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Molecular Mechanisms of the Inhibitory Effects of Propofol and Thiamylal on Sarcolemmal Adenosine Triphosphate–sensitive Potassium Channels

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Background: **Both propofol and thiamylal inhibit adenosine triphosphate–sensitive potassium (K_{ATP}) channels. In the current study, the authors investigated the effects of these anesthetics on the activity of recombinant sarcolemmal K_{ATP} channels encoded by inwardly rectifying potassium channel (Kir6.1 or Kir6.2) genes and sulfonylurea receptor (SUR1, SUR2A, or SUR2B) genes.**

Methods: **The authors used inside-out patch clamp configurations to investigate the effects of propofol and thiamylal on the activity of recombinant K_{ATP} channels using COS-7 cells transfected with various types of K_{ATP} channel subunits.**

Results: **Propofol inhibited the activities of the SUR1/Kir6.2 (EC_{50} = 77 μM), SUR2A/Kir6.2 (EC_{50} = 72 μM), and SUR2B/Kir6.2 (EC_{50} = 71 μM) channels but had no significant effects on the SUR2B/Kir6.1 channels. Propofol inhibited the truncated isoform of Kir6.2 (Kir6.2ΔC36) channels (EC_{50} = 78 μM) that can form functional K_{ATP} channels in the absence of SUR molecules. Furthermore, the authors identified two distinct mutations R31E (arginine residue at position 31 to glutamic acid) and K185Q (lysine residue at position 185 to glutamine) of the Kir6.2ΔC36 channel that significantly reduce the inhibition of propofol. In contrast, thiamylal inhibited the SUR1/Kir6.2 (EC_{50} = 541 μM), SUR2A/Kir6.2 (EC_{50} = 248 μM), SUR2B/Kir6.2 (EC_{50} = 183 μM), SUR2B/Kir6.1 (EC_{50} = 170 μM), and Kir6.2ΔC36 channels (EC_{50} = 719 μM). None of the mutants significantly affects the sensitivity of thiamylal.**

Conclusions: **These results suggest that the major effects of both propofol and thiamylal on K_{ATP} channel activity are mediated *via* the Kir6.2 subunit. Site-directed mutagenesis study suggests that propofol and thiamylal may influence Kir6.2 activity by different molecular mechanisms; in thiamylal, the SUR subunit seems to modulate anesthetic sensitivity.**

ADENOSINE triphosphate (ATP)–sensitive potassium (K_{ATP}) channels are composed of two different types of protein subunits, *i.e.*, a sulfonylurea receptor (SUR) and an inwardly rectifying K^+ channel (Kir6).[1,2] They are octamers, assembled from four SUR subunits and four Kir6.x subunits.[3] Coexpressing SUR1 and Kir6.2 (SUR1/Kir6.2) forms the pancreatic β-cell K_{ATP} channel, SUR2A and Kir6.2 (SUR2A/Kir6.2) form the cardiac K_{ATP} channel, SUR2B and Kir6.2 (SUR2B/Kir6.2) form the nonvascular smooth muscle K_{ATP} channel, and SUR2B and Kir6.1 (SUR2B/Kir6.1) form the vascular smooth muscle K_{ATP} channel.[2,4–6]

Because K_{ATP} channels are regulated by intracellular ATP, which binds to the Kir6.2 subunits,[7] they are thought to link cellular metabolism with membrane excitability.[8,9] In addition, because native K_{ATP} channel activators and inhibitors show variable tissue specificity, the different types of cloned K_{ATP} channels exhibit differential ATP sensitivity and pharmacologic properties, which are endowed by their different molecular composition of Kir6 and SUR subunits.[4] In the heart and the brain, the activation of both sarcolemmal and mitochondrial K_{ATP} channels during short periods of preconditioning with ischemia (ischemic preconditioning) protect myocardium and neural tissue from the following prolonged ischemia.[10,11] In vascular smooth muscle, activation of sarcolemmal K_{ATP} channels (SUR2B/Kir6.1) causes vasodilatation.[12] Therefore, great interest has been focused on the effects of anesthetics on K_{ATP} channel activities. In the heart, volatile general anesthetics activate K_{ATP} channels,[13–18] whereas intravenous general anesthetics except opioids[19–21] inhibit K_{ATP} channel activities.[22–25] We have studied the effects of propofol and thiamylal on sarcolemmal K_{ATP} channel activities using cell-attached and inside-out patch clamp configurations.[24,25] Propofol and thiamylal are both representative intravenous anesthetics that are used a great deal in all types of anesthesia in patients who have coronary artery disease and are undergoing a variety of surgical procedures. In our previous studies,[24,25] although both propofol and thiamylal significantly inhibited K_{ATP} channel activities at high concentrations, propofol had no significant effect, but thiamylal significantly inhibited K_{ATP} channel activities at clinically relevant concentrations in isolated rat ventricular myocardium during ischemia.

In the current study, to evaluate the differences of actions on K_{ATP} channel activities between propofol and thiamylal at the receptor level, we investigated the specificity of propofol and thiamylal on different types of reconstitute K_{ATP} channels expressed in K_{ATP}-deficient COS-7 cells (African green monkey kidney cells).

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Materials and Methods

Molecular Biology

The human Kir6.2, rat Kir6.1, rat SUR1, rat SUR2A, and rat SUR2B complementary DNAs (cDNAs) were kindly provided by Susumu Seino, M.D., Ph.D. (Professor and Chairman, Department of Cellular and Molecular Medicine, Chiba University, Chiba, Japan). A truncated form of human Kir6.2 lacking the last 36 amino acids at the C-terminus was obtained by polymerase chain reaction amplification. Polymerase chain reaction products were cloned into the pCR3.1 vector by using the TA cloning system (Invitrogen Corp., Carlsbad, CA) and then cloned into the pcDNA3.1 (−) vector (Invitrogen Corp.) for mammalian expression. Site-directed mutagenesis was performed by using the Site-Directed Mutagenesis system (Invitrogen Corp.). All Kir6.2ΔC36 DNA products were sequenced by using the BigDye terminator cycle sequencing kit and an ABI PRISM 377 DNA sequencer (Applied Biosystems, Foster City, CA) to confirm the sequence.

Cell Culture and Transfection

COS-7 cells were plated at a density of 3×10^5/dish (35 mm in 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 electrophysiologic recordings, either wild-type or mutated pCMV6c Kir alone (1 μg), or pCMV6c Kir (1 μg) plus pCMV6c SUR (1 μg) were transfected into COS-7 cells with green fluorescent protein cDNA as a reporter gene by using lipofectamine and Opti-MEN 1 reagents (Life Technologies Inc., Rockville, MD) according to the manufacturer's instructions. After transfection, cells were cultured for 48–72 h before being subjected to electrophysiologic recordings.

Superfusion System

Glibenclamide, diazoxide, or pinacidil was diluted in superfusate and directly applied to cultured cells in the glass-bottom plastic cell bath (2-ml volume) at a rate of 2–2.5 ml/min using a plastic syringe (50-ml volume), vinyl chloride tubing (0.8-mm ID, 50-cm length), and a syringe pump (Terumo STC-525, Tokyo, Japan). When the dose-dependent effects of propofol or thiamylal 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 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 electrophysiologic measurements. The average percent recovery of K_{ATP} channel activities after washout of propofol or thiamylal was 95 ± 7% of the NP_0 measured before drug treatment.

Electrophysiologic Measurements

Membrane currents were recorded in the inside-out configurations using a patch clamp amplifier as described previously.[24,25] 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) and coated with Sylgard (Dow Corning, Midland, MI). The resistance of pipettes filled with internal solution and immersed in the Tyrode's solution was 3–4 MΩ. The sampling frequency of the single-channel data was 5 KHz with a low-pass filter (1 KHz).

Electrophysiologic Data Analysis

Channel currents were recorded with a patch clamp amplifier (CEZ 2200; Nihon Kohden, Tokyo, Japan) and stored in a personal computer (Aptiva; International Business Machine Corporation, Armonk, NY) with an analog-to-digital converter (DigiData 1200; Axon Instruments, Foster, 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:

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

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 NP_o. Changes of channel activity in the presence of drugs were calculated as the relative channel activity between the values obtained before and after drug treatment.

When the inside-out patches in the ATP-free bath solution are formed, recombinant K_{ATP} channel activity gradually decreases with time. This phenomenon is known as *run-down*. Consequently, data obtained from such experiments with inside-out patches may not accurately represent the relation between a drug and K_{ATP} channel activity. To minimize this time-dependent decrease of the channel activity, we determined the effect of a single concentration of propofol and thiamylal from each inside-out patch within 3 min of patch excision. Under these conditions, the average percent recovery of

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K_{ATP} channel activities after drug washout was 96 ± 8% of the NP_o measured before drug treatment. The drug concentration needed to induce half-maximal inhibition of the channels (EC_{50}) and the Hill coefficient were calculated as follows:

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

where y is the relative NP_o, [D] is the concentration of drug, K_i is the EC_{50}, and H is the Hill coefficient. To analyze of channel kinetics, unitary events were detected using a 50% threshold level method.

Drugs

The following drugs were used: propofol (2,6-diisopropylphenol; Aldrich Chemical Co., Milwaukee, WI), thiamylal sodium (Yoshitomi Chemical, St. Louis, MO), glibenclamide, diazoxide, and pinacidil (Sigma-Aldrich Japan, Tokyo, Japan). Propofol, glibenclamide, diazoxide, and pinacidil were dissolved in dimethyl sulfoxide (the final concentration of solvent was 0.01%), which at a twofold higher concentration did not affect K_{ATP} channel currents. The thiamylal sodium ampule was opened just before use.

Statistics

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

Results

Single-channel Characteristics of Four Different Types of Recombinant K_{ATP} Channel Currents

Four types of reconstituted recombinant K_{ATP} channels were transiently expressed in COS-7 cells, inside-out patches were excised, and the K_{ATP} channel currents were recorded. Figure 1a shows representative examples of these currents. We did not observe channel openings in the cell-attached configuration in any case. However, when the patch was excised into a nucleotide-free solution, the K_{ATP} channels composed of Kir6.2 in combination with SUR1, SUR2A, or SUR2B showed marked current increases. These currents were blocked by 1 mM ATP, which shows that COS-7 cells cotransfected with wild-type Kir6.2 and SUR express functional ATP-sensitive K^+ channels. The SUR2B/Kir6.1 channel was not spontaneously activated by patch excision in the absence of intracellular ATP. Diazoxide (300 μM), a potent opener of K_{ATP} channels, activated the SUR1/Kir6.2, SUR2B/Kir6.2, and SUR2B/Kir6.1 channels with high potency but activated SUR2A/Kir6.2 channels with only lower potency. In all cases, the currents were completely blocked by 3 μM glibenclamide, the sulfonylurea that blocks K_{ATP} channels. The current–voltage relations showed inward rectification and a reversal potential of 0 mV (fig. 1b). Single-channel conductance calculations of the SUR1/Kir6.2, SUR2A/Kir6.2, SUR2B/Kir6.2, and SUR2B/Kir6.1 channels were 72 ± 2 (n = 21), 69 ± 3 (n = 18), 62 ± 2 (n = 20), and 26 ± 2 pS (n = 22) at −60 mV membrane potential, respectively.

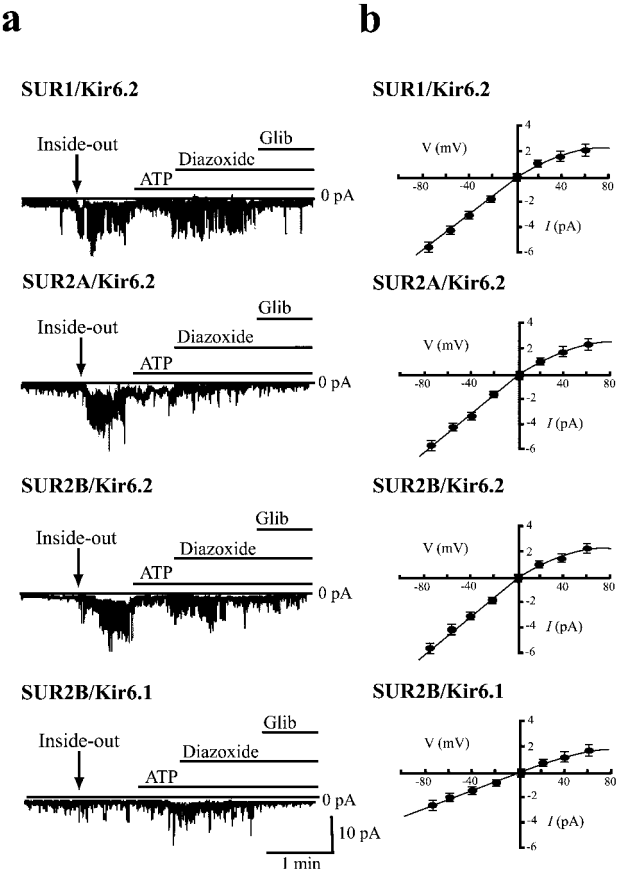

Fig. 1. Single-channel characteristics of reconstituted adenosine triphosphate (ATP)–sensitive potassium (K_{ATP}) channels in the inside-out configurations. (*a*) Representative traces of channel currents recorded from COS-7 cells coexpressing human inwardly rectifying potassium channel (Kir) 6.2 and rat sulfonylurea receptor (SUR) 1 (SUR1/Kir6.2), human Kir6.2 and rat SUR2A (SUR2A/Kir6.2), human Kir6.2 and rat SUR2B (SUR2B/Kir6.2), or rat Kir6.1 and rat SUR2B (SUR2B/Kir6.1) are shown. Membrane potential was clamped at −60 mV. Zero current levels are indicated by the *horizontal lines* marked *0 pA*. ATP (100 μM), diazoxide (300 μM), and glibenclamide (Glib) (3 μM) were added to the intracellular solution as indicated by the *horizontal solid bars*. (*b*) Current–voltage relations for SUR1/Kir6.2 (n = 7), SUR2A/Kir6.2 (n = 9), SUR2B/Kir6.2 (n = 8), and SUR2B/Kir6.1 (n = 9) currents. The curve is linear in the negative membrane potential range but shows rectification with depolarization beyond zero. Data points (*vertical bars*) are presented as mean ± SD.

These functional properties of the reconstituted SUR1/Kir6.2, SUR2A/Kir6.2, SUR2B/Kir6.2, and SUR2B/Kir6.1 channels do represent those of the native K_{ATP} channels that are present on pancreatic β cells, sarcolemmal cardiomyocytes, nonvascular smooth muscle cells, and vas-

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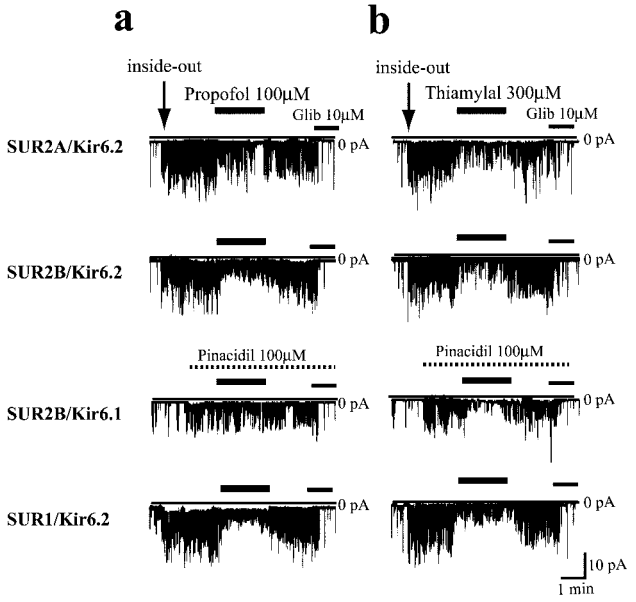

Fig. 2. Effects of propofol and thiamylal on the currents of different reconstituted adenosine triphosphate–sensitive potassium channels in the excised inside-out configuration. Membrane potentials were clamped at −60 mV. Shown are representative examples of sulfonylurea receptor (SUR) 1/inwardly rectifying potassium channel (Kir) 6.2, SUR2A/Kir6.2, SUR2B/Kir6.2, and SUR2B/Kir6.1 currents obtained before and after the application of propofol (100 μM; *a*) or thiamylal (300 μM; *b*). Because SUR2B/Kir6.1 channels are not activated in the inside-out patch clamp configurations, pinacidil (100 μM) was used to activate them. This figure shows that washout of propofol and thiamylal restores channel activities. In all cases, the channel activity was inhibited by glibenclamide (Glib; 10 μM). The periods of propofol or thiamylal administration are marked with *horizontal solid bars*. The periods of pinacidil administration are marked with *horizontal dashed bars*.

cular smooth muscle cells, respectively.[2,4–6] Consequently, these recombinant channels were used as experimental models to characterize the function of the native K_{ATP} channels in greater detail.

Effect of Anesthetics on Recombinant K_{ATP} Channels

To assess the effects of propofol or thiamylal on recombinant K_{ATP} channels, we measured single-channel currents on inside-out patch configurations in the presence of these drugs. The SUR1/Kir6.2, SUR2A/Kir6.2, and SUR2B/Kir6.2 channel currents were inhibited by application of 100 μM propofol to the intracellular membrane surface, with relative channel activities decreasing to 0.40 ± 0.11, 0.39 ± 0.07, and 0.37 ± 0.09 of control, respectively (fig. 2a). However, propofol did not significantly inhibit the SUR2B/Kir6.1 channel currents. Thiamylal at 300 μM blocked the SUR1/Kir6.2, SUR2A/Kir6.2, SUR2B/Kir6.2, and SUR2B/Kir6.1 channel currents, with relative channel activities decreasing to 0.71 ± 0.12, 0.28 ± 0.11, 0.22 ± 0.08, and 0.24 ± 0.06 of control, respectively (fig. 2b). The inhibitory effects of thiamylal and propofol on K_{ATP} channel activities were reversible

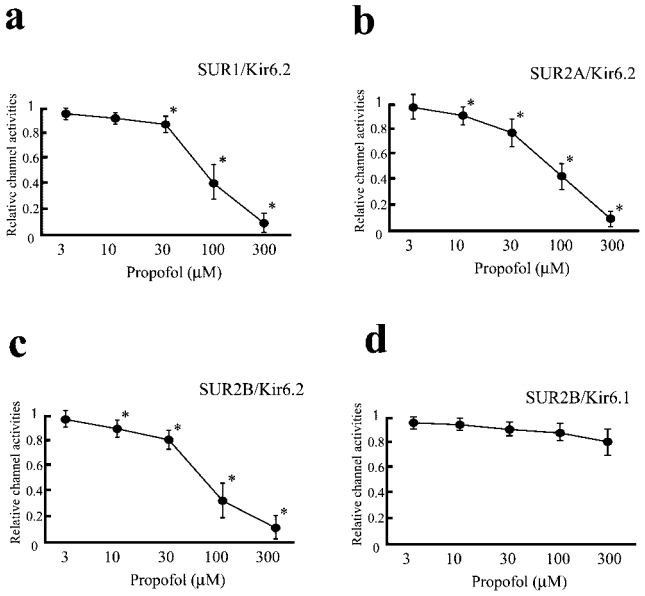

Fig. 3. Dose-dependent effects of propofol on the activities of reconstituted sulfonylurea receptor (SUR) 1/inwardly rectifying potassium channel (Kir) 6.2 (*a*), SUR2A/Kir6.2 (*b*), SUR2B/Kir6.2 (*c*), and SUR2B/Kir6.1 (*d*) channels. Each *vertical bar* constitutes measurements from 16–23 patches (mean ± SD). * $P < 0.05$ *versus* control (before propofol).

because the channel activities recovered after washout (fig. 2).

The dose-dependent effects of thiamylal and propofol on K_{ATP} channel currents are shown in figures 3 and 4, respectively. The EC_{50}s and Hill coefficients of propofol and thiamylal for different types of K_{ATP} channels are

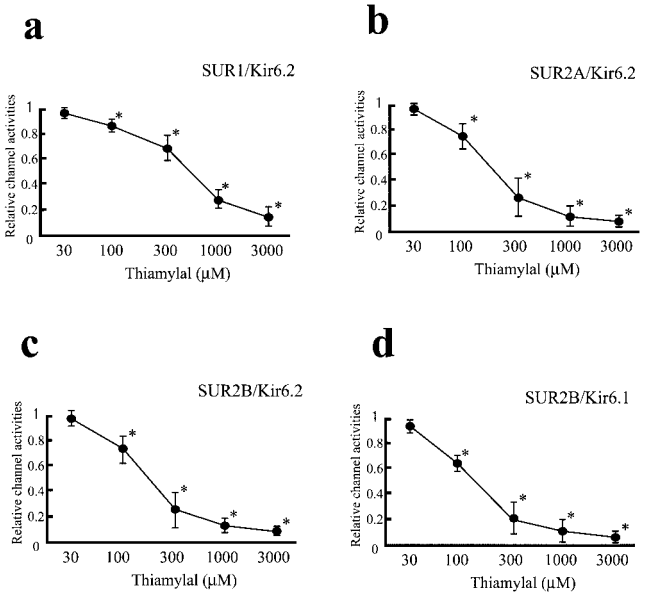

Fig. 4. Dose-dependent effects of thiamylal on the activities of reconstituted sulfonylurea receptor (SUR) 1/inwardly rectifying potassium channel (Kir) 6.2 (*a*), SUR2A/Kir6.2 (*b*), SUR2B/Kir6.2 (*c*), and SUR2B/Kir6.1 (*d*) channels. Each *vertical bar* constitutes measurements from 15–21 patches (mean ± SD). * $P < 0.05$ *versus* control (before thiamylal).

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Table 1. Effects of Propofol and Thiamylal on Differential Type of K_{ATP} Channels

	Propofol			Thiamylal		
	EC_{50}, μM	Hill Coefficient	Conductance (pS) in Presence of Propofol (100 μM)	EC_{50}, μM	Hill Coefficient	Conductance (pS) in Presence of Thiamylal (300 μM)
SUR1/Kir6.2	76.60 ± 2.20 (n = 15)	1.10	74 ± 1 (n = 8)	541 ± 46 (n = 17)*	1.12	70 ± 1 (n = 9)
SUR2A/Kir6.2	72.00 ± 3.12 (n = 12)	1.08	68 ± 3 (n = 7)	248 ± 30 (n = 19)†	1.21	71 ± 2 (n = 8)
SUR2B/Kir6.2	70.66 ± 1.60 (n = 14)	1.12	65 ± 2 (n = 8)	183 ± 23 (n = 12)†	1.09	66 ± 2 (n = 9)
SUR2B/Kir6.1	No effect	No effect	32 ± 3 (n = 10)	170 ± 22 (n = 12)†	1.08	29 ± 6 (n = 7)
Kir6.2ΔC36	79.50 ± 5.08 (n = 18)	1.11	78 ± 1 (n = 9)	719 ± 68 (n = 13)	1.12	77 ± 2 (n = 8)
Native rat cardiac K_{ATP} channel	63.10 ± 1.12 (n = 21)‡	1.06‡	75 ± 1 (n = 15)‡	234 ± 19 (n = 15)§	1.10§	75 ± 2 (n = 17)§

* $P < 0.05$, † $P < 0.01$ *vs.* Kir6.2ΔC36 (for EC_{50} value). ‡ Data from Kawano *et al.*[25] § Data from Tsutsumi *et al.*[24]
K_{ATP} = adenosine triphosphate–sensitive potassium.

summarized in table 1. Propofol inhibited the SUR1/Kir6.2, SUR2A/Kir6.2, and SUR2B/Kir6.2 channel activities with equivalent potencies, whereas even high concentrations of propofol had no significant inhibitory effects on the SUR2B/Kir6.1 channels. Thiamylal inhibits the SUR2A/Kir6.2, SUR2B/Kir6.2, and SUR2B/Kir6.1 channel activities with high affinity, but inhibits the SUR1/Kir6.2 channel activities with lower potency. In all cases, the blockades by thiamylal and propofol did not significantly change the conductance of the K_{ATP} channels, and the Hill coefficients were close to unity, which indicates that only a single propofol or thiamylal molecule has to interact with the channel to inhibit it. Table 1 also indicates that the inhibitory effects of propofol and thiamylal for the SUR2A/Kir6.2 channels are similar to those previously reported for the native rat cardiac K_{ATP} channels.[24,25]

Single-channel Characteristics of Kir6.2ΔC36 Currents

It has been previously reported that although wild-type Kir6.2 alone does not show functional channel activity, removal of the last 26 or 36 amino acids at the C-terminus of Kir6.2 (Kir6.2ΔC26 or Kir6.2ΔC36) results in channels that show significant currents in the absence of SUR.[7] We confirmed this observation by using a Kir6.2ΔC36 mutant, which showed single-channel currents (fig. 5a). Coexpression of SUR1 enhanced the Kir6.2ΔC36 currents. The Kir6.2ΔC36 currents were blocked by ATP, which confirms that Kir6.2 bears an intrinsic ATP-inhibitory site. The current–voltage relation for the Kir6.2ΔC36 currents was the same as those of SUR1/Kir6.2ΔC36.

Effect of Anesthetics on Kir6.2ΔC36 Channel Activity

Propofol at 100 μM and thiamylal at 1,000 μM inhibited the Kir6.2ΔC36 currents, with relative channel activities decreasing to 0.35 ± 0.12 ms, and 0.27 ± 0.09 of control, respectively (fig. 5b). The dose-dependent effects of thiamylal and propofol on Kir6.2ΔC36 currents are shown in figure 5c. The half-maximal blocks (EC_{50}) of propofol and thiamylal are summarized in table 1. These observations suggest that both propofol and thiamylal target the Kir6.2 subunit. SUR does not enhance the propofol sensitivity of Kir6.2, but the inhibitory effect of thiamylal was enhanced by coexpression with SUR, especially coexpression with the SUR2 subunit. The Hill coefficients of propofol and thiamylal for Kir6.2ΔC36 did not change in comparison with the other reconstituted K_{ATP} channels (table 1).

Effect of Anesthetics on Mutations in Kir6.2 Molecules

We next identified the regions of Kir6.2 that play critical roles in the inhibition of Kir6.2ΔC36 channel activity mediated by propofol or thiamylal using systematically mutating Kir6.2ΔC36. The effects of propofol (100 μM), thiamylal (1,000 μM) or ATP (1 mM), which all inhibit wild-type Kir6.2ΔC36 currents by less than 50%, were tested on each mutant (fig. 6).

It has been previously reported that the mutations that affect ATP sensitivity are located in two distinct Kir6.2 lesions, namely, the R50D lesion in the N-terminus and a lesion in the C-terminus that includes C166S, T171A, K185Q, and G334D.[26,27] We confirmed these observations (fig. 6). Interestingly, propofol sensitivity was also decreased by the C166S, T171A, and K185Q mutations. We identified another mutation, R31E, that also suppressed the ability of propofol to inhibit Kir6.2ΔC36 currents ($P < 0.001$ *vs.* wild-type Kir6.2ΔC36). Several mutations, including C166S and T171A, also caused smaller but nonsignificant shifts in the ability of 1,000 μM thiamylal to inhibit Kir6.2ΔC36 currents (fig. 6).

Analysis of Single-channel Currents

Recent mutagenesis studies have suggested that C166 or T171 in Kir6.2 plays a role in the intrinsic gating of the channel, possibly by influencing a gate located at the intracellular end of the pore.[26–28] We found that thiamylal but not propofol increases the long closed time and

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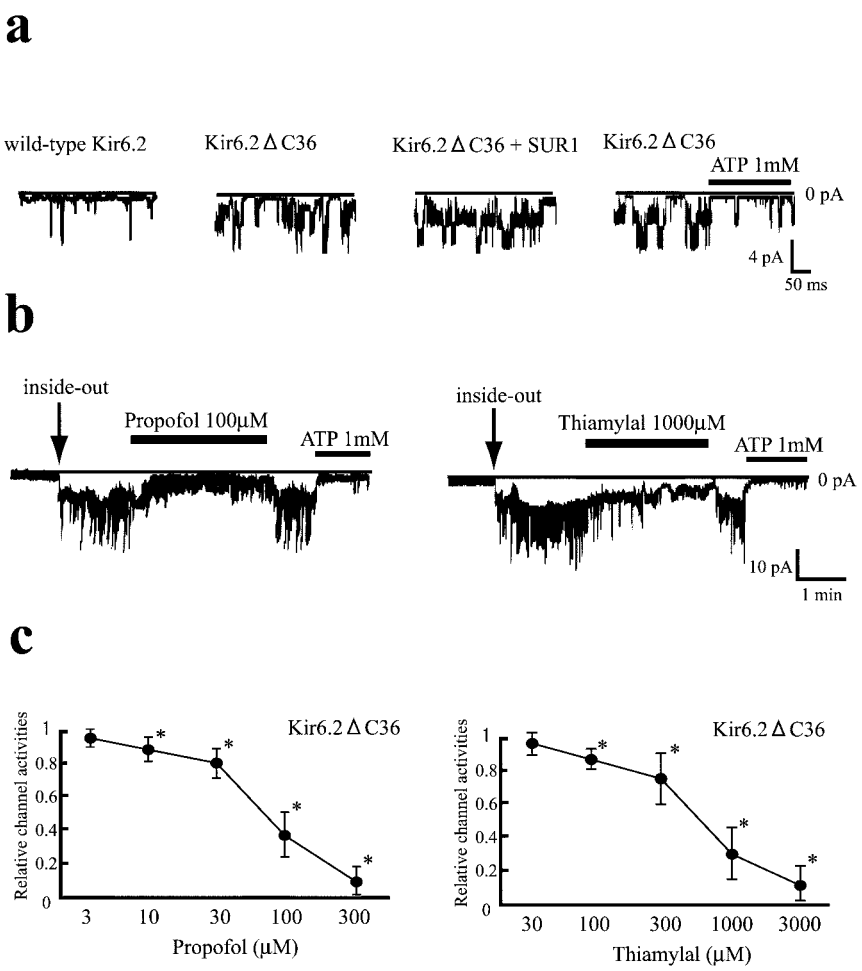

Fig. 5. Effects of propofol and thiamylal on the channel activities of the truncated isoform of inwardly rectifying potassium channel (Kir) 6.2 (Kir6.2ΔC36), which can form functional adenosine triphosphate (ATP)–sensitive potassium channels in the absence of sulfonylurea receptor (SUR) molecules, in the excised inside-out configuration. Membrane potentials were clamped at −60 mV. (*a*) Single-channel currents recorded from COS-7 cells transfected with complementary DNA encoding wild-type Kir6.2, Kir6.2ΔC36, or Kir6.2ΔC36 + SUR1 in the excised inside-out configuration. (*b*) Representative examples of Kir6.2ΔC36 currents obtained before and after the application of propofol (100 μM) or thiamylal (1,000 μM). The periods of drug treatment are marked with *horizontal bars*. (*c*) The dose dependence of Kir6.2ΔC36 channel activities on the dose of propofol and thiamylal. Each *vertical bar* constitutes measurements from 10–12 patches (mean ± SD). * $P < 0.05$ *versus* control (before propofol or thiamylal).

decreases the channel P_o of the C166S and T171A mutants (fig. 7 and table 2).

Discussion

We have demonstrated here by using K_{ATP} channels reconstituted in COS-7 cells that the intravenous anesthetics propofol and thiamylal specifically inhibit particular K_{ATP} channels. Propofol inhibits Kir6.2-containing channels combined with any of the three SUR molecules tested (SUR1, SUR2A, and SUR2B) but has no effect on SUR2B/Kir6.1 channels, whereas thiamylal strongly blocks SUR2A/Kir6.2, SUR2B/Kir6.2, and SUR2B/Kir6.1 channels but has a weaker effect on SUR1/Kir6.2 channels. These observations suggest that propofol and thiamylal could have tissue-specific inhibitory actions *in vivo*. These observations are also consistent with our previous findings that both propofol and thiamylal inhibit the native rat cardiac K_{ATP} channel (SUR2A/Kir6.2) in patch clamp configuration.[24,25]

That propofol selectively blocks Kir6.2-containing channels and also inhibits Kir6.2ΔC36 currents in the concentration range tested supports the notion that Kir6.2 may be the primary target of propofol (table 1). In addition, that propofol does not significantly inhibit SUR2B/Kir6.1 channels suggests that the Kir6.1 does not bear the propofol inhibitory site found on Kir6.2. This makes propofol the first drug reported to selectively block Kir6.2 but not Kir6.1.

The isoforms of Kir6.2 that lack the C-terminal 26 or 36 amino acids retain their sensitivity to ATP as an intrinsic property.[7] In the study reported here, we demonstrated that the K185Q mutation in Kir6.2ΔC36 eliminates the abilities of both ATP and propofol to inhibit channel activity without noticeably affecting the single-channel kinetics (fig. 6). This indicates that the site by which propofol mediates K_{ATP} channel inhibition is at least partly identical to that involved in the ATP block. Recent studies have also suggested that apart from the C-terminal K185 residue, the distal part of the C-terminal region (amino acids 333–338) and the N-terminal R50 residue participate in ATP sensitivity.[26,28] Although mutations of these regions (R50G, G334D) did not abrogate propofol-mediated channel inhibition, we did identify another mutation in the N-terminus, namely, R31E, which significantly reduces the inhibitory effects of propofol (fig. 6). These results indicate that both the N- and C-termini of Kir6.2 participate in the inhibition mediated by propofol as well as that induced by ATP.

In contrast with propofol, we found that thiamylal

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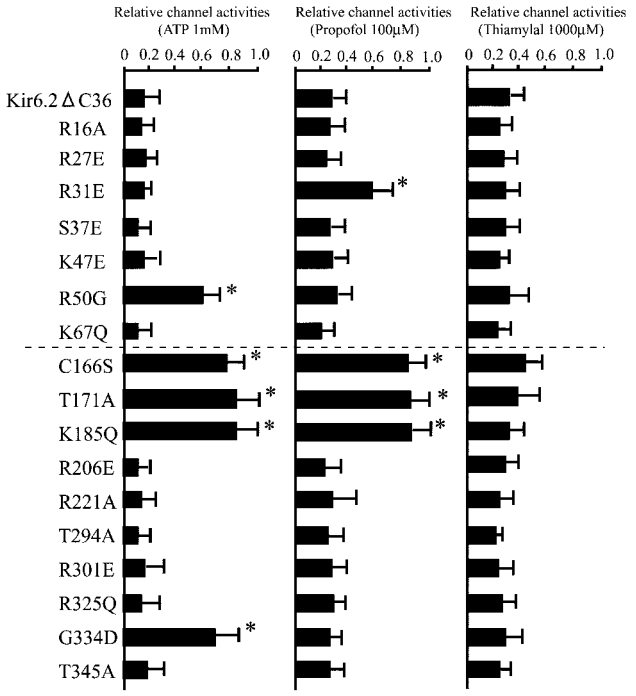

Fig. 6. Effect of mutations in the truncated isoform of inwardly rectifying potassium channel (Kir) 6.2 (Kir6.2ΔC36) on channel inhibition mediated by adenosine triphosphate (ATP; 1 mM), propofol (100 μM), or thiamylal (1,000 μM). The relative channel activities were calculated by dividing the channel activity in the presence of an inhibitor with the activity in the absence of ATP. Amino acids are denoted by the *single-letter codes*. Each *horizontal bar* constitutes measurements from 8–12 patches (mean ± SD). * $P < 0.05$ *versus* wild-type Kir6.2ΔC36.

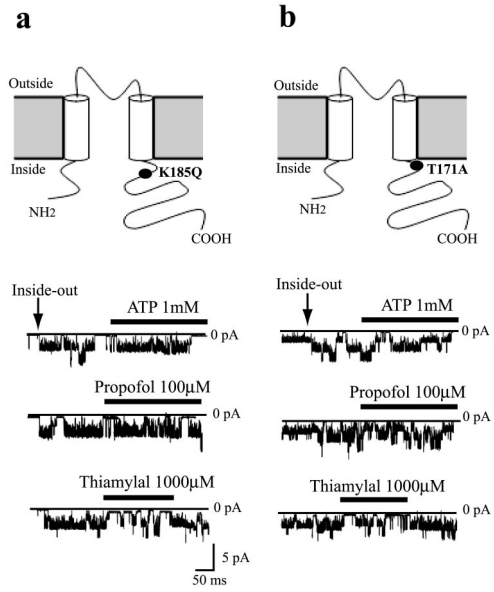

Fig. 7. Effects of adenosine triphosphate (ATP; 1 mM), propofol (100 μM), or thiamylal (1,000 μM) on single-channel currents of the truncated isoform of inwardly rectifying potassium channel 6.2 (Kir6.2ΔC36) molecules bearing the K185Q and T171A mutations. K185Q (lysine residue at position 185 to glutamine) currents (*a*) and T171A (threonine residue at position 185 to alanine) currents (*b*) recorded at −60 mV from inside-out patches excised from COS-7 cells. The periods of drug treatment are marked with *horizontal bars*. COOH and NH_2 indicate the C- and N-terminus of Kir6.2ΔC36 channel, respectively.

inhibits all four of the recombinant sarcolemmal K_{ATP} channels (albeit SUR1/Kir6.2 less potently; fig. 4 and table 1) as well as native rat cardiac K_{ATP} channels[24] and the Kir6.2ΔC36 channels. In addition, although SUR molecules did not enhance the propofol sensitivity of Kir6.2ΔC36 channels, the thiamylal sensitivity of Kir6.2ΔC36 channels was enhanced by coexpression with SUR, especially SUR2, suggesting that thiamylal likely has tissue-specific effects based on differential sensitivities to thiamylal exhibited by the various types of the K_{ATP} channels (table 1, EC_{50} values). Furthermore, the current study indicates more important findings regarding the molecular mechanisms of thiamylal actions on various types of the K_{ATP} channels. One possibility is that thiamylal may bind to both the SUR and Kir molecules. Another plausible possibility is that thiamylal acts on the Kir subunit, but its action is modulated by the SUR, because the Hill coefficients of approximately 1.1–1.2 suggest that the binding of one thiamylal is sufficient to result in the inhibition of channel activity. In addition, the notion that SUR modulates thiamylal sensitivity is also supported from the EC_{50} value where the Kir6.2ΔC36, in the absence of SUR, is the least sensitive to thiamylal. Our data also show that for the SUR2B/Kir6.2 and SUR2B/Kir6.1 channels, the EC_{50} values are similar despite differences in the Kir subunit. On the other hand, for the SUR1/Kir6.2 and SUR2A/Kir6.2 channels, the EC_{50} values are not similar, likely because of the different SURs. Again, this can be accounted for in the absence of thiamylal binding to SUR.

It has been reported that the cytosolic end of the second transmembrane domain of Kir6.2 may play an important role in the gating of the K_{ATP} channel pore.[26–28] In agreement with these reports, we showed here that mutations in this region, namely Kir6.2ΔC36–C166S and –T171A, markedly increase the channel P_o by reducing the long close time (table 2). The finding that the inhibitory effect of propofol is also reduced by these mutations suggests that these mutations affect the ability of propofol to block channel activity by changing the channel gating kinetics rather than by altering the affinity of propofol for its binding site. In contrast, thiamylal increased the long closed times with all of the Kir6.2ΔC36 mutants. In particular, thiamylal converted the long burst kinetics of Kir6.2ΔC36-T171A currents to the long closed kinetics that were typically observed with the Kir6.2ΔC36 channels (fig. 7b and percent long closed time of Kir6.2ΔC36-T171A in table 2). It is therefore possible that thiamylal acts as an open channel blocker of the Kir6 channel.

Recent investigations have established that K_{ATP} channel activation plays an important role in ischemic preconditioning of myocardium and neural tissue, during skeletal muscle ischemia, and in the regulation of vascu-

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Table 2. Single-channel Kinetics of Kir6.2ΔC36 and Kir6.2ΔC36-T171A Currents

	No.	Mean Open Time, ms	Mean Short Closed Time, ms	Mean Long Closed Time, ms	Mean Burst Duration Time, ms	Percent Long Closed Time
Kir6.2ΔC36						
Control	5	1.2 ± 0.2	0.34 ± 0.02	5.8 ± 0.6	2.8 ± 0.5	42.8 ± 12.6
Propofol	4	0.9 ± 0.2	0.29 ± 0.05	17.8 ± 3.2	2.6 ± 0.4	62.8 ± 15.2
Thiamylal	6	1.1 ± 0.3	0.36 ± 0.06	20.5 ± 6.4	2.2 ± 0.4	68.5 ± 12.0
Kir6.2ΔC36-T171A						
Control	5	3.0 ± 1.1	0.38 ± 0.14	18.5 ± 7.2	96 ± 21	5.2 ± 1.2
Propofol	5	2.9 ± 0.7	0.42 ± 0.10	19.6 ± 6.8	90 ± 19	5.5 ± 0.7
Thiamylal	6	2.7 ± 0.9	0.54 ± 0.21	35.9 ± 10.2	16 ± 8	32.8 ± 12.3

lar smooth muscle tone.[10–12] In addition, it has been shown that these desirable endogenous effects of K_{ATP} channel activation can be induced pharmacologically by K_{ATP} channel openers.[29,30] These observations suggest new therapeutic intervention strategies that may specifically benefit patients who are at risk for development of untoward ischemic events during cardiac, vascular, or neurologic surgery. In addition, it seems that volatile anesthetics, including isoflurane, desflurane, and sevoflurane, can also protect the myocardium against stunning and infarction by activating K_{ATP} channels.[14–16] In contrast to the volatile anesthetics, however, we demonstrate here that two representatives intravenous anesthetics, propofol and thiamylal, interact with one or both of the Kir6 subunits to block the K_{ATP} channel currents in a concentration-dependent manner. Recent functional studies have provided direct evidence that each Kir6.1 and Kir6.2 play separate physiologic roles.[31–34] Kir6.2 forms the pore region of the K_{ATP} channels in the heart, brain, and skeletal muscle and activation of these channels has shown to be important for cell protection.[10–12] In contrast, the Kir6.1-containing K_{ATP} channel is critical in the regulation of vascular tonus, especially in the coronary arteries, and it is known that it protects against vasospasm during and after myocardial ischemia.[31] Therefore, our results indicate that intravenous anesthesia with propofol and thiamylal may impair the beneficial effects mediated by K_{ATP} channel activation in various organs. However, it is possible that propofol may not significantly inhibit channel activity at the concentrations that are generally used in the clinical setting. Plasma concentrations of propofol up to 50 μM after clinical intravenous induction administration have been reported.[35] If protein binding is taken into account, the clinically relevant concentration of propofol is less than 2 μM.[25] The concentrations of propofol needed to inhibit K_{ATP} channel activity *in vitro* are higher than these postulated free plasma concentrations, which suggests that propofol at the concentrations used clinically may not affect K_{ATP} channel activity. In the current study, the differential propofol effects on Kir6.1 and Kir6.2 are evident at concentrations greater than 10–30 μM (fig. 3); it is unlikely that this differential effect will be encountered in the clinical setting. However, because propofol is the first drug reported to selectively block Kir6.2 but not Kir6.1, it may be useful in other experimental settings that require modulation of the functions induced by Kir6.2.

Unlike propofol, thiamylal may well significantly depress K_{ATP} channel activity when it is used as an anesthetic. Plasma concentrations of thiamylal up to 0.5 mM after clinical intravenous induction administration have been reported.[36] If protein binding is taken into account, the clinically relevant concentrations of thiamylal range from 0.05 to 0.08 mM.[24] Thiamylal inhibits all four recombinant K_{ATP} channels at these clinical concentrations (fig. 4). Therefore, it is likely that when thiamylal is used as an intravenous anesthetic, it may inhibit the K_{ATP} channel activities in the patient. These results may suggest that thiamylal impairs the endogenous organ-protective mechanism mediated by K_{ATP} channels against intraoperative ischemic or hypoxic injury. However, there are other well-established mechanisms of organ protection that do not involve K_{ATP} channel activities. Thiamylal is a likely candidate for neuroprotection and has been used as such in our country.[37]

Our study has several limitations. First, we combined cDNAs from different species (human and rat) to reconstitute K_{ATP} channels. Sequence differences between human and rat cDNAs may induce possible influences on the electrophysiologic properties of K_{ATP} channels. However, in most previous studies,[2,4] K_{ATP} channels were reconstituted by the combination of Kir and SUR cDNAs from different species (rat or mouse), and it has been confirmed that the electrophysiologic properties of all kinds of reconstructed K_{ATP} channels are similar to those of the native K_{ATP} channels. In addition, 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 electrophysiologic 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 the current study, we confirmed that the sensitivity to ATP, diazoxide, and glibenclamide and the single-channel conductance of all kinds of reconstituted K_{ATP} channels were similar to

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those of native K_{ATP} channels (fig. 1). Therefore, we expect that the reconstituted 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, we studied the effects of propofol and thiamylal on sarcolemmal K_{ATP} channels because mitochondrial K_{ATP} channels have not been cloned. However, in the heart and brain, mitochondrial rather than sarcolemmal K_{ATP} channels might play an important role for the protection of these tissues. In the future, we must study the molecular mechanisms of these anesthetics on reconstituted mitochondrial K_{ATP} channels.

In conclusion, propofol inhibits all channels with Kir6.2 but does not inhibit SUR2B/Kir6.1, which is the vascular smooth muscle channel. In contrast, thiamylal inhibits all channels with either Kir6.1 or Kir6.2. These results, as well as site-directed mutagenesis studies, suggest that propofol and thiamylal may act *via* the Kir6.2 subunit, albeit by different molecular mechanisms. The N- and C-termini of Kir6.2 participate in the inhibition of K_{ATP} channel by propofol. In the case of thiamylal, the SUR subunit seems to modulate anesthetic activity on the Kir subunit.

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