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Molecular Mechanisms Underlying Ketamine-mediated Inhibition of Sarcolemmal Adenosine Triphosphate-sensitive Potassium Channels

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Background: **Ketamine inhibits adenosine triphosphate-sensitive potassium (K_{ATP}) channels, which results in the blocking of ischemic preconditioning in the heart and inhibition of vasorelaxation induced by K_{ATP} channel openers. In the current study, the authors investigated the molecular mechanisms of ketamine's actions on sarcolemmal K_{ATP} channels that are reassociated by expressed subunits, inwardly rectifying potassium channels (Kir6.1 or Kir6.2) and sulfonylurea receptors (SUR1, SUR2A, or SUR2B).**

Methods: **The authors used inside-out patch clamp configurations to investigate the effects of ketamine on the activities of reassociated Kir6.0/SUR channels containing wild-type, mutant, or chimeric SURs expressed in COS-7 cells.**

Results: **Ketamine racemate inhibited the activities of the reassociated K_{ATP} channels in a SUR subtype-dependent manner: SUR2A/Kir6.2 (IC_{50} = 83 μM), SUR2B/Kir6.1 (IC_{50} = 77 μM), SUR2B/Kir6.2 (IC_{50} = 89 μM), and SUR1/Kir6.2 (IC_{50} = 1487 μM). S-(+)-ketamine was significantly less potent than ketamine racemate in blocking all types of reassociated K_{ATP} channels. The ketamine racemate and S-(+)-ketamine both inhibited channel currents of the truncated isoform of Kir6.2 (Kir6.2ΔC36) with very low affinity. Application of 100 μM magnesium adenosine diphosphate significantly enhanced the inhibitory potency of ketamine racemate. The last transmembrane domain of SUR2 was essential for the full inhibitory effect of ketamine racemate.**

Conclusions: **These results suggest that ketamine-induced inhibition of sarcolemmal K_{ATP} channels is mediated by the SUR subunit. These inhibitory effects of ketamine exhibit specificity for cardiovascular K_{ATP} channels, at least some degree of stereoselectivity, and interaction with intracellular magnesium adenosine diphosphate.**

ADENOSINE triphosphate-sensitive potassium (K_{ATP}) channels are inhibited by intracellular adenosine triphosphate (ATP) and activated by magnesium adenosine diphosphate (MgADP) and thus provide a link between the cellular metabolic state and excitability.[1,2] K_{ATP} channels are composed of an ATP-binding cassette protein, sulfonylurea receptor (SUR), and an inwardly rectifying K^+ channel (Kir) subunit, Kir6.0; SUR acts as a regulatory subunit whereas Kir subunits form the ATP-sensitive channel pore.[3] These channels, as metabolic sensors, are associated with such cellular functions as insulin secretion, cardiac preconditioning, vasodilatation, and neuroprotection.[4–7]

In cardiac myocytes, intravenous general anesthetics, such as ketamine racemate, propofol, and thiamylal, directly inhibit native sarcolemmal K_{ATP} channels.[8–10] Although these observations suggest that intravenous anesthetics may impair the endogenous organ protective mechanisms mediated by K_{ATP} channels, the possibility that K_{ATP} channel inhibition by intravenous anesthetics might have adverse consequences in clinical practice remains controversial. Indeed, propofol and thiamylal are known to possess cardioprotective and neuroprotective properties, respectively, with these being mediated by other well-established mechanisms that do not involve K_{ATP} channels.[11,12] In recent *in vitro* studies, however, ketamine racemate, but not the stereoisomer S-(+)-ketamine, was found to block early and late preconditioning in rabbit hearts and inhibit vasorelaxation induced by a K_{ATP} channel opener.[13–16] It is therefore possible that the mechanisms underlying ketamine-induced inhibition of K_{ATP} channel activity may differ from those of propofol and thiamylal. A previous mutagenesis study demonstrated that the major effects of both propofol and thiamylal on K_{ATP} channel activity are mediated *via* the Kir6.2 subunit.[17] However, organ specificity and the molecular site of action of ketamine have not been investigated in detail. In addition, although it is now well established that intracellular MgADP can modulate the sensitivity of K_{ATP} channel activators and inhibitors,[18,19] there is no evidence that intracellular MgADP modulates intravenous anesthetics inhibitory effects on sarcolemmal K_{ATP} channels.

In the current study, we used patch clamp techniques to examine the electrophysiological effects and molecular mechanisms of racemic ketamine and S-(+)-ketamine on different types of reassociated K_{ATP} channels containing wild-type, mutant, or chimeric SURs expressed in COS-7 cells (African green monkey kidney cells). We also investigated the effects of intracellular MgADP on the inhibitory actions of ketamine racemate.

Materials and Methods

Molecular Biology

cDNAs (The human Kir6.2, rat Kir6.1, rat SUR1, rat SUR2A, and rat SUR2B) and expression vector pCMV6C

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were kindly provided by Susumu Seino, MD., Ph.D. (Professor and Chairman, Department of Cellular and Molecular Medicine, Chiba University, Chiba, Japan). Coexpressing SUR1 and Kir6.2 (SUR1/Kir6.2) forms the pancreatic β cell K_{ATP} channel, SUR2A and Kir6.2 (SUR2A/Kir6.2) forms the cardiac K_{ATP} channel, SUR2B and Kir6.2 (SUR2B/Kir6.2) forms the nonvascular smooth muscle K_{ATP} channel, and SUR2B and Kir6.1 (SUR2B/Kir6.1) forms the vascular smooth muscle K_{ATP} channel.[3,20,21] A truncated form of human Kir6.2 lacking the last 36 amino acids at the C terminus was obtained by polymerase chain reaction amplification as previously described.[17] Chimeric cDNA constructs were produced by splicing, using the overlap extension polymerase chain reaction technique.[22] The exact amino acid composition of the SUR1-SUR2A chimeric constructs was: chimera SUR1–2A = (1–1035, SUR1)-(1013–1261, SUR2A)-(1297–1581, SUR1); SUR2A-1 = (1–1013, SUR2A)-(1035–1277, SUR1)-(1241–1545, SUR2A). All DNA products were sequenced using BigDye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA), and an ABI PRISM 377 DNA sequencer (Applied Biosystems) was used to confirm the sequence.

Cell Culture and Transfection

K_{ATP} channel-deficient COS-7 cells were plated at a density of 3×10^5 per dish (35 mm diameter) and cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. A full-length Kir cDNA and a full-length SUR cDNA were subcloned into the mammalian expression vector pCMV6c. For electrophysiological recordings, mutated pCMV6c Kir alone (1 μg) or either wild-type or mutated pCMV6c Kir (1 μg) plus pCMV6c SUR (1 μg) were transfected into COS-7 cells with green fluorescent protein cDNA (pEGFP-N1; Clontech Laboratories, Palo Alto, CA) as a reporter gene by using lipofectamine and Opti-MEM 1 reagents (Life Technologies Inc., Rockville, MD) according to the manufacturer's instructions. After transfection, cells were cultured for 48 to 72 h before being subjected to electrophysiological recordings.

Electrophysiological Measurements

Membrane currents were recorded in the inside-out configurations using a patch-clamp amplifier as described previously.[9,10,17] Transfected cells were identified by their green fluorescence under a microscope. The intracellular solution contained 140 mM KCl, 2 mM EGTA, 2 mM $MgCl_2$, and 10 mM HEPES (pH = 7.3). The pipette solution contained 140 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM HEPES (pH = 7.4). Recordings were made at 36° ± 0.5°C. Patch pipettes were pulled with an electrode puller (PP-830; Narishige, Tokyo, Japan). The resistance of pipettes filled with internal solution and immersed in the Tyrode's solution was 5–7 MΩ. The sampling frequency of the single-channel data were 5 KHz with a low-pass filter (1 KHz).

Electrophysiological Data Analysis

Channel currents were recorded with a patch clamp amplifier (CEZ 2200; Nihon Kohden, Tokyo, Japan) and stored in a personal computer (Aptiva; IBM, Armonk, NY) with an analog-to-digital converter (DigiData 1200; Axon Instruments, Foster City, CA). pClamp version 7 software (Axon Instruments) was used for data acquisition and analysis. The open probability (P_o) was determined from current amplitude histograms and was calculated as follows:

$$P_o = \frac{\left(\sum_{j=1}^{N} t_j \cdot j\right)}{T_d \cdot N} \quad (1)$$

where t_j is the time spent at current levels corresponding to j = 0, 1, 2, N channels in the open state, T_d is the duration of the recording, and N is the number of the channels active in the patch. Recordings of 2–3 min were analyzed to determine P_o. The channel activity was expressed as NPo. The NPo in the presence of drugs was normalized to the baseline NPo value obtained before drug administration and presented as the relative channel activity. When the concentration-dependent effects of drugs were studied, the superfusion was stopped for approximately 1 min at each concentration, and these drugs were injected into the cell bath using a glass syringe to five final concentrations in a cumulative manner (total volume injected was approximately 10–20 μl). Therefore, the superfusion was stopped for approximately 5 min; preliminary studies showed that the stopping of superfusion for approximately 5 min had no significant effects on electrophysiological measurements. The average percent recovery of K_{ATP} channel activities after washout of Ketamine racemate or S-(+)-ketamine was 94 ± 6% of the NPo measured before drug treatment.

The drug concentration needed to induce half-maximal inhibition of the channels (IC_{50}) and the Hill coefficient were calculated as follows:

$$y = \frac{1}{1 + ([D]/K_i)^H} \quad (2)$$

where y is the relative NP_o, [D] is the concentration of drug, K_i is IC_{50}, and H is the Hill coefficient.

Drugs

The following drugs were used: ketamine racemate, S-(+)-ketamine, glibenclamide, and pinacidil (Sigma-

Aldrich Japan, Tokyo, Japan). Glibenclamide and pinacidil were dissolved in dimethylsulfoxide (the final concentration of solvent was 0.01%); preliminary studies showed that 0.02% of dimethylsulfoxide, a twofold higher concentration than we used in the current study, had no significant effects on all types of reassociated K_{ATP} channel currents.

Statistics

All data were presented as means ± SD. Differences between data sets were evaluated either by repeated-measures one-way analysis of variance followed by the Scheffé *F* test or by Student *t* test. $P < 0.05$ was considered significant.

Results

Sarcolemmal K_{ATP} channels SUR2A/Kir6.2 (cardiac type), SUR2B/Kir6.1 (vascular smooth muscle type), SUR2B/Kir6.2 (nonvascular smooth muscle type), and SUR1/Kir6.2 (pancreatic β-cell type) were heterologously expressed in COS-7 cells. Our previous experiments have shown that the single-channel characteristics of all types of reassociated K_{ATP} channels were similar to those of native K_{ATP} channels.[17]

Effects of Ketamine Racemate and S-(+)-ketamine on Sarcolemmal KATP Channels in the Absence of Intracellular ADP

To assess the effects of ketamine racemate and S-(+)-ketamine on reassociated K_{ATP} channels in the absence of intracellular ADP, we measured single-channel currents on inside-out patches in the presence of these drugs. Application of 100 μM ketamine racemate to the intracellular membrane surface inhibited the SUR2A/Kir6.2, SUR2B/Kir6.1, and SUR2B/Kir6.2 channel currents, with relative channel activities decreasing to 0.45 ± 0.11, 0.41 ± 0.07, and 0.47 ± 0.18, respectively (fig. 1A). However, 100 μM ketamine racemate did not significantly inhibit the SUR1/Kir6.2 channel currents. On the contrary, S-(+)-ketamine at 100 μM did not significantly inhibit all of the reassociated K_{ATP} channel currents (fig. 1B). The inhibitory effects of ketamine racemate and S-(+)-ketamine on K_{ATP} channel activities were readily reversible (fig. 1A).

The concentration-dependent effects of ketamine racemate and S-(+)-ketamine on the activities of various types of reassociated K_{ATP} channels, in the absence of intracellular ADP, are shown in figure 2. The IC_{50} values and Hill coefficients of ketamine and S-(+)-ketamine for the SUR2A/Kir6.2, SUR2B/Kir6.1, SUR2B/Kir6.2, and SUR1/Kir6.2 channels are summarized in table 1. The IC_{50} value of ketamine racemate for SUR2A/Kir6.2 (83 ± 8 μM) (table 1) was very similar to that obtained by Ko *et al.*[8] for the native cardiac K_{ATP} channel in the absence of intracellular ADP (63 μM). Ketamine racemate inhibited the activity of all types of reassociated K_{ATP} channels with higher potency than S-(+)-ketamine. In addition, ketamine inhibited the SUR2A/Kir6.2, SUR2B/Kir6.1, and SUR2B/Kir6.2 channels with higher potency than the SUR1/Kir6.2 channel.

Effects of Ketamine Racemate and S-(+)-ketamine on Kir6.2ΔC36 Channel Activity

A C-terminal truncated pore-forming subunit of Kir6.2 (Kir6.2ΔC36), lacking the last 36 amino acids, is capable of forming a functional channel in the absence of SUR.[23] This has proved to be a useful tool for discriminating the site of action of various agents on K_{ATP} channels.

Ketamine racemate and S-(+)-ketamine inhibited the Kir6.2ΔC36 channel current with very low affinity, with 1 mM concentrations of both anesthetics producing less than 30% inhibition (fig. 3, A and B). This result indicates that the SUR subunit, rather than Kir6.2, is primarily responsible for the effects of ketamine racemate and S-(+)-ketamine on wild-type K_{ATP} channels, a view that is supported by the differential actions of ketamine on K_{ATP} channels containing different types of SUR subunit.

Effects of Ketamine Racemate in Presence of Intracellular ADP

The effects of several sulfonylureas and K_{ATP} channel openers are modified by interaction of MgADP with SURs.[18,19] Indeed, it has been previously reported that MgADP simultaneously activates the K_{ATP} channels strongly *via* SUR and inhibits them weakly *via* the ATP binding site on Kir6.2.[23,24] Therefore, we next examined whether the inhibitory effects of ketamine on reassociated K_{ATP} channel currents are affected in the presence of intracellular MgADP.

Figure 4 shows the effects of 100 μM ketamine racemate on SUR1/Kir6.2, SUR2A/Kir6.2, and SUR2B/Kir6.2 channel activities in the absence or presence of 100 μM MgADP. The IC_{50} for these channels are given in table 2. MgADP was significantly enhanced the inhibitory potency of ketamine racemate on all types of reassociated K_{ATP} channels.

Effects of Ketamine Racemate on SUR Chimeras

The differential effects of ketamine on SUR1 and SUR2 enabled us to use a chimeric approach in identifying regions of SUR2 critical for inhibition by ketamine racemate. SUR is a member of the ATP-binding cassette transporter family and is predicted to possess two intracellular nucleotide binding domains and three transmembrane domains (TMD0, TMD1, and TMD2) that contain five, six, and six transmembrane helices (TMs), respectively.[25] It has been reported recently that TMD2 of SUR1 and SUR2 is crucial for the action of several SUR1-selective sulfonylureas, K_{ATP} channel blocker, and SUR2-selective K_{ATP} channel openers, respectively.[22,26]

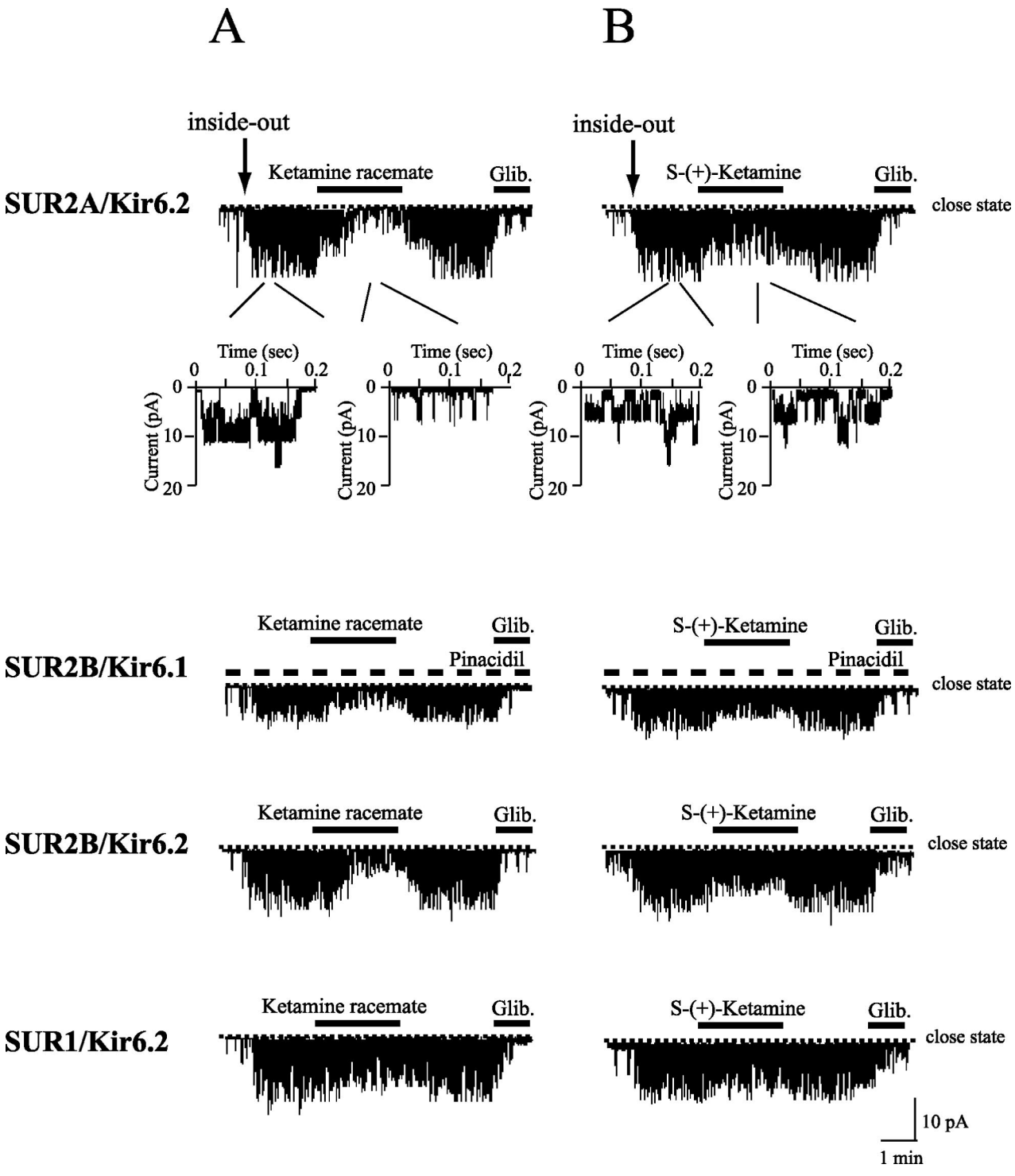

Fig. 1. Effects of ketamine racemate and S-(+)-ketamine on the currents of different reassociated adenosine triphosphate-sensitive potassium channels in the excised inside-out configuration. Membrane potentials were clamped at −60 mV. Shown are representative examples of sulfonylurea receptor (SUR) 2A/inwardly rectifying potassium channel (Kir) 6.2, SUR2B/Kir6.1, SUR2B/Kir6.2, and SUR1/Kir6.2 currents obtained before and after the application of ketamine racemate (100 μM) (*A*) or S-(+)-ketamine (100 μM) (*B*). Because SUR2B/Kir6.1 channels are not activated in the inside-out patch clam configurations, pinacidil (100 μM) was used to activate them. This figure shows that washout of ketamine racemate and S-(+)-ketamine restores channel activities. In all cases, the channel activity was inhibited by glibenclamide (Glib; 10 μM). The periods of ketamine racemate or S-(+)-ketamine administration are marked with *horizontal solid bars*. The periods of pinacidil administration are marked with *horizontal dashed bars.*

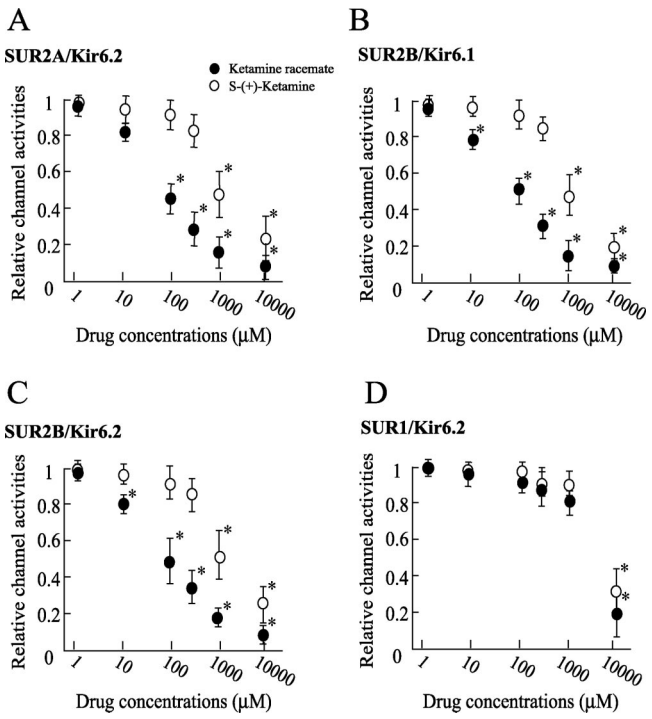

Fig. 2. Concentration-dependent effects of ketamine racemate (●) and S-(+)-ketamine (○) on the activities of reconstituted sulfonylurea receptor (SUR) 2A/inwardly rectifying potassium channel (Kir) 6.2 (*A*), SUR2B/Kir6.1 (*B*), SUR2B/Kir6.2 (*C*), and SUR1/Kir6.2 (*D*) channels. Each *vertical bar* constitutes measurements from 9–18 patches (mean ± SD). * $P < 0.05$ *versus* baseline (before drug).

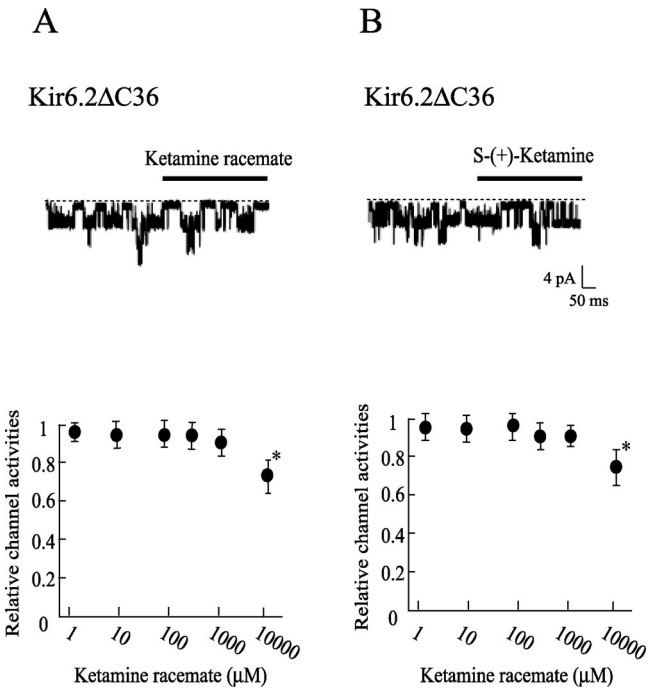

Fig. 3. Effects of ketamine racemate and S-(+)-ketamine on the channel activities of the truncated isoform of inwardly rectifying potassium channel 6.2 (Kir6.2ΔC36), which can form functional adenosine triphosphate-sensitive potassium channels in the absence of sulfonylurea receptor molecules, in the excised inside-out configuration. Membrane potentials were clamped at −60 mV. (*A*) Representative examples of Kir6.2ΔC36 currents obtained before and after the application of ketamine racemate (100 μM) and S-(+)-ketamine (100 μM). The periods of drug treatment are marked with *horizontal bars*. (*B*) Dose-dependent effects of ketamine racemate and S-(+)-ketamine on the activities of Kir6.2ΔC36 channels. Each *vertical bar* constitutes measurements from 10–15 patches (mean ± SD). *$P < 0.05$ *versus* baseline (before drug).

Thus, we hypothesized that TMD2 of SUR might be involved in the SUR2-selective inhibition of ketamine. To test this hypothesis, we constructed chimeric SURs, in which portions of TMD2 were swapped between SUR1 and SUR2A and coexpressed with Kir6.2 in COS-7 cells (fig. 5A).

Ketamine racemate sensitivity could be introduced into SUR1 by transferring TMs 13–17 from SUR2A to SUR1 (chimera SUR1–2) (fig. 5B). The reverse chimera, in which TMs 13–16 were swapped from SUR1 to SUR2A (chimera SUR2–1), was also sensitive to inhibition by ketamine racemate but to a lesser extent than wild-type SUR2A. These results suggest that the region within TM 13–17 is essential for the full inhibitory effect of ketamine racemate.

Discussion

In the current study, we transiently expressed the different subtypes of reassociated sarcolemmal K_{ATP} channels in COS-7 cells and demonstrated that ketamine

Table 1. Effects of ketamine and S-(+)-ketamine on different types of re-associated K_{ATP} channels

	Ketamine racemate			S-(+)-Ketamine		
	IC_{50} (μM)	n	*h*	IC_{50} (μM)	n	*h*
SUR2A/Kikr6.2	83 ± 8*	15	0.92	889 ± 92*	16	0.89
SUR2B/Kir6.1	77 ± 9*	12	1.03	703 ± 76*	13	0.94
SUR2B/Kir6.2	89 ± 10*	9	0.98	925 ± 101*	15	0.91
SUR1/Kir6.2	1487 ± 345	18	1.12	1826 ± 393	18	1.08

SUR2A/Kir6.2 = re-associated sulfonylurea receptor 2A and inwardly rectifying potassium channel 6.2 channels; SUR2B/Kir6.1 = re-associated sulfonylurea receptor 2B and inwardly rectifying potassium channel 6.1 channels; SUR2B/Kir6.2 = re-associated sulfonylurea receptor 2B and inwardly rectifying potassium channel 6.2 channels; SUR1/Kir6.2 = re-associated sulfonylurea receptor 1 and inwardly rectifying potassium channel 6.2 channels.

IC_{50} values are presented as mean ± SD.

h indicates the Hill coefficient value.

* $P < 0.05$ against SUR1/Kir6.2 (for IC_{50} value).

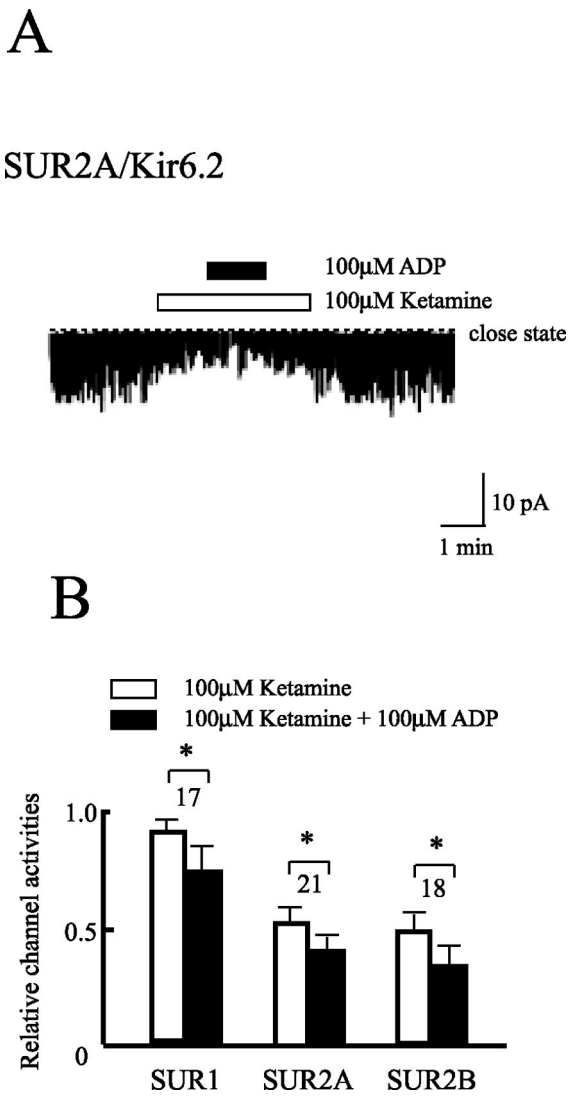

Fig. 4. Effects of anesthetics and magnesium adenosine diphosphate (MgADP) on reassociated sulfonylurea receptor (SUR) 1/inwardly rectifying potassium channel (Kir) 6.2, SUR2A/Kir6.2, and SUR2B/Kir6.2 channels in the excised inside-out configuration. Membrane potentials were clamped at −60 mV. (*A*) Representative examples of SUR2A/Kir6.2 currents in the absence or presence of anesthetics and MgADP are shown. Ketamine racemate (100 μM), propofol (100 μM), thiamylal (300 μM), and MgADP (100 μM) are added to the intracellular solution as indicated by *horizontal bars*. (*B*) The relative channel activities in the presence of anesthetics without MgADP (*open columns*) and with MgADP (*closed columns*). The relative channel activities in the presence of anesthetics are expressed as a fraction of that observed in the absence of the drugs. The number of patches is given above each bar. The *columns* and *bars* indicate the mean and SD, respectively. *$P < 0.05$ *versus* without MgADP value.

racemate and S-(+)-ketamine inhibited these channels in a concentration-dependent manner. Our results also indicated that ketamine racemate specifically inhibits cardiovascular type K_{ATP} channels. Notably, ketamine's potency in blocking K_{ATP} channels containing SUR2 was approximately 18-fold higher than its ability to block K_{ATP} channels containing SUR1. Furthermore, ketamine

Table 2. IC_{50}s of inhibition of re-associated K_{ATP} channels by ketamine racemate in the absence and presence of 100 μM magnesium adenosine diphosphate

	Ketamine racemate (μM)	
	MgADP (−)	100 μM MgADP (+)
SUR1/Kir6.2	1487 ± 345 (18)	789 ± 43 (n = 6)*
SUR2A/Kir6.2	83 ± 8 (15)	58 ± 8 (6)*
SUR2B/Kir6.2	89 ± 10 (9)	49 ± 5 (6)*

MgADP = magnesium adenosine diphosphate; SUR1/Kir6.2 = re-associated sulfonylurea receptor 1 and inwardly rectifying potassium channel 6.2 channels; SUR2A/Kir6.2 = re-associated sulfonylurea receptor 2A and inwardly rectifying potassium channel 6.2 channels; SUR2B/Kir6.2 = re-associated sulfonylurea receptor 2B and inwardly rectifying potassium channel 6.2 channels.

IC_{50} values are presented as means ± SD (n).

* $P < 0.05$ against IC_{50} value in the absence of MgADP.

racemate was significantly more potent than S-(+)-ketamine in blocking all types of reassociated K_{ATP} channels. Because ketamine racemate is a mixture of S-(+) and R-(-)-ketamine stereoisomers, this observation would suggest that block of K_{ATP} channels by ketamine enantiomers may be at least partly stereoselective.

K_{ATP} channels are formed from pore-forming Kir6.0 and regulatory SUR subunits, arranged with 4:4 stoichio-

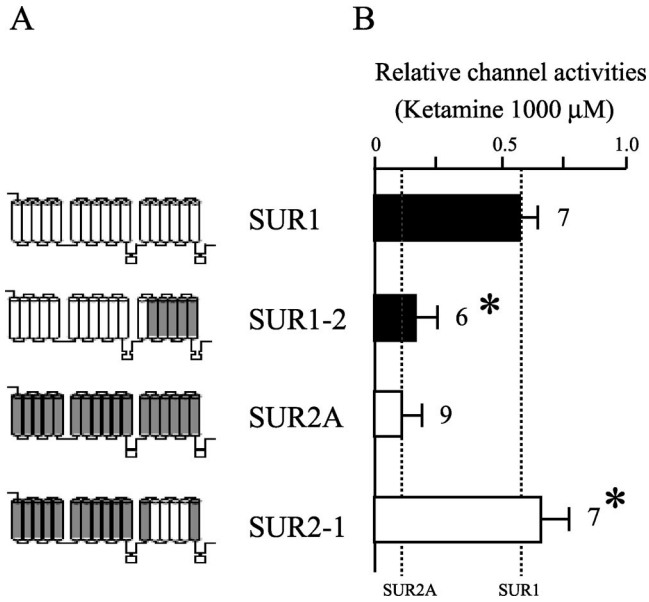

Fig. 5. Effects of ketamine racemate on sulfonylurea receptor (SUR) chimeras. (*A*) Schematic representation of wild-type and chimeric SURs. Open regions indicate SUR1 and solid regions indicate SUR2A. (*B*) Effects of ketamine racemate (1 mM) on single-channel currents of reconstituted inwardly rectifying potassium channel (Kir) 6.2 with SUR1, SUR2A, chimera SUR1–2, and chimera SUR2–1. The *dotted lines* indicate the mean inhibition of SUR1/Kir6.2 and SUR2A/Kir6.2 currents. The *numbers in brackets* indicate the number of patches. For statistical analysis, the chimeras based on SUR1 ([squlf]) were compared with SUR1, and the chimeras based on SUR2A (■) were compared with SUR2A. *$P < 0.05$.

metries.[3] The SUR subunit is encoded by two different genes, SUR1 and SUR2; SUR1 serves as the regulatory subunits of the pancreatic β-cell K_{ATP} channel, and splice variants of SUR2 act as the cardiac (SUR2A) and smooth muscle (SUR2B) SURs.[3] K_{ATP} channel activators and inhibitors show variable tissue specificity, the different types of K_{ATP} channel exhibit differential ATP sensitivity and pharmacologic properties, which are endowed by their different molecular composition of Kir6 and SUR subunits.[20] In the current study, the effects of ketamine racemate and S-(+)-ketamine were influenced by the type of SUR subunits, indicating that these anesthetics may interact with the SUR2 rather than the SUR1 subunits of the channels (table 1, figs. 1 and 2). In addition, the results obtained in the current study showing that both ketamine racemate and S-(+)-ketamine at concentrations up to 1 mM had no significant effects on the current generated by expressing Kir6.2ΔC36 in the absence of SUR (fig. 3) suggest that the inhibitory effects of these drugs on K_{ATP} channel activities are not mediated through Kir6.0 subunits. That is, it is unlikely that binding to SUR1 or Kir6.0 subunits contributes significantly to the inhibitory effect of these anesthetics. Furthermore, the inhibitory effects of ketamine racemate on SUR2/Kir6.0 channels were significantly larger than those of S-(+)-ketamine (table 1, figs. 1 and 2). It is, therefore, suggested that specific binding to SUR2A and SUR2B subunits may be the major mechanism underlying the tissue-specific and stereoselective inhibitory effects of ketamine.

The specificity of ketamine for K_{ATP} channels containing SUR2 isoforms enabled us to use a chimeric approach to identify regions of SURs important for activity of the drug. Chimeric sulfonylurea receptors were constructed in which isolated domains were swapped between SUR1 and SUR2 to identify regions of SUR2 that are required for the high affinity action of ketamine. In this way, we showed that high-affinity inhibition by ketamine racemate could be introduced into SUR1 by transferring parts of TMD2 (TMs 13–17) from SUR2A to SUR1 (fig. 5) (chimera SUR1–2). Furthermore, the reverse chimera, in which parts of TMD2 (TMs 13–16) were swapped from SUR1 to SUR2A (fig. 5) (chimera SUR2–1), abolished high-affinity ketamine racemate inhibition in SUR2. These results suggest that this region within TMD2 of SUR2A is essential for high-affinity inhibition by ketamine racemate.

It has been reported that clinical plasma concentrations for ketamine racemate are 20–50 μM[27] or 3–60 μM,[8] and the percentage of ketamine bound to plasma protein are 12%[27] or 45–50%,[8] suggesting that plasma concentrations of free ketamine racemate are 2.4–6 μM or 1.4–30 μM, respectively, during surgical anesthesia. The threshold concentrations at which ketamine racemate inhibits reassociated K_{ATP} channels containing SUR2 are close to this range (>10–100 μM) (fig. 2, A–C), but this is not the case for SUR1-containing channels (>10 mM) (fig. 2D). It is possible, therefore, that inhibition of sarcolemmal K_{ATP} channels containing SUR2 (cardiovascular type) by ketamine might have adverse consequences in clinical practice. Recently, physiologic studies on mice lacking different K_{ATP} channel subunits have begun to clarify the roles of cardiac and vascular K_{ATP} channels in cardiovascular pathophysiology. Cardiac K_{ATP} channel ($Kir6.2^{-/-}$)-deficient mice had a number of cardiac abnormalities during myocardial ischemia or severe stress, include impaired ischemic preconditioning and attenuated electrocardiographic ST changes.[28,29] Impaired vascular smooth muscle function was a feature of Kir6.1-deficient and SUR2-deficient mice and manifested as episodic coronary artery vasospasm and a high rate of sudden death.[6,30] Increased systolic and diastolic blood pressure was also observed in SUR2-deficient mice.[30] Indeed, racemic ketamine, but not the S-(+)-ketamine stereoisomer, was found to block early and late preconditioning in rabbit hearts and inhibit vasorelaxation induced by a K_{ATP} channel opener.[13–16] In heart, however, mitochondrial rather than sarcolemmal K_{ATP} channels might play an important role in ischemic and anesthetic preconditioning. Because the molecular identity of the channel has not been established, molecular biologic approaches such as reassociation of cloned channels or gene targeting technique are not yet applicable for the study of mitochondria K_{ATP} channel function. Recently, Zaugg *et al.*[31] reported that 10 μM R-(-)-ketamine, but not S-(+)-ketamine, inhibited diazoxide-induced flavoprotein oxidation of rat myocytes, an index of mitochondrial K_{ATP} channel activation. Thus, these observations may point to the similarity of ketamine's inhibitory effects on sarcolemmal and mitochondrial K_{ATP} channels.

It is well established that MgADP simultaneously activates the K_{ATP} channels strongly *via* SUR and inhibits them weakly *via* the ATP binding site on Kir6.2.[23,24] In addition, recent studies demonstrated that intracellular MgADP could modulate the sensitivity of K_{ATP} channel activators and inhibitors.[18,19] The current study indicated that ketamine racemate inhibits K_{ATP} channels *via* SUR subunits and that a physiologic concentration of MgADP (100 μM) significantly enhanced the inhibitory effects of ketamine racemate on all three types of SUR1/Kir6.2, SUR2A/Kir6.2, and SUR2B/Kir6.2 channels (table 2, fig. 4B). It is very difficult to explain the precise mechanisms of the interaction between MgADP and ketamine racemate, it might be possible to speculate that ketamine racemate attenuates (or abolishes) the MgADP-induced activation mediated by the high-affinity site on SUR, thereby exposing the inhibitory effect of MgADP on Kir6.2 such that the overall inhibitory potency is enhanced. Therefore, it might be possible to estimate that ketamine racemate may inhibit K_{ATP} channels con-

taining SUR2 when the channels are opened *in vivo* by increased ADP concentrations, such as those occurring during ischemia.

Our study has several limitations. First, although we used the same amount of SUR cDNA and Kir cDNA for transfection, the genomic integration of the various constructs may have been different, and a varying ratio of SUR *versus* Kir may affect electrophysiological findings. Therefore, it might be better for us to establish the level of expression as well as the ratio of SUR *versus* Kir subunits by polymerase chain reaction method and Western blot analyses. However, in our previous study, we confirmed that the sensitivity to ATP, diazoxide, and glibenclamide and the single-channel conductance of all kinds of reassociated K_{ATP} channels were similar to those of native K_{ATP} channels.[17] Therefore, we expect that the reassociated K_{ATP} channels in the current study can be used as experimental models to characterize the function of the native K_{ATP} channels and that we can draw conclusions from our experimental model. Second, as we discussed above, the threshold concentrations at which ketamine racemate inhibited reassociated K_{ATP} channels containing SUR2 (10–100 μM) are close to free plasma concentrations for ketamine (1.4–30 μM). In contrast, considering that intracellular concentrations of ketamine racemate should be much lower than this range, the concentrations of ketamine racemate we used in the current study should be much higher than the clinically relevant concentrations, suggesting the inhibitory effects of ketamine racemate on sarcolemmal K_{ATP} channels were observed at very high concentrations in the inside-out patch clamp configurations. Third, although we studied the effects of ketamine racemate on sarcolemmal K_{ATP} channels using the inside-out patch clamp configurations, there is a possibility that ketamine racemate acts on these channels from outside of the membrane. To confirm this possibility, it is necessary to study the effects of ketamine racemate using outside-out patch clamp configurations. Fourth, although we studied direct effects of ketamine racemate on sarcolemmal K_{ATP} channel activities, it should be noted that ketamine could affect K_{ATP} channel activities through alteration in nitric oxide concentrations, for example.[32]

In conclusion, ketamine-induced inhibition of sarcolemmal K_{ATP} channels is mediated by SUR subunits. These inhibitory effects of ketamine exhibit a high degree of specificity for cardiovascular K_{ATP} channels, the most critical binding site for ketamine being in the C-terminal set of TMs of SUR2A, and they exhibit at least some degree of stereoselectivity; ketamine racemate was more potent than S-(+)-ketamine. Our results further suggest that MgADP enhances the inhibitory effects of ketamine racemate on sarcolemmal K_{ATP} channel activity under conditions of metabolic inhibition, for example, during ischemia.

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