with intracellular nucleotides.

Nicorandil is a nicotinamide ester with vasodilatory and cardioprotective effects and clinically useful therapeutics for ischemic heart diseases (The IONA Study Group, 2002). Its main target is vascular nucleoside diphosphate-dependent K^+ channels and cardiac ATP-sensitive K^+ (K_{ATP}) channels (Yamada et al., 1999). These channels open under metabolic stress to regulate vascular tonus and suppress cardiac excitability, respectively. They are composed of a pore-forming subunit Kir6.x and its regulator, a sulfonylurea receptor (SUR) (Babenko et al., 1998; Seino, 1999). SUR is an ABC protein that allosterically induces pore opening by interacting with intracellular ATP and ADP with its two cytoplasmic nucleotide-binding domains (NBD1 and NBD2) (Ashcroft and Gribble, 1998) (Fig. 6A). There are two genes for SUR that encode SUR1 and SUR2 (Aguilar-Bryan et al., 1995; Inagaki et al., 1996) and two splice variants of SUR2 (SUR2A and SUR2B), which differ only in the C-terminal 42 amino acids (C42) (Isomoto et al., 1996). There are also two genes for Kir6.x that encode Kir6.1 and Kir6.2 (Inagaki et al., 1995a,b). Cardiac K_{ATP} channels are composed of SUR2A and Kir6.2 (Inagaki et al., 1996), whereas vascular nucleoside diphosphate-dependent K^+ channels are formed from SUR2B and Kir6.1 (Yamada et al., 1997; Miki et al., 2002).

SUR2A/Kir6.2 and SUR2B/Kir6.1 channels differ in single-channel conductance and spontaneous activity recorded in the absence of intracellular nucleotides caused by the different Kir6.x subunits (Yamada et al., 1997). However, both SUR2A and SUR2B form K_{ATP} channels with Kir6.2 with identical single-channel conductance and characteristic spontaneous activity, which is inhibited by intracellular ATP (Inagaki et al., 1996; Isomoto et al., 1996). This inhibition is a hallmark of K_{ATP} channels and arises from binding of ATP to Kir6.2 (Tucker et al., 1997; Trapp et al., 2003).

K^+ channel openers such as nicorandil bind to SUR and

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ABBREVIATIONS: K_{ATP} channels, ATP-sensitive K^+ channels; SUR, sulfonylurea receptor; C42, the C-terminal 42 amino acids of the sulfonylurea receptor; NBD, nucleotide-binding domain; R conformation, the conformation of SUR2 that is able to induce opening of the Kir6.2 channel pore; T conformation, the conformation of SUR2 that is unable to induce opening of the Kir6.2 channel pore; HEK, human embryonic kidney.

activate K_{ATP} channels in concert with intracellular nucleotides (Inagaki et al., 1996; Shyng et al., 1997; Schwanstecher et al., 1998; D'hahan et al., 1999; Hambrock et al., 1999; Ashcroft and Gribble, 2000; Bienengraeber et al., 2000; Moreau et al., 2000; Reimann et al., 2001). Although SUR2A and SUR2B share a common receptor site for nicorandil in their 17th transmembrane domain (Reimann et al., 2001) (Fig. 6A), nicorandil ~30 to 100 times more potently activates SUR2B/Kir6.2 than SUR2A/Kir6.2 channels (Shindo et al., 1998). The mechanism underlying this difference has not been clearly identified (Reimann et al., 2001).

In this study, we examined the effects of the point mutations in NBDs that inhibit binding and hydrolysis of nucleotides on nicorandil-induced activation of K_{ATP} channels containing SUR2. We found that NBDs play very different roles in SUR2A and SUR2B. Because the amino acid sequences of the NBDs and the nicorandil-binding site are identical in SUR2A and SUR2B, their different reactions must reside in the functional roles of C42.

Materials and Methods

Site-Directed Mutagenesis and Expression of Recombinant ATP-Sensitive K^+ Channels. Site-directed mutagenesis was carried out on cDNAs of mouse SUR2A and SUR2B subcloned into the expression vector pcDNA3 with QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). Human embryonic kidney (HEK) 293T cells were cotransfected with either pcDNA3 containing mouse Sur2, Kir6.2, or pd2EGFP (BD Biosciences Clontech, Palo Alto, CA) by using LipofectAMINE (Invitrogen, Carlsbad, CA) according to the manufacturer's instruction as described previously (Matsushita et al., 2002). The cells that expressed green fluorescent protein were identified by fluorescence microscopy and were used for electrophysiology.

Electrophysiology. ATP-sensitive K^+ channels expressed in HEK 293T cells were analyzed with the inside-out configuration of the patch-clamp method as described previously (Matsushita et al., 2002). The pipette solution contained 140 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 5 mM HEPES, pH adjusted to 7.3 with KOH. Unless otherwise indicated, the internal side of inside-out patch membranes was perfused with internal solution containing 140 mM KCl, 5 mM EGTA, 2 mM $MgCl_2$, and 5 mM HEPES, pH adjusted to 7.3 with KOH, in which free Mg^{2+} concentration was 1.4 mM. When ATP or ADP was added to the internal solution, $MgCl_2$ was added to maintain this free Mg^{2+} concentration. To remove Mg^{2+} from the internal solution, $MgCl_2$ was omitted, and EGTA was replaced with equimolar EDTA. Nicorandil was a gift from Chugai Pharmaceutical Co. Ltd. (Tokyo, Japan). It was dissolved at 2 M in glacial acetic acid and diluted before use to the desired concentration in internal solution. When nicorandil was used at final concentration of 3 and 10 mM, ~25 and ~70 mM of KOH was required to adjust the pH to 7.3, respectively. In these experiments, the appropriate amount of KCl was removed from the internal solution, and ATP and ADP were added to these solutions after adjustment of pH to prevent hydrolysis of the nucleotides at acidic pH. Then, the pH was readjusted. Acetic acid alone did not significantly affect ATP-sensitive K^+ channels at a concentration (~85 mM) equivalent to that used in the solution containing 10 mM nicorandil.

Statistical Analysis. All statistical values are presented as mean ± S. E. The statistical difference was evaluated by Student's t test. For multiple comparison between pairs, difference was assessed with analysis of variance and the Bonferroni method. A value of $p < 0.05$ was considered statistically significant.

Results

Effect of the Walker A Lysine Mutation on Nicorandil-Induced Activation of SUR2A/Kir6.2 Channels in the Presence of ATP Alone. We analyzed the concentration-dependent effect of nicorandil on SUR2A/Kir6.2 K_{ATP} channels expressed in HEK 293T cells (Fig. 1). K_{ATP} channel currents were measured in inside-out membrane patches voltage-clamped at −60 mV with the symmetrical ~140 mM K^+. In the presence of 1 mM intracellular ATP, nicorandil activated wild-type SUR2A/Kir6.2 channels in a concentration-dependent manner over the range of 10 μM to 10 mM (Fig. 1, Aa and B). This concentration-response relationship was fit with the following Hill equation:

$$y = a + b/(1 + (K_d / [Nico])^{n_H}) \quad (1)$$

where y is the channel activity normalized to the maximum activity recorded in the absence of ATP (relative channel activity), a is a basal relative channel activity in the absence of nicorandil, b is the maximum relative channel activity induced by nicorandil; K_d is the dissociation constant, [*Nico*] is the concentration of nicorandil in molar equivalents, and n_H is the Hill coefficient. The a, b, K_d, and n_H values were estimated as 0.01, 0.98, 1.65 mM, and 0.63, respectively (Table 1). The effect of nicorandil was sometimes biphasic with a rapid increase in current followed by a slow increase that did not reach stationarity within the time of the application (Fig. 1, Aa and Ac). We estimated the error on the measurements caused by this phenomenon by fitting the time-dependent change in the current amplitude with a single or double exponential function. By comparing the asymptote of the function and the measured current amplitude, we estimate that the error occurring when the biphasic response appeared was 5.4 ± 1.3%.

When an invariant lysine in the Walker A motif of NBD1 was substituted with alanine (K708A), the response of SUR2A/Kir6.2 channels to nicorandil was completely abolished (Fig. 1, Ab and B). The corresponding mutation in NBD2 (K1349A), however, partially suppressed the response (Fig. 1, Ac and B). These mutations have been shown to impair nucleotide binding and hydrolysis at the corresponding NBD of SUR (Ueda et al., 1997, 1999; Bienengraeber et al., 2000). The relationship between concentrations of nicorandil and SUR2A(K1349A)/Kir6.2 channel activity was fit with eq. 1. The a, b, K_d, and h values were estimated as 0.01, 0.50, 1.78 mM, and 2.63, respectively (Table 1). SUR2A carrying both mutations [SUR2A(K708A, K1349A)] formed with Kir6.2 K_{ATP} channels that were not responsive to nicorandil (Fig. 1, Ad and B). These results indicate that ATP binding to NBD1 is essential for nicorandil to activate SUR2A/Kir6.2 channels, whereas binding and/or hydrolysis of ATP at NBD2 modestly increases the response of SUR2A/Kir6.2 channels to the agent.

Effect of the Walker A Lysine Mutation on Nicorandil-Induced Activation of SUR2A/Kir6.2 Channels in the Presence of ATP and ADP. Nicorandil activated wild-type SUR2A/Kir6.2 channels more potently in the presence of ATP (1 mM) and ADP (100 μM) than ATP (1 mM) alone (Fig. 2, Aa and B) as reported previously with native cardiac K_{ATP} channels (Shen et al., 1991). The concentration-response relationship was fit with eq. 1 with a, b, K_d, and h values of 0.08, 0.79, 80.2 μM and 0.85, respectively (Table 1). Again,

SUR2A(K708A)/Kir6.2 and SUR2A(K708A, K1349A)/Kir6.2 channels did not respond to nicorandil (Fig. 2, Ab, Ad, and B). SUR2A(K1349A)/Kir6.2 channels showed an impaired response to nicorandil (Fig. 2, Ac and B). The concentration-response relationship was fit with eq. 1 with a, b, K_d, and h values of 0.04, 0.39, 1.58 mM, and 1.78, respectively (Table 1). The relative channel activity of SUR2A(K1349A)/Kir6.2 channels at each concentration of nicorandil was not significantly different in the presence and absence of ADP (Figs. 1B and 2B). Therefore, ADP seems to potentiate the response of wild-type SUR2A/Kir6.2 channels to nicorandil mainly through NBD2.

Effect of the Walker A Lysine Mutation on Nicorandil-Induced Activation of SUR2B/Kir6.2 Channels in the Presence of ATP Alone. Spontaneous activity of wild-type SUR2B/Kir6.2 channels was almost completely inhibited by 1 mM Mg-free ATP (ATP^{4-}), whereas in the presence of MgATP, their activity gradually increased (Fig. 3Aa) (Reimann et al., 2000). On average, this basal activity reached a steady-state value of 0.30 ± 0.05 (n = 8). This value was significantly higher than that for SUR2A/Kir6.2 channels in the presence of ATP (1 mM) alone (0.02 ± 0.01, $p = 4.97 \times 10^{-9}$, n = 20) or in the presence of ATP (1 mM) and ADP (100 μM) (0.09 ± 0.03, p = 0.001, n = 7). Nicorandil further increased the activity of wild-type SUR2B/Kir6.2 channels in a concentration-dependent manner over the range of 1 μM to 10 mM (Fig. 3B). Fitting the concentration-response relationship with eq. 1 yielded a, b, K_d, and h values of 0.26, 0.64, 23.4 μM, and 0.47, respectively (Table 1). Thus, nicorandil much more potently activated wild-type SUR2B/Kir6.2 channels than SUR2A/Kir6.2 channels in the presence of ATP (1 mM) alone as reported previously (Shindo et al., 1998).

Either SUR2B(K708A), SUR2B(K1349A), or SUR2B(K708A, K1349A) with Kir6.2 formed K_{ATP} channels with much lower basal activity than wild-type SUR2B/Kir6.2 channels [0.007 ± 0.004 ($p = 3.6 \times 10^{-4}$, n = 12), 0.002 ± 0.002 ($p = 4.0 \times 10^{-4}$, n = 5), and 0.001 ± 0.0002 ($p = 7.0 \times 10^{-3}$, n =

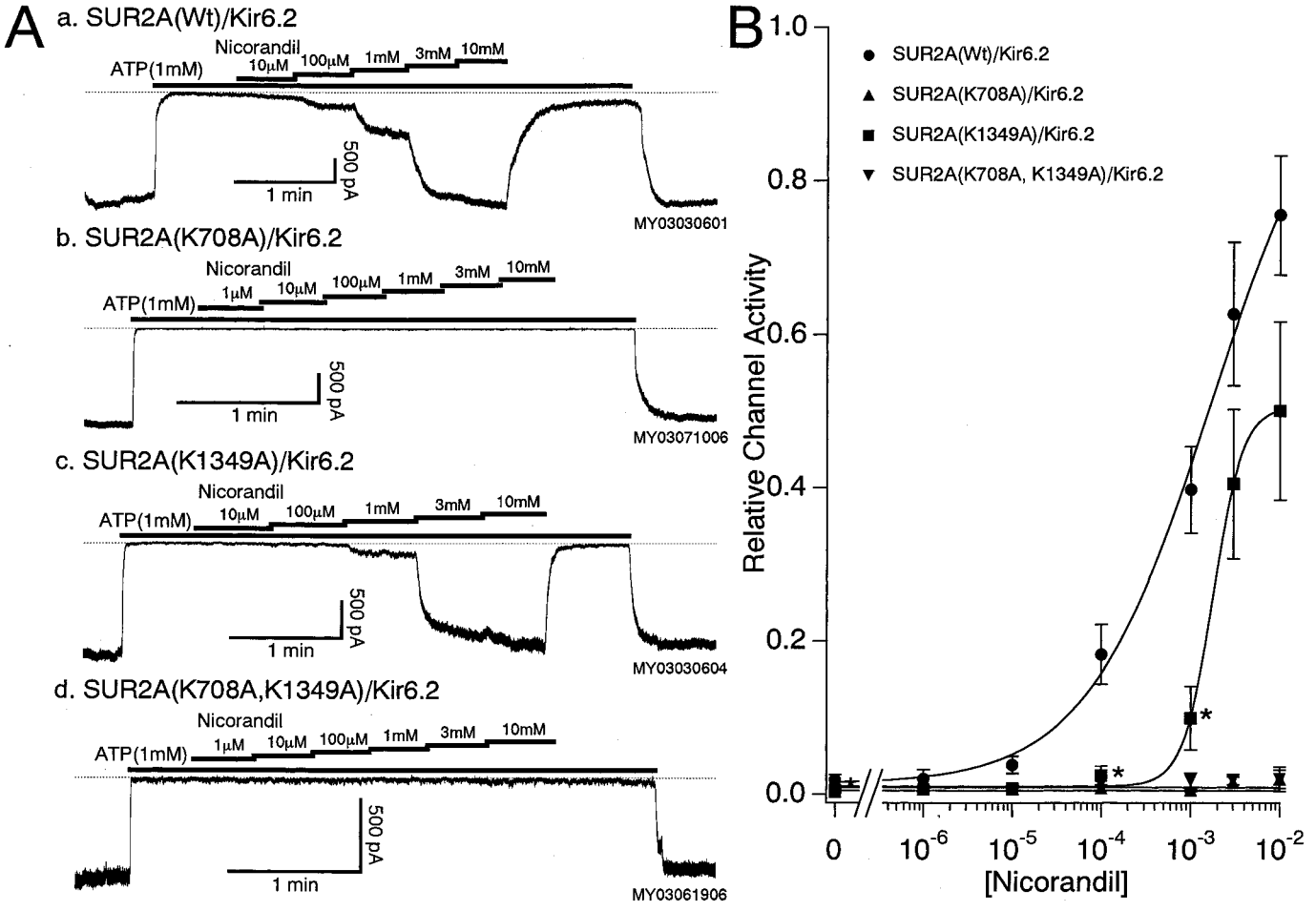

Fig. 1. Effect of the Walker A mutation on concentration-dependent activation of SUR2A/Kir6.2 channels by nicorandil recorded in the presence of MgATP. A, representative current traces showing the effect of nicorandil in the presence of 1 mM ATP on K_{ATP} channels composed of Kir6.2 and either wild-type SUR2A (a), SUR2A whose invariant lysine in the Walker A motif of NBD1 was substituted with alanine [SUR2A(K708A)] (b), SUR2A whose invariant lysine in the Walker A motif of NBD2 was substituted with alanine [SUR2A(K1349A)] (c), or SUR2A carrying both mutations [SUR2A(K708A, K1349A)] (d). The currents were measured at −60 mV in symmetrical ~140 mM KCl in inside-out membrane patches. The zero current level is indicated by dotted lines. Perfusion protocol is indicated by bars above the current traces. B, concentration-response relationships for the effect of nicorandil on wild-type SUR2A/Kir6.2 (●) (n = 15–20), SUR2A(K708A)/Kir6.2 (▲) (n = 10), SUR2A(K1349A)/Kir6.2 (■) (n = 8–12), and SUR2A(K708A, K1349A)/Kir6.2 (▼) channels (n = 6). The ordinate indicates channel activity normalized to that recorded in the absence of ATP (relative channel activity). Symbols and bars represent the mean and S.E., respectively. Lines indicate the fit of the data with eq. 1 in the text. ⋆, significantly different from corresponding wild-type SUR2A/Kir6.2 channel current amplitude. The amplitudes of SUR2A(K708A)/Kir6.2 or SUR2A(K708A, K1349A)/Kir6.2 currents were significantly different from that of wild-type SUR2A/Kir6.2 currents at every concentration.

TABLE 1

Parameters used for fitting of the concentration-response relationships

The concentration-response relationships of the K_{ATP} channels composed of Kir6.2 and SUR2 with or without different mutations (Figs. 1–5) were fit with eq. 1 with the parameters listed.

Nucleotides[a] and Types of SUR2	*a*	*b*	K_d	n_H
			mM	
ATP (1 mM)				
SUR2A(wt)	0.01	0.98	1.65	0.63
SUR2A(K1349A)	0.01	0.50	1.78	2.63
SUR2A(D1470N)	0.02	0.80	0.34	0.57
SUR2B(wt)	0.26	0.64	0.02	0.47
SUR2B(K708A)	0.01	0.60	0.67	0.96
SUR2B(K1349A)	0.01	0.40	1.70	1.07
SUR2B(D1470N)	0.05	0.37	0.79	1.19
ATP (1 mM) +ADP (100 μM)				
SUR2A(wt)	0.08	0.79	0.08	0.85
SUR2A(K1349A)	0.04	0.39	1.58	1.78
SUR2A(D1470N)	0.07	0.78	0.40	0.57
SUR2B(wt)	0.57	0.38	0.18	0.41
SUR2B(K708A)	0.03	0.33	1.03	1.61
SUR2B(K1349A)	0.05	0.63	0.49	0.90
SUR2B(D1470N)	0.05	0.38	0.50	1.22

wt, wild type.

[a] Nucleotides added to the internal side of the inside-out patch membranes.

3), respectively]. The K708A and K1349A mutations also almost equally impaired the response of SUR2B/Kir6.2 channels to nicorandil. The concentration-response relationships were fit with eq. 1 with a, b, K_d, and h values of 0.01, 0.60, 672 μM, and 0.96 for SUR2B(K708A)/Kir6.2 channels and 0.01, 0.40, 1.70 mM, and 1.07 for SUR2B(K1349A)/Kir6.2 channels, respectively (Table 1). Thus, although ATP-binding to either NBD1 or NBD2 can independently support the channels' response to nicorandil, the two reactions seem to cooperatively form the basal and nicorandil-induced activity of wild-type SUR2B/Kir6.2 channels.

Effect of the Walker A Lysine Mutation on Nicorandil-Induced Activation of SUR2B/Kir6.2 Channels in the Presence of ATP and ADP. When ADP (100 μM) was added to ATP (1 mM), the basal activity of wild-type SUR2B/Kir6.2 channels was further increased to 0.58 ± 0.07 (p = 0.006 versus that in the presence of 1 mM ATP alone). Under this condition, nicorandil again increased channel activity in a concentration-dependent manner. This relationship was fit with eq. 1 with a, b, K_d, and h values of 0.57, 0.38, 181 μM, and 0.41, respectively (Table 1). Thus, in contrast to SUR2A/

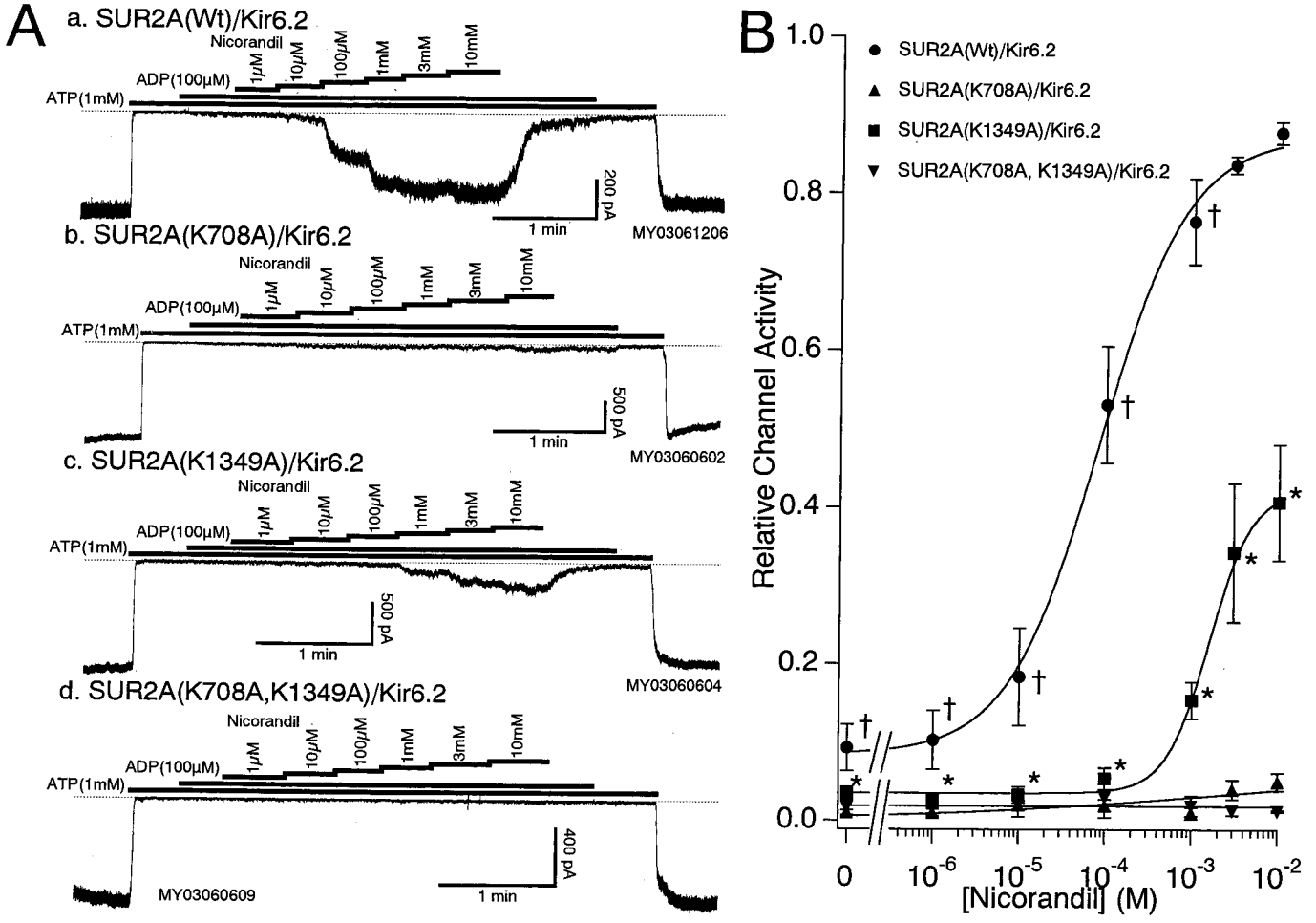

Fig. 2. Effect of the Walker A mutation on concentration-dependent activation of SUR2A/Kir6.2 channels by nicorandil recorded in the presence of ATP and ADP. A, representative current traces showing the effect of nicorandil in the presence of 1 mM ATP and 100 μM ADP on K_{ATP} channels composed of Kir6.2 and either wild-type SUR2A (a), SUR2A(K708A) (b), SUR2A(K1349A) (c), or SUR2A(K708A, K1349A) (d). B, concentration-response relationships for the effect of nicorandil on wild-type SUR2A/Kir6.2 (●) (n = 7), SUR2A(K708A)/Kir6.2 (▲) (n = 8), SUR2A(K1349A)/Kir6.2 (■) (n = 18–21), and SUR2A(K708A, K1349A)/Kir6.2 (▼) channels (n = 3). All data were fit with eq. 1. ⋆, significantly different from corresponding wild-type SUR2A/Kir6.2 channel current amplitude. The amplitudes of SUR2A(K708A)/Kir6.2 or SUR2A(K708A, K1349A)/Kir6.2 currents were significantly different from that of wild-type SUR2A/Kir6.2 currents at every concentration. †, significantly different from corresponding current amplitudes measured in the presence of ATP alone (Fig. 1B).

Kir6.2 channels, SUR2B/Kir6.2 channels were not sensitized but rather were slightly desensitized to nicorandil by ADP.

Each of the K708A, K1349A, and K708A plus K1349A mutations greatly reduced the basal activity of SUR2B/Kir6.2 channels in ATP and ADP to 0.02 ± 0.01 ($p = 4.8 \times 10^{-8}$, $n = 8$), 0.04 ± 0.01 ($p = 1.1 \times 10^{-5}$, $n = 6$) and 0.02 ± 0.002 ($p = 6.7 \times 10^{-3}$, $n = 4$), respectively. As was the case in the presence of ATP (1 mM) alone, the K708A and K1349A mutations almost equally impaired the effect of nicorandil on SUR2B/Kir6.2 channels. The concentration-response relationships were fit with eq. 1 with a, b, K_d, and h values of 0.03, 0.33, 1.03 mM, and 1.61 for SUR2B(K708A)/Kir6.2 channels and 0.05, 0.63, 493 μM, and 0.90 for SUR2B(K1349A)/Kir6.2 channels, respectively (Table 1). Therefore, both in the presence of ATP alone and ATP plus ADP, NBD1 and NBD2 seem to equally and cooperatively support the response of SUR2B/Kir6.2 channels to nicorandil. The effect of nicorandil between 10 μM and 1 mM on SUR2B(K1439A)/Kir6.2 channels was significantly larger in the presence of ATP plus ADP than with ATP alone, indicating that in SUR2B, theADP-NBD1 interaction may more efficiently support the action of nicorandil than the ATP-NBD1 interaction.

Effect of a Mutation in the Walker B Motif of NBD2 on Nicorandil-Induced Activation of SUR2A/Kir6.2 and SUR2B/Kir6.2 Channels in the Presence of ATP Alone or ATP and ADP. We finally examined the effect of nicorandil on K_{ATP} channels containing SUR2A whose invariant aspartate at 1470 at the C-terminal end of the Walker B motif in NBD2 was substituted with aspargine [SUR2A(D1470N)]. This mutation has been shown to impair hydrolysis of ATP at NBD2 (Bienengraeber et al., 2000). Nicorandil concentration-dependently activated SUR2A(D1470N)/Kir6.2 channels in the presence of ATP alone (Fig. 5Aa). The amplitude of the response of SUR2A(D1470N)/Kir6.2 channels to each concentration of nicorandil was not significantly different from that of the wild-type SUR2A/Kir6.2 channels (Fig. 5Ac). ADP (100 μM) did not significantly increase the potency of nicorandil (Fig. 5, Ab and Ac). The fitting of the concentration-response relationships with eq. 1 yielded a, b, K_d, and h values of 0.02, 0.80, 340 μM, and

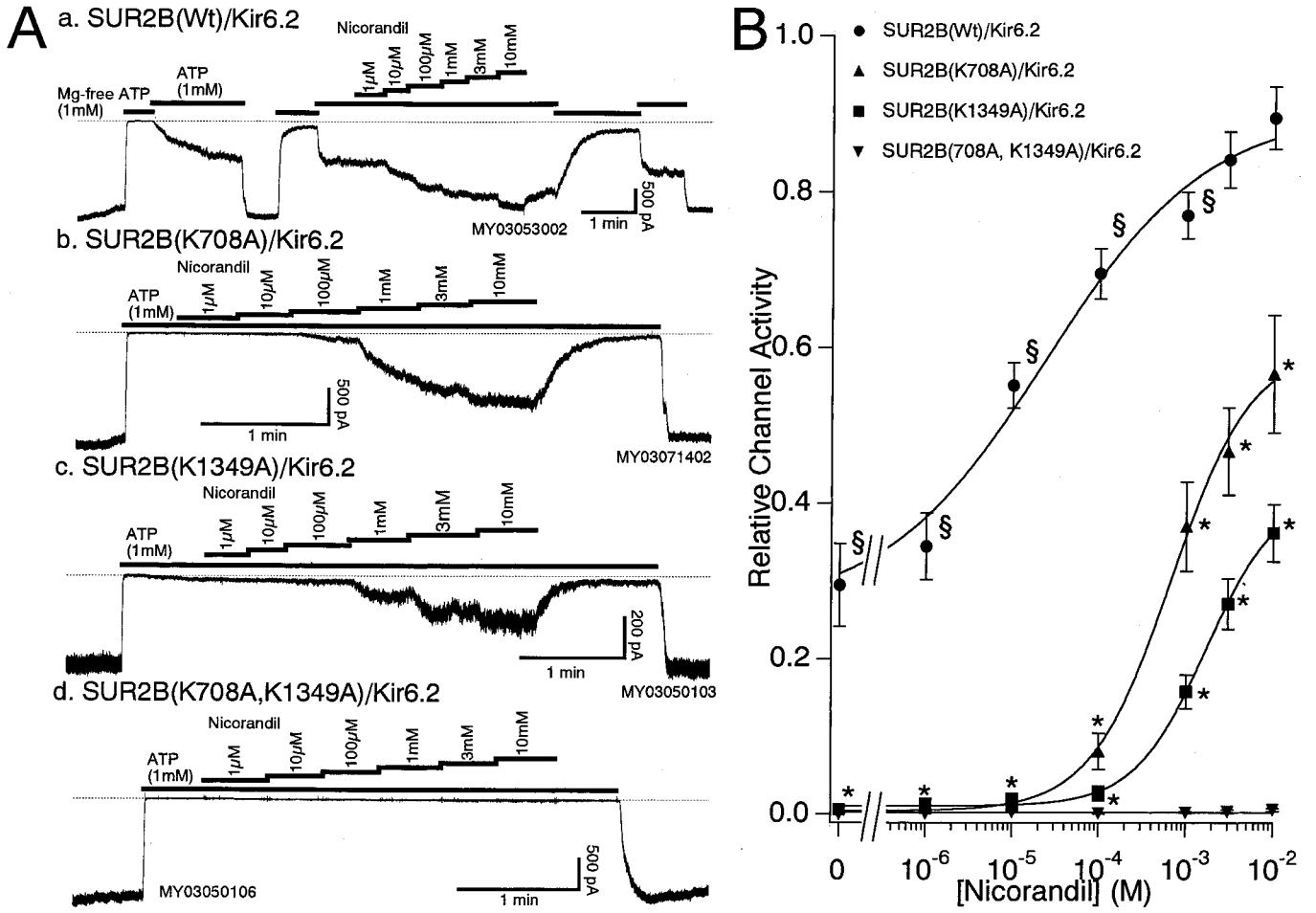

Fig. 3. Effect of the Walker A mutation on concentration-dependent activation of SUR2B/Kir6.2 channels by nicorandil recorded in the presence of ATP. A, representative current traces showing the effect of nicorandil in the presence of 1 mM ATP on the K_{ATP} channels composed of Kir6.2 and either wild-type SUR2B (a), SUR2B whose invariant lysine in the Walker A motif of NBD1 was substituted with alanine [SUR2B(K708A)] (b), SUR2B whose invariant lysine in the Walker A motif of NBD2 was substituted with alanine [SUR2B(K1349A)] (c), or SUR2B carrying both mutations [SUR2B(K708A, K1349A)] (d). B, concentration-response relationships for the effect of nicorandil on wild-type SUR2B/Kir6.2 (●) ($n = 7$), SUR2B(K708A)/Kir6.2 (▲) ($n = 6$), SUR2B(K1349A)/Kir6.2 (■) ($n = 5$), and SUR2B(K708A, K1349A)/Kir6.2 (▼) channels ($n = 3$). All data were fit with eq. 1. ⋆, significantly different from corresponding wild-type SUR2B/Kir6.2 channel current amplitude. The amplitude of SUR2B(K708A, K1349A)/Kir6.2 currents was significantly different from that of wild-type SUR2B/Kir6.2 currents at every concentration. §, significantly different from corresponding wild-type SUR2A/Kir6.2 current amplitudes in the presence of ATP alone (Fig. 1B).

0.57 in the presence of ATP (1 mM) alone and 0.07, 0.78, 403 μM, and 0.57 in the presence of ATP (1 mM) and ADP (100 μM), respectively (Table 1). Thus, this mutation seems to impair ADP-NBD2 interaction. This is consistent with the notion that ADP sensitizes SUR2A/Kir6.2 channels to nicorandil through NBD2 (Fig. 2). This result further suggests that the ADP-NBD2 interaction hardly participates in the response of wild-type SUR2A/Kir6.2 channels to nicorandil when this is recorded in the presence of ATP alone.

In contrast, the same mutation strongly suppressed the basal and nicorandil-induced activities of SUR2B/Kir6.2 channels both in the presence of ATP (1 mM) alone and ATP (1 mM) plus ADP (100 μM) (Fig. 5B). The fitting of the concentration-response relationships with eq. 1 yielded a, b, K_d, and h values of 0.05, 0.37, 792 μM, and 1.19 in the presence of ATP (1 mM) alone and 0.05, 0.38, 497 μM, and 1.22 in the presence of ATP (1 mM) and ADP (100 μM), respectively (Table 1). Thus, it is likely that ATP hydrolysis and ADP binding at NBD2 are critical for the basal and nicorandil-induced activity of wild-type SUR2B/Kir6.2 but not SUR2A/Kir6.2 channels.

Discussion

Our major finding is that the functional roles of NBDs in nicorandil-induced activation of K_{ATP} channels are very different between SUR2A and SUR2B, although their amino acid sequences are identical. Because the C42 are the only difference between SUR2A and SUR2B, this part of the protein is likely to be responsible for the difference in behavior (Fig. 6A).

To consider the functional difference between SUR2A and SUR2B in more detail and thereby delineate the role of C42, we evaluated our allosteric model of SUR2 (Fig. 6B). A K_{ATP} channel is a hetero-octamer composed of four SUR and four Kir6.2 subunits (Clement et al., 1997; Shyng and Nichols, 1997). Physiologically, SUR regulates the Kir6.2 channel pore in response to intracellular nucleotides (Ashcroft and Gribble, 1998). It is plausible that this reaction arises from a conformational change of SUR (Monod et al., 1965; Yuan et al., 2001; Jones and George, 2002; Smith et al., 2002; Chang, 2003). For the sake of simplicity, we assume that SUR2 can adopt two distinct conformations which are able (R conformation) and unable (T conformation) to induce pore opening

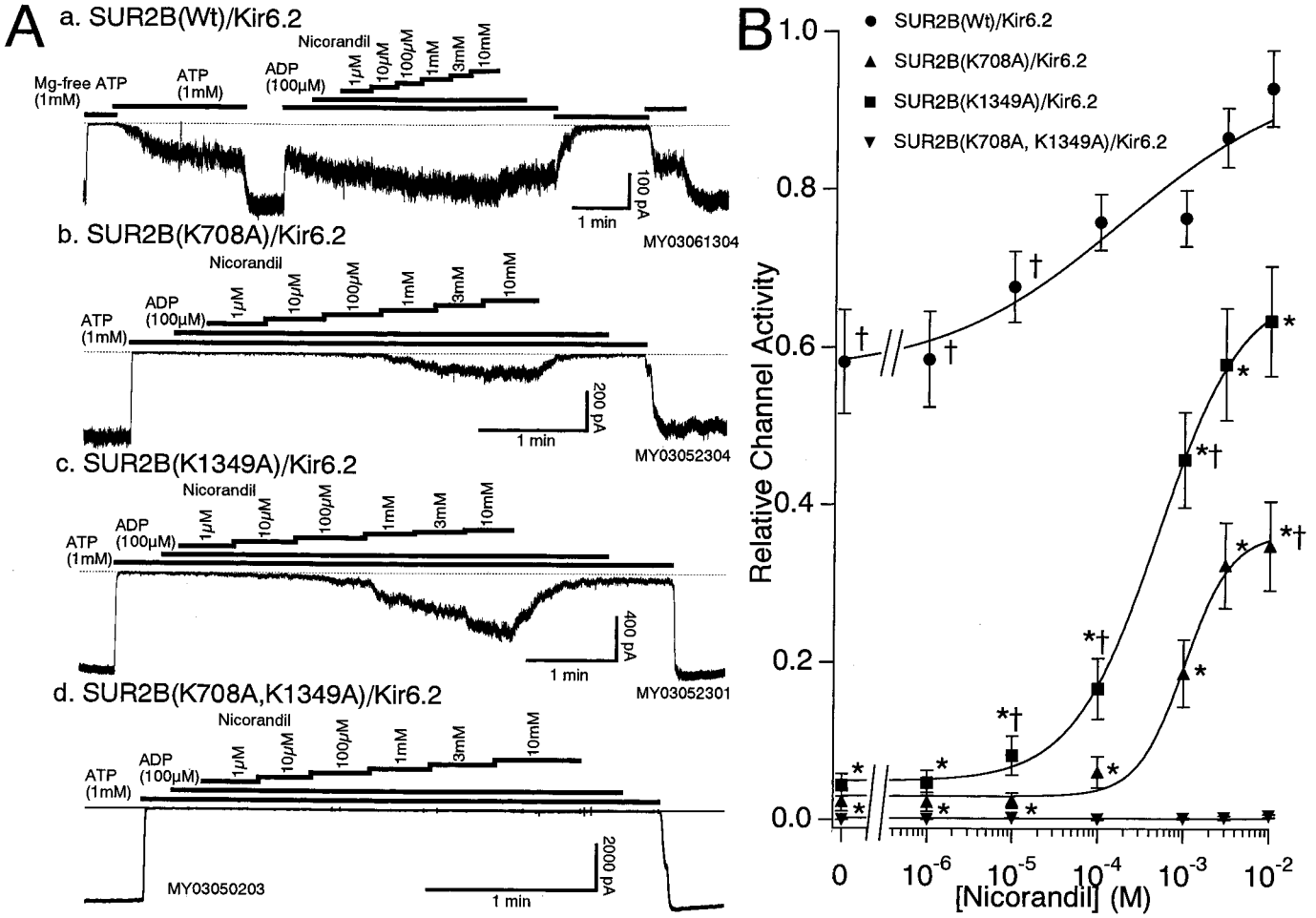

Fig. 4. Effect of the Walker A mutation on concentration-dependent activation of SUR2B/Kir6.2 channels by nicorandil recorded in the presence of ATP and ADP. A, representative current traces showing the effect of nicorandil in the presence of 1 mM ATP and 100 μM ADP on K_{ATP} channels composed of Kir6.2 and either wild-type SUR2B (a), SUR2B(K708A) (b), SUR2B(K1349A) (c), or SUR2B(K708A, K1349A) (d). B, concentration-response relationship for the effect of nicorandil on wild-type SUR2B/Kir6.2 (●) (n = 5), SUR2B(K708A)/Kir6.2 (▲) (n = 8), SUR2B(K1349A)/Kir6.2 (■) (n = 6), and SUR2B(K708A, K1349A)/Kir6.2 (▼) channels (n = 3). All data were fit with eq. 1. ⋆, significantly different from corresponding wild-type SUR2B/Kir6.2 channel current amplitude. The amplitude of SUR2B(K708A, K1349A)/Kir6.2 currents was significantly different from that of wild-type SUR2B/Kir6.2 currents at every concentration. †, significantly different from corresponding current amplitudes measured in the presence of ATP alone (Fig. 3B).

and that they are in equilibrium determined by the allosteric constant L (Monod et al., 1965).

ATP and ADP activate K_{ATP} channels through NBDs, whereas nicorandil does so through its receptor in the 17th transmembrane domain of SUR2 (Fig. 6A) (Moreau et al., 2000; Reimann et al., 2001). Thus, they may preferentially bind to their receptors in the R conformation, thereby shifting the equilibrium toward this conformation. The premise of this hypothesis is that the conformational change affects the structure of NBDs and the drug receptor and thus their sensitivities to the ligands (Schwanstecher et al., 1998; Hambrock et al., 1999; Ashcroft and Gribble, 2000; Jones and George, 2002).

If nucleotides and nicorandil displace the same equilibrium through distinct receptors, they should cause the heterotropic effect on each other (Monod et al., 1965). Neither SUR2A(K708A, K1349A)/Kir6.2 nor SUR2B(K708A, K1349A)/Kir6.2 channels responded to nicorandil (Figs. 1–4). This result can be interpreted as indicating that in the absence of nucleotide-NBD interactions, T is so strongly favored that up to 10 mM nicorandil cannot significantly shift the equilibrium toward R. The biphasic response of SUR2/Kir6.2 channels may also indicate that nicorandil and nucleotides cooperatively regulate the same equilibrium with distinct kinetics.

Allosteric Interaction Between Nucleotides and Nicorandil in SUR2A. Figure 1B indicates that ATP binding to NBD1 more efficiently promotes the T to R conformational change of SUR2A than ATP or ADP binding to NBD2. ADP significantly increased the basal activity and the sensitivity to nicorandil of the wild-type channels (Fig. 2B), indicating that ADP promotes the T-R transition. Under this condition, the nucleotide-NBD1 interaction was again critical for the channels' response to nicorandil. The effect of nicorandil on SUR2A(K1349A)/Kir6.2 channels was not significantly different in the presence and absence of ADP (Figs. 1B and 2B). Thus, ADP would facilitate the T-R transition by mainly acting through NBD2, which is consistent with the observation that the D1470N mutation abolished ADP-induced sensitization (Fig. 5Ac). The nonsignificant effect of D1470N mutation on the response of SUR2A/Kir6.2 channels to nicorandil in the presence of ATP alone (Fig. 5Ac) indicates that

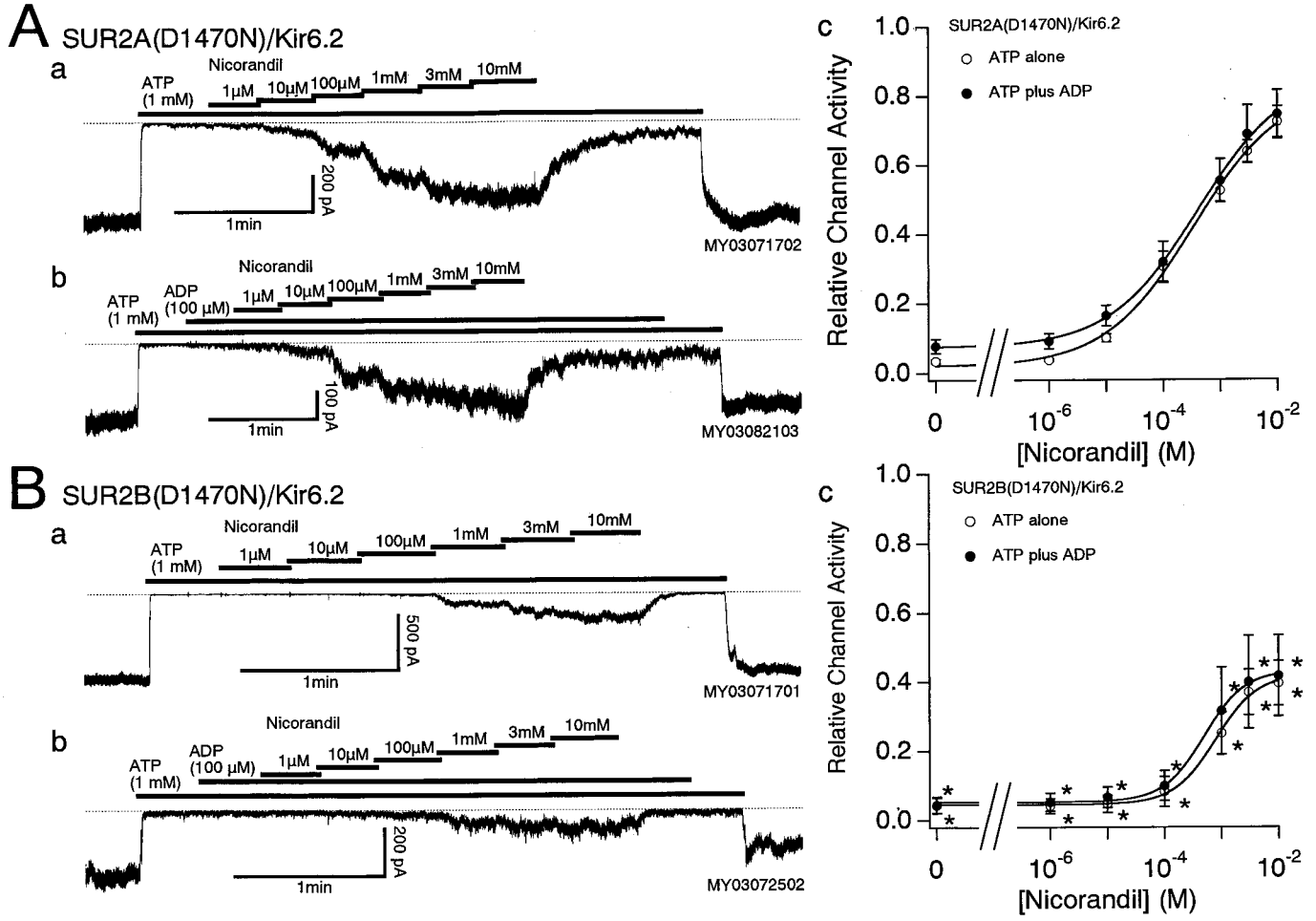

Fig. 5. Effect of the Walker B mutation on concentration-dependent activation of SUR2A/Kir6.2 and SUR2B/Kir6.2 channels by nicorandil recorded in the presence of ATP alone or ATP plus ADP. A, effect of nicorandil on K_{ATP} channels composed of Kir6.2 and SUR2A whose invariant aspartate in the Walker B motif was substituted with aspargine [SUR2A(D1470)] in the presence of 1 mM ATP alone (a) and 1 mM ATP plus 100 μM ADP (b). c, concentration-response relationship for the effect of nicorandil on SUR2A(D1470N)/Kir6.2 channels in the presence of ATP alone (○) (n = 6) or ATP plus ADP (●) (n = 6). B, effect of nicorandil on K_{ATP} channels composed of Kir6.2 and SUR2B whose invariant aspartate in the Walker B motif was substituted with aspargine [SUR2B(D1470N)] in the presence of 1 mM MgATP alone (a) and 1 mM MgATP plus 100 μM MgADP (b). c, concentration-response relationship for the effect of nicorandil on SUR2B(D1470N)/Kir6.2 channels in the presence of ATP alone (○) (n = 6) or ATP plus ADP (●) (n = 6). All data were fit with eq. 1. ⋆, significantly different from corresponding wild-type SUR2B/Kir6.2 channel current amplitude.

the ADP-NBD2 interaction does not significantly participate in the conformation change of SUR2A. In other words, hydrolysis of ATP at NBD2 of SUR2A is not significant. Therefore, in the presence of the interaction ATP-NBD1, the T-R transition of SUR2A is facilitated by the interaction ATP-NBD2 in the presence of ATP alone but more efficiently by the interaction of ADP-NBD2 in the presence of ATP plus ADP.

Reimann et al. (2001) reported that ADP did not significantly augment the effect of nicorandil on SUR2A/Kir6.2 channels. However, their representative current traces clearly show a larger response to nicorandil in the presence of ATP plus ADP than with ATP alone. The discrepancy might have occurred because they normalized the current recorded in the presence of nicorandil plus nucleotides to that recorded in the presence of nucleotides alone. The larger basal current recorded in the presence of ATP plus ADP than in ATP alone may then have arithmetically negated the effect of ADP on the nicorandil-induced response.

Allosteric Interaction between Nucleotides and Nicorandil in Nucleotides in SUR2B. The large basal activity of wild-type SUR2B/Kir6.2 channels seems to result from ATP hydrolysis because it did not exist in the absence of Mg^{2+} and built up over several minutes after the addition of Mg^{2+} (Fig. 3Aa) (Bienengraeber et al., 2000; Reimann et al., 2000). NBD2 has higher ATPase activity than NBD1 (Bienengraeber et al., 2000; Matsuo et al., 2000), which is consistent with significantly lower basal activity of SUR2B(K1349A)/Kir6.2 and SUR2B(D1470N)/Kir6.2 channels than wild-type SUR2B/Kir6.2 channels (Figs. 3B and 5B). In this case, SUR2B may have higher probability of R than SUR2A in the presence of ATP alone because of ATP hydrolysis in NBD2. In consequence, C42 seems to regulate ATPase activity of NBD2.

Nicorandil much more potently activated wild-type SUR2B/Kir6.2 than SUR2A/Kir6.2 channels (Figs. 1B and 3B). The significantly impaired response of SUR2B(D1470N)/Kir6.2 channels to ADP or nicorandil (Fig. 5Bc) again sug-

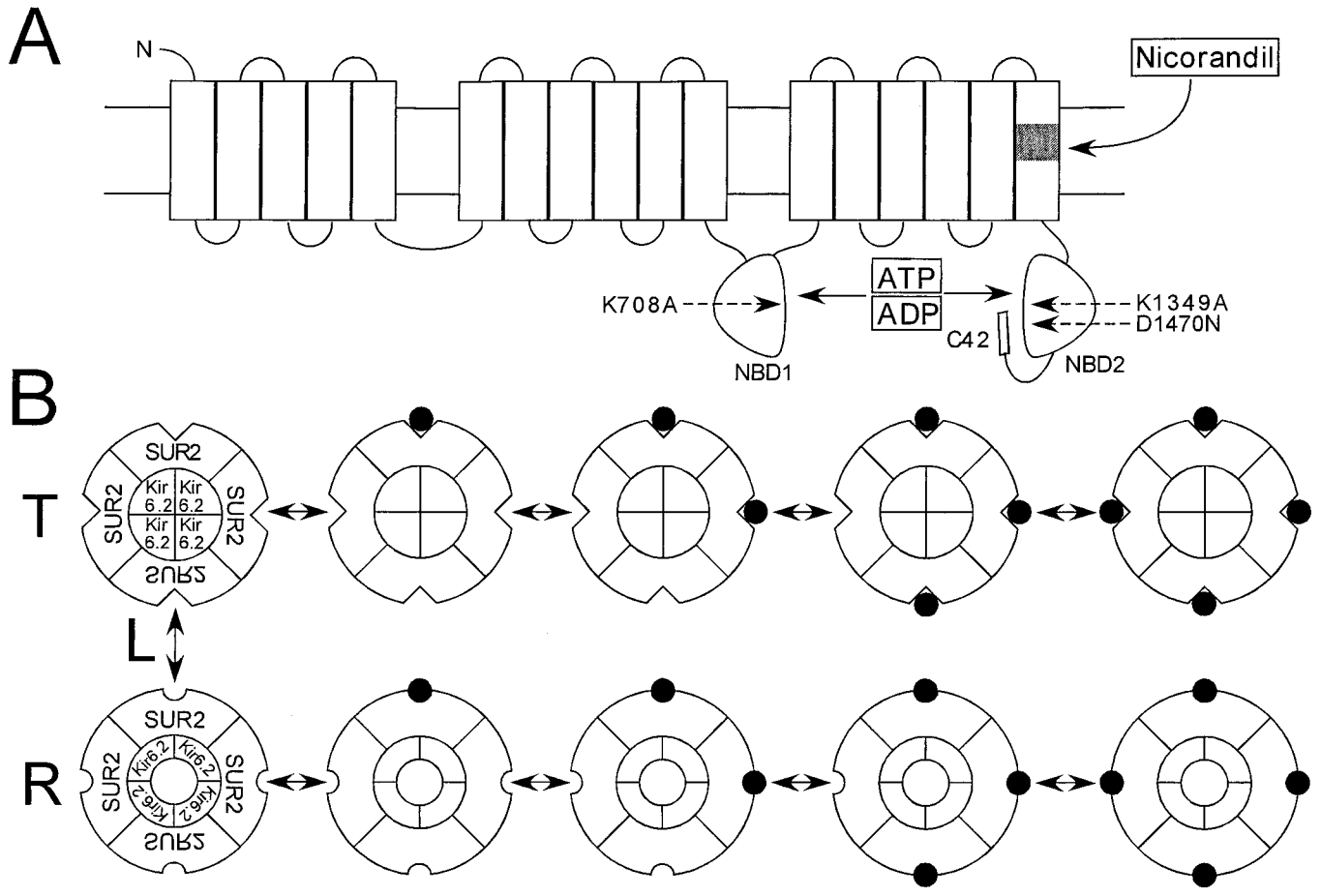

Fig. 6. Allosteric model of SUR2/Kir6.2 channels. A, schematic representation of SUR2. The upper and lower horizontal lines indicate the external and internal surface of the plasma membrane, respectively. SUR2 has 17 transmembrane domains, with NBD1 between the 11th and 12th transmembrane domains, NBD2 in the C terminus, and the receptor for nicorandil (▨) in the 17th transmembrane domain. SUR2A and SUR2B differ only in the C42 amino acids. NBD1 and NBD2 serve as the receptor for intracellular ATP and ADP, and NBD2 has higher ATPase activity than does NBD1. Approximate locations where the K708A, K1349A, and D1470N mutations were made are also depicted. B, an allosteric model of SUR2/Kir6.2 channels. Here, we assume the following: 1) an SUR2/Kir6.2 channel has a hetero-octameric structure composed of four SUR2 and four Kir6.2 subunits; 2) each SUR2 has a receptor (dent) for a ligand (●); 3) SUR2 has two distinct conformations that are able (R conformation) and unable (T conformation) to open the channel pore formed from Kir6.2 subunits; 4) the T and R conformations are in equilibrium determined by the allosteric constant L, where L is the ratio of the concentration of R to that of T in the absence of any ligands; 5) the structure of the receptor with regard to the ligand is distinct in the R and T conformations and has higher affinity for the ligand in the R than the T conformation; and 6) as a result, the ligand shifts the T-R equilibrium toward the R conformation. The ligand can be regarded as either nicorandil, ATP, or ADP. In the case of nicorandil, the dent represents the drug receptor. For ATP and ADP, the dent corresponds to either NBD1 or NBD2.

gests that SUR2B takes the R conformation strongly dependent on ATP hydrolysis and ADP binding in NBD2. Exogenously added ADP further increased the basal activity but not the sensitivity to nicorandil of wild-type SUR2B/Kir6.2 channels (Fig. 4B). Thus, nicorandil may increase ATPase activity of SUR2B (Bienengraeber et al., 2000) to a saturating level, thereby maximizing the probability of R in the presence of ATP alone.

Either the K708A or K1349A mutation greatly reduced the basal activity and the potency and efficacy of nicorandil in the presence of ATP alone (Fig. 3B). A possible decrease in the ATPase activity caused by these mutations does not fully account for the observed changes because exogenously added ADP did not restore the basal or nicorandil-induced activity of the mutant channels (Fig. 4B). From our model and comparison of the concentration-dependent effects of nicorandil on the channels with or without the K708A or K1349A mutation, it is possible to assess the heterotropic effect of nucleotides binding to NBDs. Our preliminary analysis indicated that the interaction of nucleotides with NBD1 and NBD2 apparently reduces L, respectively, by a factor of 0.02 and 0.03 in SUR2B and 0.07 and 0.34 in SUR2A in the presence of ATP plus ADP, whereas, under these conditions, comparable concentrations of nucleotides may be available in NBDs of SUR2A and SUR2B. Therefore, binding of the nucleotides to either NBD1 or NBD2 more strongly promotes the T-R transition in SUR2B than in SUR2A and almost equally contributes to this transition in SUR2B. In consequence, C42 modulates the interaction of both NBD1 and NBD2 with nucleotides. The different response of SUR2A(K708A)/Kir6.2 (Figs. 1B and 2B) and SUR2B(K708A)/Kir6.2 (Figs. 3B and 4B) indicates that the interaction of nucleotides and the remaining NBD2 more strongly promotes the T-R transition in SUR2B than SUR2A.

Reimann et al. (2001) proposed that an interaction between ATP-NBD1 but not ATP-NBD2 was essential for the action of nicorandil on SUR2B/Kir6.2 channels. However, a single concentration (100 μM) of nicorandil they examined has only a slight effect on SUR2B(K708A)/Kir6.2 and SUR2B(K1349A)/Kir6.2 channels (Fig. 3B), which probably led to the misinterpretation.

Comparison of SUR2A and SUR2B and Functional Roles of the C-terminal 42 Amino Acids. To summarize, NBD1 and NBD2 cooperatively induce the T-R transition in both SUR2A and SUR2B. NBD1 plays a significantly more important role than NBD2 in SUR2A, whereas both NBDs contribute almost equally to this phenomenon in SUR2B. In SUR2A, interaction occurs with ATP-binding to both NBDs in the presence of ATP alone but with ATP-binding to NBD1 and ADP-binding to NBD2 in the presence of ATP plus ADP. Here, NBD2 serves as a regulator of SUR2A/Kir6.2 channels by sensing ADP concentration. In SUR2B, NBD2 effectively hydrolyzes ATP, and this reaction is further stimulated by nicorandil. The formed ADP interacts with NBD2 and probably also NBD1. The interaction of ATP and ADP with either NBD more strongly contributes to the conformational change in SUR2B than SUR2A. These distinctions probably underlie both the higher basal activity and the greater nicorandil sensitivity of SUR2B/Kir6.2 than SUR2A/Kir6.2 channels in the presence of ATP alone.

Taken together, C42 modulates the interactions between nucleotides and NBDs. We proposed that steric interaction between the central part of C42 and the Walker A motif of NBD2 determines the ADP-NBD2 interaction (Matsushita et al., 2002). Although it is unknown whether NBDs of SUR2 form a dimer (Hopfner et al., 2000; Locher et al., 2002; Smith et al., 2002; Chang, 2003), homology modeling of the three-dimensional structure of NBDs from HisP, MJ1267, and MJ0796 (Hung et al., 1998; Karpowich et al., 2001; Smith et al., 2002) allocates the C-terminal half of C42 to the dimer interface close to the Walker A motif of NBD2 (data not shown). Such a structural basis could underlie the effect of C42 on both NBDs. However, further studies are clearly needed to explicitly identify the molecular mechanism by which C42 determines the physiological and pharmacological characteristics of SUR2A and SUR2B.

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References

Aguilar-Bryan L, Nichols CG, Wechsler SW, Clement IVJP, Boyd AE III, González G, Herrera-Sosa H, Nguy K, Bryan J, and Nelson DA (1995) Cloning of the β cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. *Science (Wash DC)* **268:**423–426.

Ashcroft FM and Gribble FM (1998) Correlating structure and function in ATP-sensitive K^+ channels. *Trends Pharmacol Sci* **21:**288–294.

Ashcroft FM and Gribble FM (2000) New windows on the mechanism of action of K_{ATP} channel openers. *Trends Pharmacol Sci* **21:**439–445.

Babenko AP, Aguilar-Bryan L, and Bryan J (1998) A view of SUR/K_{IR}6. X, KATP channels. *Annu Rev Physiol* **60:**667–687.

Bienengraeber M, Alekseev AE, Abraham MT, Carrasco AJ, Moreau C, Vivaudou M, Dzeja PR, and Terzic A (2000) ATPase activity of the sulfonylurea receptor: a catalytic function for the K_{ATP} channel complex. *FASEB J* **14:**1943–1952.

Chang G (2003) Structure of MsbA from Vibrio cholera: a multidrug resistance ABC transporter homolog in a closed conformation. *J Mol Biol* **330:**419–430.

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

D'hahan N, Moreau C, Prost A-L, Jacquet H, Alekseev AE, Terzic A, and Vivaudou M (1999) Pharmacological plasticity of cardiac ATP-sensitive potassium channels toward diazoxide revealed by ADP. *Proc Natl Acad Sci USA* **96:**12162–12167.

Hambrock A, Löffler-Walz C, Kloor D, Delabar U, Horio Y, Kurachi Y, and Quest U (1999) ATP-sensitive K^+ channel modulator binding to sulfonylurea receptors SUR2A and SUR2B: opposite effects of MgADP. *Mol Pharmacol* **55:**832–840.

Hopfner K-P, Karcher A, Shin DS, Craig L, Arthur M, Carney JP, and Tainer JA (2000) Structural biology of Rad50 ATPase: ATP-driven conformational control in DNA double-strand break repair and the ABC-ATPase superfamily. *Cell* **101:**789–800.

Hung L-W, Wang IX, Nikaido K, Liu P-Q, Ames GF-L, and Kim S-H (1998) Crystal structure of the ATP-binding subunit of an ABC transporter. *Nature (Lond)* **396:**703–707.

Inagaki N, Gonoi T, Clement IVJP, Namba N, Inazawa J, Gonzalez G, Aguilar-Bryan L, Seino S, and Bryan J (1995a) Reconstitution of I_{KATP}: an inward rectifier subunit plus the sulfonylurea receptor. *Science (Wash DC)* **270:**1166–1170.

Inagaki N, Gonoi T, Clement IVJP, Wang C-Z, Aguilar-Bryan L, Bryan J, and Seino S (1996) A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K^+ channels. *Neuron* **16:**1011–1017.

Inagaki N, Tsuura Y, Namba N, Masuda K, Gonoi T, Horie M, Seino T, Mizuta M, and Seino S (1995b) Cloning and functional characterization of a novel ATP-sensitive potassium channel ubiquitously expressed in rat tissues, including pancreatic islets, pituitary, skeletal muscle and heart. *J Biol Chem* **270:**5691–5694.

The IONA Study Group (2002) Effect of nicorandil on coronary events in patients with stable angina: the Impact of Nicorandil in Angina (IONA) randomised trial. *Lancet* **359:**1269–1275.

Isomoto S, Kondo C, Yamada M, Matsumoto S, Higashiguchi O, Horio Y, Matsuzawa Y, and Kurachi Y (1996) A novel sulfonylurea receptor forms with BIR (Kir6.2) a smooth muscle type ATP-sensitive K^+ channel. *J Biol Chem* **271:**24321–24324.

Jones PM and George AM (2002) Mechanism of ABC transports: a molecular dynamics simulation of a well characterized nucleotide-binding subunit. *Proc Natl Acad Sci USA* **99:**12639–12644.

Karpowich N, Martsinkevich O, Millen L, Yuan Y-R, Dai PL, MacVey K, Thomas PJ, and Hunt PJ (2001) Crystal structures of the MJ1267 ATP binding cassette reveal an induced-fit effect at the ATPase active site of an ABC transporter. *Structure* **9:**571–586.

Locher KP, Lee AT, and Rees DC (2002) The *E. coli* BtuCD structure: a framework for ABC transporter architecture and mechanism. *Science (Wash DC)* **296:**1091–1098.

Matsuo M, Tanabe K, Kioka N, Amachi T, and Ueda K (2000) Different binding properties and affinities for ATP and ADP among sulfonylurea receptor subtypes, SUR1, SUR2A and SUR2B. *J Biol Chem* **275:**28757–28763.
Matsushita K, Kinoshita K, Matsuoka T, Fujita A, Fujikado T, Tano Y, Nakamura H, and Kurachi Y (2002) Intramolecular interaction of SUR2 subtypes for intracellular ADP-induced differential control of K_{ATP} channels. *Circ Res* **90:**554–561.
Miki T, Suzuki M, Shibasaki T, Uemura H, Sato T, Yamagichi K, Koseki H, Iwanaga T, Nakaya H, and Seino S (2002) Mouse model of Prinzmetal angina by disruption of the inward rectifier Kir6.1. *Nat Med* **5:**466–472.
Monod J, Wyman J, and Changeux J-P (1965) On the nature of allosteric transitions: a plausible model. *J Mol Biol* **12:**88–118.
Moreau C, Jacquet H, Prost A-N, D'hahan N, and Vivaudou M (2000) The molecular basis of the specificity of action of K_{ATP} channel openers. *EMBO (Eur Mol Biol Organ) J* **24:**6644–6651.
Reimann F, Ashcroft FM, and Gribble FM (2001) Structural basis for the interference between nicorandil and sulfonylurea action. *Diabetes* **50:**2253–2259.
Reimann F, Gribble FM, and Ashcroft FM (2000) Differential response of K_{ATP} channels containing SUR2A or SUR2B subunits to nucleotide and pinacidil. *Mol Pharmacol* **58:**1318–1325.
Schwanstecher M, Sieverding C, Dörschner H, Gross I, Aguilar-Bryan L, Schwanstecher C, and Bryan J (1998) Potassium channel openers require ATP to bind to and act through sulfonylurea receptors. *EMBO (Eur Mol Biol Organ) J* **19:**5529–5535.
Seino S (1999) ATP-sensitive potassium channels: a model of heteromultimeric potassium channel/receptor assemblies. *Annu Rev Physiol* **61:**337–362.
Shen WK, Tung RT, Machulda MM, and Kurachi Y (1991) Essential role of nucleotide diphosphates in nicorandil-mediated activation of cardiac ATP-sensitive K^+ channels. *Circ Res* **69:**1152–1158.
Shindo T, Yamada M, Isomoto S, Horio Y, and Kurachi Y (1998) SUR2 subtype (A and B)-dependent differential activation of the cloned ATP-sensitive K^+ channels by pinacidil and nicorandil. *Br J Pharmacol* **124:**985–991.
Shyng S-L, Ferrigni T, and Nichols CG (1997) Regulation of K_{ATP} channel activity by diazoxide and MgADP. Distinct functions of the two nucleotide binding folds of sulfonylurea receptor. *J Gen Physiol* **110:**643–654.
Shyng S-L and Nichols CG (1997) Octameric stoichiometry of the K_{ATP} channel complex. *J Gen Physiol* **110:**655–664.
Smith PC, Karpowich N, Millen L, Moody JE, Rosen J, Thomas PJ, and Hunt JF (2002) ATP binding to the motor domain from an ABC transporter drives formation of a nucleotide sandwich dimmer. *Mol Cell* **10:**139–149.
Trapp S, Haider S, Jones P, Sansom MSP, and Ashcroft FM (2003) Identification of residues contributing to the ATP binding site of Kir6.2. *EMBO (Eur Mol Biol Organ) J* **22:**2903–2912.
Tucker SJ, Gribble FM, Zhao C, Trapp S, and Ashcroft FM (1997) Truncation of Kir6.2 produces ATP-sensitive K^+ channels in the absence of the sulphonylurea receptor. *Nature (Lond)* **387:**179–183.
Ueda K, Inagaki N, and Seino S (1997) MgADP antagonism to Mg^{2+}-independent ATP binding of the sulfonylurea receptor SUR1. *J Biol Chem* **37:**22983–22986.
Ueda K, Komine J, Matsuo M, Seino S, and Amachi T (1999) Cooperative binding of ATP and MgADP in the sulfonylurea receptor is modulated by glibenclamide. *Proc Natl Acad Sci USA* **96:**1268–1272.
Yamada M, Isomoto S, Matsumoto S, Kondo C, Shindo T, Horio Y, and Kurachi Y (1997) Sulfonylurea receptor 2B and Kir6.1 form a sulfonylurea-sensitive but ATP-sensitive K^+ channel. *J Physiol (Lond)* **499:**715–720.
Yamada M, Satoh E, Kondo C, Repunte VP, and Kurachi Y (1999) Potassium ion channels: molecular structure, function, and disease, in *Current Topics in Membranes* (Kurachi Y, Jan LY, and Lazdunski M eds) vol 46, pp 387–415, Academic Press, San Diego.
Yuan Y-R, Blecker S, Martsinkevich O, Millen L, Thomas PJ, and Hunt JF (2001) The crystal structure of the MJ0796 ATP-binding cassette: implications for the structural consequences of ATP hydrolysis in the active site of an ABC transporter. *J Biol Chem* **276:**32313–32321.

Address correspondence to: Dr. Yoshihisa Kurachi, Department of Pharmacology II, Graduate School of Medicine Osaka University, 2-2 Yamada-oka, Suita, Osaka 565-0871 Japan. E-mail: ykurachi@pharma2.med.osaka-u.ac.jp