J Anesth (2007) 21:472–479
DOI 10.1007/s00540-007-0551-9

Journal of
Anesthesia

© JSA 2007

Effects of intracellular MgADP and acidification on the inhibition of cardiac sarcolemmal ATP-sensitive potassium channels by propofol

Hirohide Yamada[1], Takashi Kawano[1], Katsuya Tanaka[1], Sonoko Yasui[2], Kazuaki Mawatari[2], Akira Takahashi[2], Yutaka Nakaya[2], and Shuzo Oshita[1]

[1] Department of Anesthesiology, Tokushima University School of Medicine, 3-18-15 Kuramoto, Tokushima 770-8503, Japan
[2] Department of Nutrition and Metabolism, Tokushima University School of Medicine, Tokushima, Japan

Abstract
Purpose. Propofol inhibits adenosine triphosphate-sensitive potassium (K_{ATP}) channels, which may result in the blocking of ischemic preconditioning in the heart. During cardiac ischemia, sarcolemmal K_{ATP} channel activity is regulated by the increased levels of cytosolic metabolites, such as adenosine diphosphate (ADP) and protons. However, it remains unclear whether these cytosolic metabolites modulate the inhibitory action of propofol. The aim of this study was to investigate the effects of intracellular MgADP and acidification on K_{ATP} channel inhibition by propofol.
Methods. We used inside-out patch-clamp configurations to investigate the effects of propofol on the activities of recombinant cardiac sarcolemmal K_{ATP} channels, which are reassociated by expressed subunits, sulfonylurea receptor (SUR) 2A, and inwardly rectifying potassium channels (Kir6.2).
Results. In the absence of MgADP, propofol inhibited the SUR2A/Kir6.2 channel currents in a concentration-dependent manner, and an IC_{50} of 78 μM. Increasing the intracellular MgADP concentrations to 0.1 and 0.3 mM markedly attenuated the inhibitory potency of propofol, and shifted the IC_{50} to 183 and 265 μM, respectively. Moreover, decreasing the intracellular pH from 7.4 to 6.5 attenuated the inhibitory potency of propofol, and shifted the IC_{50} to 277 μM. In addition, propofol-induced inhibition of truncated Kir6.2ΔC36 currents, which form a functional channel without SUR2A, was not affected by an increase in intracellular MgADP. However, intracellular acidification (pH 6.5) significantly reduced the propofol sensitivity of Kir6.2ΔC36 channels.
Conclusion. Our results demonstrated that the existence of intracellular MgADP and protons attenuated the direct inhibitory potency of propofol on recombinant cardiac sarcolemmal K_{ATP} channels, via SUR2A and Kir6.2 subunits, respectively.

Key words Propofol · Intravenous anesthetics · Potassium channel · Adenosine diphosphate · Acidification

Address correspondence to: T. Kawano
Received: October 23, 2006 / Accepted: June 29, 2007

Introduction

In cardiomyocytes, activation of sarcolemmal adenosine triphosphate-sensitive potassium (K_{ATP}) channels protects the heart against myocardial ischemia by shortening the duration of the cardiac action potential, resulting in reduced mechanical contraction and energy sparing [1,2]. During myocardial ischemia, the opening of K_{ATP} channels is induced not only by a reduction in cytosolic ATP levels but also by an increase in MgADP or protons, as principal physiologic regulators [3,4]. It is well established that the sensitivity of some pharmacological K_{ATP} channel regulators is also modulated by these cytosolic metabolic factors [5–7].

Our previous studies demonstrated that propofol directly inhibited native and recombinant cardiac sarcolemmal K_{ATP} channel activities during simulated ischemia [8, 9]. These observations suggest that intravenous anesthetics may impair the endogenous organ protective mechanisms mediated by K_{ATP} channels. In our previous experimental model, however, simulated ischemia was induced by lowering the intracellular ATP concentration, and there was no evidence that intracellular MgADP and protons modulated propofol-inhibitory effects on sarcolemmal K_{ATP} channels. Therefore, it is important, in representing in vivo conditions, to examine the effect of changes in intracellular concentrations of MgADP and protons on the inhibition of K_{ATP} channels by propofol.

Cardiac sarcolemmal K_{ATP} channels are composed of an ATP-binding cassette protein, sulfonylurea receptor (SUR) 2A, and an inwardly rectifying K^+ channel (Kir) subunit, Kir6.2; SUR acts as a regulatory subunit while Kir subunits form the ATP and pH-sensitive channel pore [10–12].

In the present study, we used patch-clamp techniques to examine the effects and molecular mechanisms of either intracellular MgADP or acidification-induced

alteration of propofol in recombinant cardiac sarcolemmal K_{ATP} channels expressed in COS-7 cells.

Materials and methods

Molecular biology

cDNAs (Kir6.2 and SUR2A) and the expression vector pCMV6C were kindly provided by Dr. Susumu Seino, MD., Ph.D. (Professor and Chairman, Department of Cellular and Molecular Medicine, Chiba University, Chiba, Japan). Co-expression of SUR2A and Kir6.2 (SUR2A/Kir6.2) forms the cardiac K_{ATP} channel. A truncated form of Kir6.2 lacking the last 36 amino acids at the C terminus was obtained by polymerase chain reaction (PCR) amplification, as previously described [9]. All DNA products were sequenced using a BigDye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). In addition, an ABI PRISM 377 DNA sequencer (Applied Biosystems) was used to confirm the sequence.

Cell culture and transfection

COS-7 cells, which natively lack K_{ATP} channels [13], were plated at a density of 3×10^5 per dish (35-mm diameter) and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum. A full-length Kir6.2 cDNA and SUR2A cDNA were subcloned into the mammalian expression vector pCMV6c. For electrophysiological recordings, mutated pCMV6c Kir6.2 alone (1 µg) or pCMV6c Kir6.2 (1 µg) and pCMV6c SUR2A (1 µg) was transfected into COS-7 cells with green fluorescent protein cDNA (pEGFP-N1; Clontech Laboratories, Palo Alto, CA, USA) as a reporter gene, using lipofectamine and Opti-MEM 1 reagents (Life Technologies, Rockville, MD, USA) according to the manufacturer's instructions. After transfection, cells were cultured for 48–72 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 [14,15]. Transfected cells were identified by their green fluorescence under a microscope. The intracellular solution contained 140 mM KCl, 2 mM ethyleneglycol tetraacetic acid (EGTA), 2 mM $MgCl_2$, and 10 mM hydroxy ethylenepiperazine ethanesulfonic acid (HEPES; pH = 7.4). The pH of the intracellular solution was adjusted with HCl and the pH inside the bath was continuously measured with a pH meter (model 611; Orion Research Instruments, Cambridge, MA, USA). 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 bath temperature 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 Tyrode's solution was 5–7 MΩ. The sampling frequency of the single-channel data was 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; International Business Machine, Armonk, NY, USA) with an analog-to-digital converter (DigiData 1200; Axon Instruments, Foster, CA, USA). 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 $j = 0, 1, 2, \ldots, N$ 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. The *NP*o in the presence of drugs was normalized to the baseline *NP*o value obtained before drug administration and presented as the relative channel activity. To prevent propofol adsorption into the delivery system minimally, propofol was diluted in superfusate and directly applied to the cells in the glass cell bath (2-ml volume) at a rate of $3\,ml \cdot min^{-1}$, using a glass syringe (30-ml volume), vinyl chloride tubing (0.8-mm internal diameter [ID]; 50-cm length), and a syringe pump (Terumo STC-525; Tokyo, Japan); preliminary studies showed that propofol concentrations of superfusate in the cell bath were not significantly influenced by this propofol delivery system. 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 have shown that this stopping had no significant effects on electrophysiological measurements. The average percent recovery of K_{ATP} channel activities after the

washout of study drugs was 96 ± 5% of the *NP*o measured before drug treatment.

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

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

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

Drugs

The following drugs were used: propofol (2, 6-diisopropylphenol; Aldrich Chemical, Milwaukee, WI, USA), glibenclamide, and pinacidil (Sigma-Aldrich Japan, Tokyo, Japan). Propofol, glibenclamide, and pinacidil were dissolved in dimethylsulfoxide (DMSO) (the final concentration of solvent was 0.01%), which, at a two-fold higher concentration than the final concentration, did not affect K_{ATP} channel currents.

Statistics

All data values were presented as means ± SDs. Differences between data sets were evaluated by analysis of variance (ANOVA) followed by the Scheffé *F* test. $P < 0.05$ was considered significant.

Results

Sarcolemmal cardiac K_{ATP} channels were heterologously expressed in COS-7 cells. Our previous experiments have shown that the single-channel characteristics of recombinant SUR2A/Kir6.2 channels were similar to those of native K_{ATP} channels [9,14,15].

Effects of intracellular MgADP on propofol-induced inhibition of SUR2A/Kir6.2 channels

In Fig. 1, the single K_{ATP} channel current is shown in inside-out membrane patches. We did not observe any

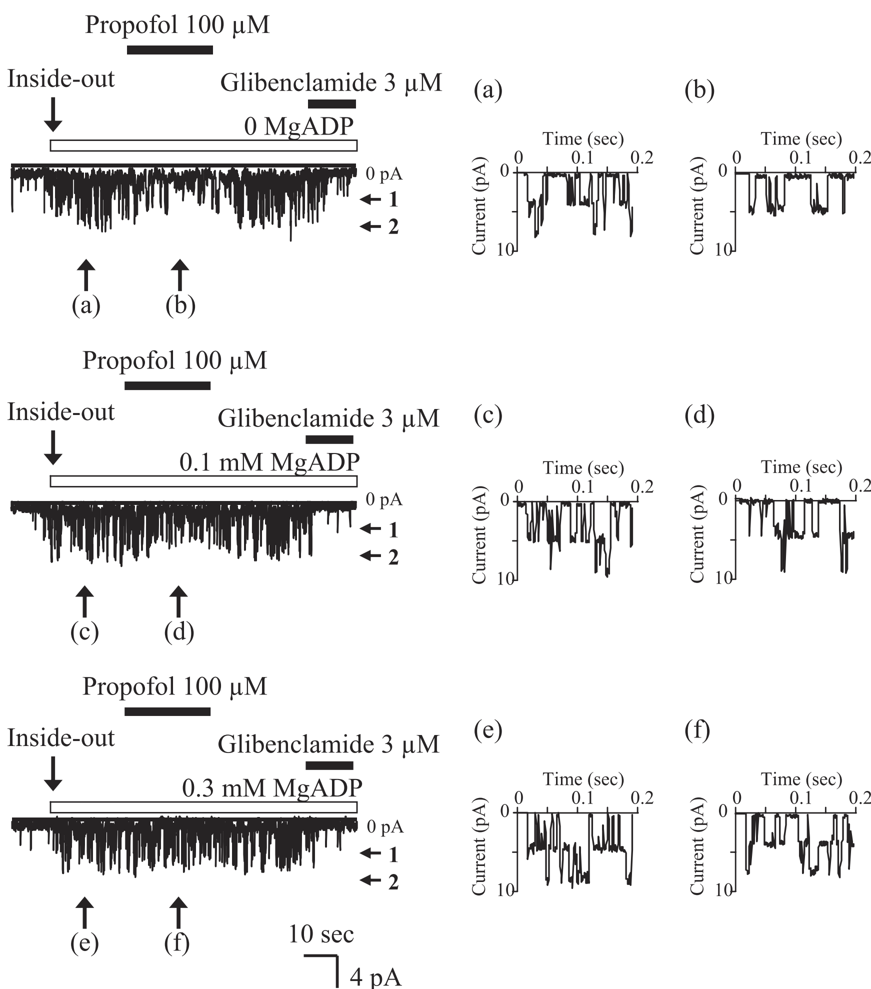

Fig. 1. Effects of intracellular MgADP on the propofol-induced inhibition of sulfonylurea receptor (SUR) 2A/inwardly rectifying potassium channel (Kir) 6.2 channels. Intracellular MgADP lowers the inhibitory effects of propofol on the SUR2A/Kir6.2 channels. Single-SUR2A/Kir6.2 currents recorded from the inside-out patches are illustrated. Membrane potential was clamped at −60 mV. Zero current levels are indicated by the *horizontal lines marked "0 pA"*. The number of open channels is shown on the right side of the traces, *with arrows*. The *bars* shown above each trace indicate the period of exposure of membrane patches to the test solution containing propofol or MgADP. Enlarged records are shown on the *right*

channel openings in the cell-attached configuration. However, when the patch was excised into an ATP-free solution, the SUR2A/Kir6.2 channels showed marked current increases. In Fig. 1, 100 μM propofol was superfused to test whether the drug-induced channel inhibition was modified by the presence of various concentrations of MgADP. Exposure to 100 μM propofol inhibited the opening of the channel. Propofol significantly inhibited the activities of the SUR2A/Kir6.2 channel in the absence of MgADP, with relative channel activities decreasing to 0.47 ± 0.18 of the control (Fig. 1, top trace). However, the channel inhibition induced by 100 μM propofol was weaker in the presence of 0.1 mM MgADP than in 0 mM MgADP, with relative channel activities decreasing to 0.71 ± 0.12 of the control (middle trace). Furthermore, 100 μM propofol failed to inhibit the opening of the channel during superfusion of 0.3 mM MgADP, with relative channel activities decreasing to 0.91 ± 0.11 of the control (bottom trace).

The concentration-inhibition relationship between propofol and the SUR2A/Kir6.2 channel activities was determined by normalizing the channel activities obtained in the test solutions to the control current in which propofol was absent (Fig. 2). Increasing the MgADP concentration from 0 to 0.1 mM and 0.3 mM shifted the IC_{50} from 78 μM to 183 and 265 μM, respectively. The Hill coefficient was 1.03, 1.12, and 1.14, respectively.

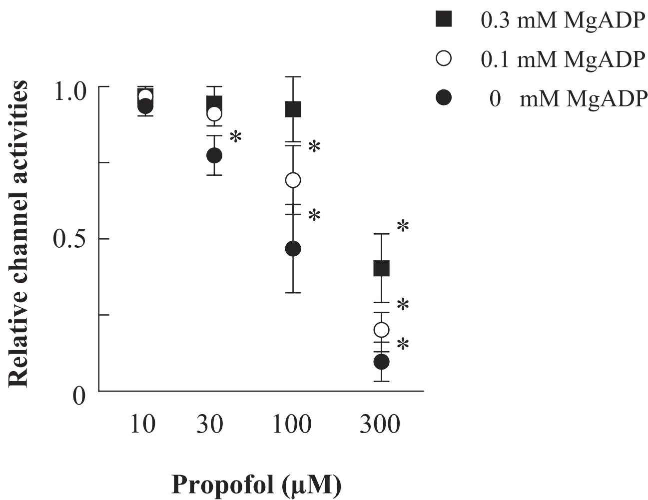

Fig. 2. Concentration-inhibition relationship of sulfonylurea receptor (SUR) 2A/inwardly rectifying potassium channel (Kir) 6.2 channel activity for propofol obtained during superfusion of various concentrations of intracellular MgADP. *Each vertical bar* constitutes measurements from 12 to 15 patches (mean ± SD). *$P < 0.05$ versus baseline (before propofol)

Effects of intracellular acidification on propofol-induced inhibition of SUR2A/Kir6.2 channels

Next, we examined whether intracellular acidification also modified the inhibitory effects on SUR2A/Kir6.2 channels by propofol, using an inside-out patch-clamp configuration. Representative examples for the effects of propofol are shown in Fig. 3. Application of 100 μM propofol at normal pH bath solution to the inside of the membrane surface significantly inhibited the inside-out SUR2A/Kir6.2 currents in the patch area, with relative

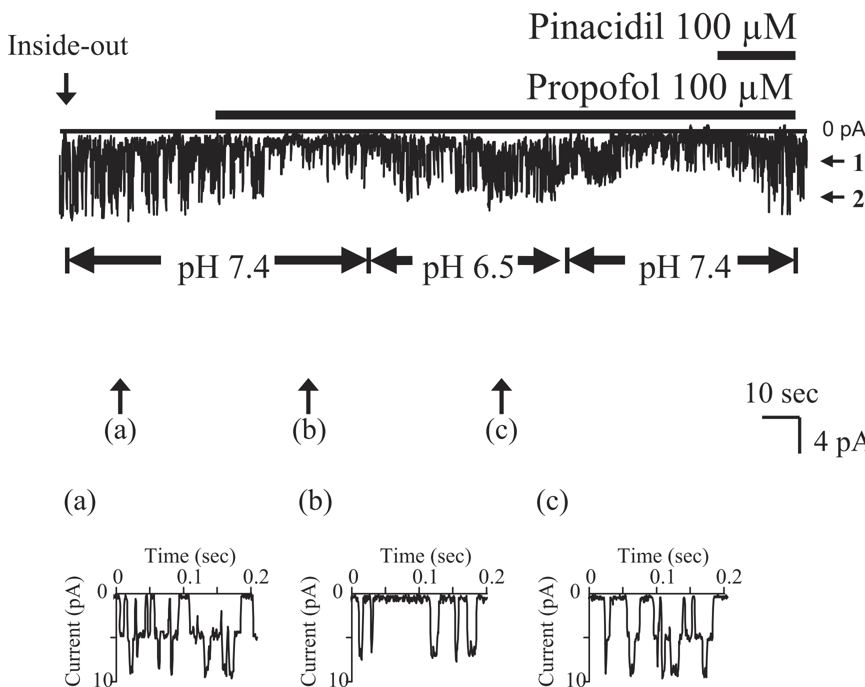

Fig. 3. Effects of intracellular acidification on the propofol-induced inhibition of sulfonylurea receptor (SUR) 2A/inwardly rectifying potassium channel (Kir) 6.2 channels in the excised inside-out configuration. Propofol and pinacidil, in solutions buffered at the indicated external pH values, were superfused in the bath solution. The pipette solution was maintained at pH 7.4. Membrane potential was clamped at −60 mV. Zero current levels are indicated by the *horizontal lines marked "0 pA"*. The number of open channels is shown on the right side of the traces, *with arrows*. The *bars* shown above each trace indicate the period of exposure of membrane patches to the test solution containing propofol or MgADP. Enlarged records are shown on the right

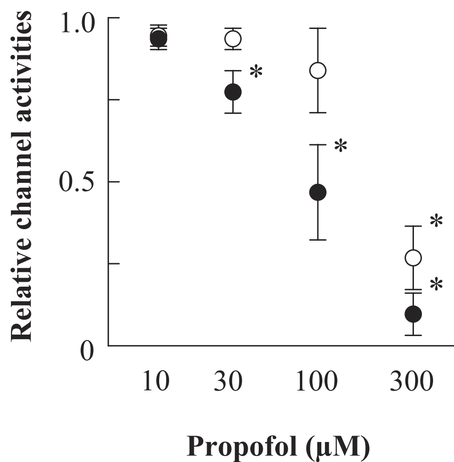

Fig. 4. Concentration-inhibition relation of activity of sulfonylurea receptor (SUR) 2A/inwardly rectifying potassium channel (Kir) 6.2 channels for propofol obtained during superfusion of either external pH 7.4 (*closed circles*) or 6.5 (*open circles*). *Each vertical bar* constitutes measurements from 15 patches (mean ± SD). $*P < 0.05$ versus baseline (before propofol)

channel activities decreasing to 0.45 ± 0.12 (n = 15). However, switching from an internal pH of 7.4 to 6.5, propofol failed to significantly inhibit the inside-out SUR2A/Kir6.2 currents, with relative channel activity of 0.88 ± 0.19 of control. Moreover, Fig. 3 shows that channel activities were re-inhibited when propofol was superfused in solution at normal pH.

The concentration-inhibition relationship between propofol and the SUR2A/Kir6.2 channel activities was determined by normalizing the channel activities obtained in test solutions to the control current in which propofol was absent (Fig. 4). Decreasing the intracellular pH from 7.4 to 6.5 shifted the IC_{50} from 78 to 277 μM. The Hill coefficient was 1.03 and 1.21, respectively.

Effects of intracellular MgADP on propofol-induced inhibition of Kir6.2ΔC36 channels

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 [11]. This has proved to be a useful tool for discriminating the site of action of various agents on K_{ATP} channels.

Our previous study has demonstrated that the Kir6.2 subunit is the primary binding site of K_{ATP} channels of propofol. Therefore, we investigated whether either intracellular MgADP or acidification influenced the propofol-Kir6.2 channel interactions. As shown in Fig. 5A, propofol (100 μM) at normal pH in the absence of MgADP inhibited Kir6.2ΔC36 channel currents, consistent with our previous study. Intracellular MgADP failed to attenuate the inhibitory action of propofol (Fig. 5B, top trace). On the other hand, intracellular acidification (pH 6.5) significantly reduced the propofol sensitivity of Kir6.2ΔC36 channels (Fig. 5B, bottom trace). The propofol-induced changes in *NP*o in the absence or presence of either intracellular MgADP or acidification are summarized in Fig. 5C.

Discussion

Recently, we demonstrated that propofol inhibited recombinant cardiac sarcolemmal K_{ATP} channels in the absence of intracellular MgADP [9]. Here, we further demonstrated that the inhibitory potency of propofol was attenuated by an increase in intracellular MgADP. Intracellular acidification also reduced propofol sensitivity to these channels. In addition, our results showed that the inhibitory action of propofol on Kir6.2ΔC36 channels in the absence of SUR2A was modulated by intracellular acidification but not by MgADP. These results suggest that intracellular MgADP and protons modify the inhibitory sensitivity of propofol to cardiac sarcolemmal K_{ATP} channels mediated by SUR2A and Kir6.2 subunits, respectively.

K_{ATP} channels exist in the plasma membranes of various tissues. Their gating is highly responsive to metabolic changes; the K_{ATP} channels also act as sensors of cell energy metabolism [16]. In cardiac muscle cells, under normal physiologic conditions, K_{ATP} channels exist mainly in a closed, inactive form [1]. However, during myocardial ischemia, as the intracellular ATP concentration falls, they open, resulting in an enhanced outward repolarizing flow of K^+ and cell membrane hyperpolarization. Consequently, the action potential duration is shortened; the voltage-dependent calcium current and contractility are decreased, thereby leading to ATP preservation during ischemia [1,2,16]. Thus, it is thought that K_{ATP} channels exert a protective property in myocardial ischemic diseases [16].

Interestingly, experimental as well as clinical observations have demonstrated that pre-ischemic exposure to volatile anesthetics improves ischemic outcome [17–19]. These properties have been attributed to an anesthetic preconditioning [17]. Although the precise mechanisms remain unclear, several studies suggest that the activation of cardiac sarcolemmal K_{ATP} channels plays a pivotal role in anesthetic preconditioning [20,21]. Indeed, electrophysiological studies using patch-clamp techniques demonstrate that isoflurane may activate K_{ATP} channels directly via the Kir6.2 subunit [22], or facilitate pharmacologically activated channels via protein kinase C [23]. In contrast, our previous studies in the absence of MgADP, at physiological pH, indicated that propofol

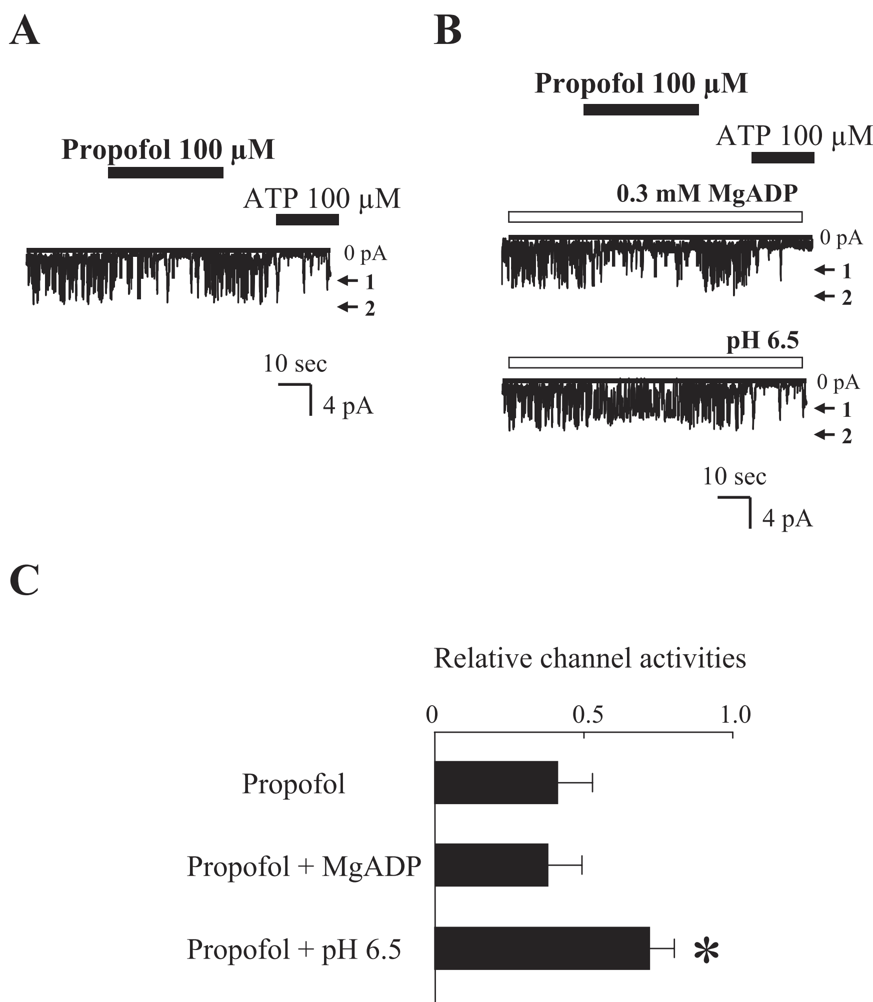

Fig. 5A–C. Effects of intracellular MgADP or acidification on the interaction between propofol and the truncated form of, inwardly rectifying potassium channel (Kir) 6.2, Kir6.2ΔC36, in the excised inside-out configuration. **A** Representative examples of Kir6.2ΔC36 currents in the absence or presence of anesthetics are shown. **B** MgADP (100 μM) and acidic solution (pH 6.5) are superfused to the intracellular bath as indicated by *horizontal bars*. Membrane potentials were clamped at –60 mV. Zero current levels are indicated by the *horizontal lines marked "0 pA"*. The number of open channels is shown on the right side of the traces, *with arrows*. **C** Relative channel activities in the presence of propofol with or without intracellular MgADP and acidification. The relative channel activities in the presence of propofol are expressed as a fraction of that observed in the absence of the drug. *Each horizontal bar* constitutes measurements from 10 to 12 patches (mean ± SD). $*P < 0.05$ versus control (propofol without intracellular MgADP and acidification)

at supraclinical high concentrations directly inhibited native and recombinant cardiac sarcolemmal K_{ATP} channel activities induced by the depletion of intracellular ATP [9, 15]. In cardiac ischemia, the concentration of intracellular ATP drops while the levels of intracellular ADP and protons rise. In this study, we examined the effect of changes in intracellular concentrations of MgADP and protons on the inhibition of K_{ATP} channels by propofol. We found the inhibition of propofol on cardiac sarcolemmal K_{ATP} channels was markedly reduced in the presence of intracellular MgADP and protons. These results suggested that propofol may preserve the endogenous organ protective mechanisms mediated by K_{ATP} channels against intraoperative ischemic or hypoxic injury. However, we cannot completely exclude the possibility that differential concentrations of propofol affect K_{ATP} channel in vivo conditions, because the concentrations of propofol we used in the present in vitro experimental model were higher than clinically relevant free-plasma concentrations (<2 μM). Further investigation seems to be required to clarify the influence of propofol associated with K_{ATP} channels in clinical practice.

In addition to ATP depletion, the ischemic myocardium is characterized by increased levels of cytosolic ischemic metabolites, such as MgADP. An increasing number of experiments have indicated that MgADP serves as a cofactor to define the responsiveness of cardiac sarcolemmal K_{ATP} channels toward several types of both K_{ATP} channel activators and inhibitors [5–7]. It has been reported that diazoxide, a K_{ATP} channel opener, activates SUR2A-containing recombinant K_{ATP} channels, only when sufficient internal MgADP is present [5]. On the other hand, intracellular MgADP reportedly enhances the blocking efficacy of glibenclamide in β-cell-type K_{ATP} channels (Kir6.2/SUR1), whereas MgADP decreases this efficacy in the cardiac-type K_{ATP} channels [7]. Along with previous reports of the nucleotide diphosphate-dependent action of K_{ATP} channel modulators, the present study demonstrated that the K_{ATP} channel-blocking efficacy of propofol was reduced by intracellular MgADP (Fig. 2). In contrast, the present

results also indicated that the inhibitory effects of propofol on the current generated by expressing Kir6.2ΔC36 in the absence of SUR were not affected by intracellular MgADP (Fig. 5B,C). MgADP activates the K_{ATP} channels strongly via SUR [24], whereas the site for channel inhibition by propofol is located on the Kir6.2 subunit [9]. It is therefore suggested that the MgADP-induced changes in the channel-blocking efficacy by propofol may not be a consequence of the interactions between MgADP and propofol bindings at the same time, but instead, may be due to an allosteric modification from specific interactions between MgADP and SUR to the propofol-effective site.

Our results also indicated that the propofol sensitivity of SUR2A/Kir6.2 channels was significantly reduced during intracellular acidification (Fig. 4). In addition, the inhibitory efficiency of propofol on Kir6.2ΔC36 channels was similarly reduced during intracellular acidification (Fig. 5B,C). Previous reports have demonstrated that protons directly bind on the Kir6.2 subunit, and interact with ATP in regulating K_{ATP} channel activity; a decrease in intracellular pH reduces the ATP sensitivity, leading to recovery from channel inhibition by ATP [12]. Further, our previous study showed that the site by which propofol mediates K_{ATP} channel inhibition is at least partly identical to that involved in the ATP block [9]. Accordingly, these results suggest the possibility that common mechanisms of proton-ATP interactions may be associated with the intracellular pH-dependent modulation of propofol on the Kir6.2 subunit.

In conclusion, our results provide an underlying basis for the important pathophysiological findings that intracellular MgADP and protons modulate the direct inhibitory effects of propofol on recombinant cardiac sarcolemmal K_{ATP} channels via SUR2A and Kir6.2 subunits, respectively. Under conditions of metabolic inhibition, an increase of intracellular MgADP and protons markedly decreased the sensitivity of cardiac sarcolemmal K_{ATP} channels to propofol.

Acknowledgments. We thank Dr. Susumu Seino, MD., Ph.D. (Professor and Chairman, Department of Cellular and Molecular Medicine, Chiba University, Chiba, Japan) for providing cDNAs (Kir6.2 and SUR2A) and expression vector pCMV6C. This study was supported by a Grant-in-Aid for Scientific Research (C, 15591636) from the Japan Society for the Promotion of Science, Tokyo, Japan.

References

1. Noma A (1983) ATP-regulated K^+ channels in cardiac muscle. Nature 305:147–148
2. Suzuki M, Li RA, Miki T, Uemura H, Sakamoto N, Ohmoto-Sekine Y, Tamagawa M, Ogura T, Seino S, Marban E, Nakaya H (2001) Functional roles of cardiac and vascular ATP-sensitive potassium channels clarified by Kir6.2-knockout mice. Circ Res 88:570–577
3. Kakei M, Kelly RP, Ashcroft SJ, Ashcroft FM (1986) The ATP-sensitivity of K^+ channels in rat pancreatic B-cells is modulated by ADP. FEBS Lett 208:63–66
4. Dunne MJ, Petersen OH (1986) Intracellular ADP activates K^+ channels that are inhibited by ATP in an insulin-secreting cell line. FEBS Lett 208:59–62
5. D'hahan N, Moreau C, Prost AL, Jacquet H, Alekseev AE, Terzic A, Vivaudou M (1999) Pharmacological plasticity of cardiac ATP-sensitive potassium channels toward diazoxide revealed by ADP. Proc Natl Acad Sci USA 96:12 162–12 167
6. Jahangir A, Terzic A, Kurachi Y (1994) Intracellular acidification and ADP enhance nicorandil induction of ATP sensitive potassium channel current in cardiomyocytes. Cardiovasc Res 28: 831–835
7. Gribble FM, Tucker SJ, Seino S, Ashcroft FM (1998) Tissue specificity of sulfonylureas: studies on cloned cardiac and beta-cell K_{ATP} channels. Diabetes 47:1412–1418
8. Kawano T, Oshita S, Tsutsumi Y, Tomiyama Y, Kitahata H, Kuroda Y, Takahashi A, Nakaya Y (2002) Clinically relevant concentrations of propofol have no effect on adenosine triphosphate-sensitive potassium channels in rat ventricular myocytes. Anesthesiology 96:1472–1477
9. Kawano T, Oshita S, Takahashi A, Tsutsumi Y, Tomiyama Y, Kitahata H, Kuroda Y, Nakaya Y (2004) Molecular mechanisms of the inhibitory effects of propofol and thiamylal on sarcolemmal adenosine triphosphate-sensitive potassium channels. Anesthesiology 100:338–346
10. Aguilar-Bryan L, Clement JP 4th, Gonzalez G, Kunjilwar K, Babenko A, Bryan J (1998) Toward understanding the assembly and structure of K_{ATP} channels. Physiol Rev 78:227–245
11. Tucker SJ, Gribble FM, Zhao C, Trapp S, Ashcroft FM (1997) Truncation of Kir6.2 produces ATP-sensitive K^+ channels in the absence of the sulphonylurea receptor. Nature 387:179–183
12. Xu H, Cui N, Yang Z, Wu J, Giwa LR, Abdulkadir L, Sharma P, Jiang C (2001) Direct activation of cloned K_{ATP} channels by intracellular acidosis. J Biol Chem 276:12898–12902
13. Inagaki N, Gonoi T, Clement JP 4th, Namba N, Inazawa J, Gonzalez G, Aguilar-Bryan L, Seino S, Bryan J (1995) Reconstitution of IKATP: an inward rectifier subunit plus the sulfonylurea receptor. Science 270:1166–1170
14. Kawano T, Oshita S, Takahashi A, Tsutsumi Y, Tomiyama Y, Kitahata H, Kuroda Y, Nakaya Y (2004) Molecular mechanisms of the inhibitory effects of bupivacaine, levobupivacaine, and ropivacaine on sarcolemmal adenosine triphosphate-sensitive potassium channels in the cardiovascular system. Anesthesiology 101:390–398
15. Kawano T, Oshita S, Takahashi A, Tsutsumi Y, Tanaka K, Tomiyama Y, Kitahata H, Nakaya Y (2005) Molecular mechanisms underlying ketamine-mediated inhibition of sarcolemmal adenosine triphosphate-sensitive potassium channels. Anesthesiology 102:93–101
16. Zhuo ML, Huang Y, Liu DP, Liang CC (2005) K_{ATP} channel: relation with cell metabolism and role in the cardiovascular system. Int J Biochem Cell Biol 37:751–764
17. Tanaka K, Ludwig LM, Kersten JR, Pagel PS, Warltier DC (2004) Mechanisms of cardioprotection by volatile anesthetics. Anesthesiology 100:707–721
18. De Hert SG, Cromheecke S, ten Broecke PW, Mertens E, De Blier IG, Stockman BA, Rodrigus IE, Van der Linden PJ (2003) Effects of propofol, desflurane, and sevoflurane on recovery of myocardial function after coronary surgery in elderly high-risk patients. Anesthesiology 99:314–323
19. Cromheecke S, Pepermans V, Hendrickx E, Lorsomradee S, Ten Broecke PW, Stockman BA, Rodrigus IE, De Hert SG (2006) Cardioprotective properties of sevoflurane in patients undergoing aortic valve replacement with cardiopulmonary bypass. Anesth Analg 103:289–296

20. Toller WG, Gross ER, Kersten JR, Pagel PS, Gross GJ, Warltier DC (2000) Sarcolemmal and mitochondrial adenosine triphosphate-dependent potassium channels: mechanism of desflurane-induced cardioprotection. Anesthesiology 92:1731–1739
21. Marinovic J, Bosnjak ZJ, Stadnicka A (2006) Distinct roles for sarcolemmal and mitochondrial adenosine triphosphate-sensitive potassium channels in isoflurane-induced protection against oxidative stress. Anesthesiology 105:98–104
22. Stadnicka A, Marinovic J, Bienengraeber M, Bosnjak ZJ (2006) Impact of in vivo preconditioning by isoflurane on adenosine triphosphate-sensitive potassium channels in the rat heart: lasting modulation of nucleotide sensitivity during early memory period. Anesthesiology 104:503–510
23. Fujimoto K, Bosnjak ZJ, Kwok WM (2002) Isoflurane-induced facilitation of the cardiac sarcolemmal K_{ATP} channel. Anesthesiology 97:57–65
24. Nichols CG, Shyng SL, Nestorowicz A, Glaser B, Clement JP 4th, Gonzalez G, Aguilar-Bryan L, Permutt MA, Bryan J (1996) Adenosine diphosphate as an intracellular regulator of insulin secretion. Science 272:1785–1787