Intravenous Anesthetic Propofol Inhibits Multiple Human Cardiac Potassium Channels

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ABSTRACT

Background: Propofol is widely used clinically for the induction and maintenance of anesthesia. Clinical case reports have shown that propofol has an antitachycardia/fibrillation effect; however, the related ionic mechanisms are not fully understood. The current study investigates the effects of propofol on human cardiac potassium channels.

Methods: The whole cell patch voltage clamp technique was used to record transient outward potassium current ($I_{to}$) and ultrarapidly activating delayed rectifier potassium current ($I_{Kur}$) in human atrial myocytes and hKv1.5, human ether-à-go-go-related gene (hERG), and hKCNQ1/hKCNE1 channels stably expressed in HEK 293 cells. Current clamp mode was used to record action potentials in human atrial myocytes.

Results: In human atrial myocytes, propofol inhibited $I_{to}$ in a concentration-dependent manner ($IC_{50} = 33.5 ± 2.0 \mu M$ for peak current, n = 6) by blocking open channels without affecting the voltage-dependent kinetics or the recovery time constant; propofol decreased $I_{Kur}$ ($IC_{50} = 35.3 ± 1.9 \mu M$, n = 6) in human atrial myocytes and inhibited hKv1.5 current expressed in HEK 293 cells by preferentially binding to the open channels. Action potential duration at 90% repolarization was slightly prolonged by 30 μM propofol in human atrial myocytes. In addition, propofol also suppressed hERG and hKCNQ1/hKCNE1 channels expressed in HEK 293 cells.

Conclusion: Propofol inhibits multiple human cardiac potassium channels, including human atrial $I_{to}$ and $I_{Kur}$, as well as hKv1.5, hERG, and hKCNQ1/hKCNE1 channels stably expressed in HEK 293 cells, and slightly prolongs human atrial action potential duration, which may contribute to the antitachycardia/fibrillation effects observed in patients who receive propofol. (Anesthesiology 2015; 122:571-84)

Propofol is an intravenously administered anesthetic that is commonly used clinically to induce and/or maintain anesthesia. This anesthetic has multiple advantages, for example, quick onset and rapid recovery, with minimal side effects. In addition, propofol has been reported to have both proarrhythmic and antiarrhythmic effects.1 It has been reported that a slowed atrioventricular conduction was frequently observed in children with paroxysmal supraventricular tachycardia undergoing radiofrequency catheter ablation,2 and a completed atrioventricular block was found in an elderly patient undergoing knee replacement arthroplasty.3 Sinus arrest,4 severe bradycardia, atrioventricular blockade,5 polymorphic ventricular tachycardia,6,7 and some particular arrhythmias similar to Brugada syndrome8 have been reported in patients receiving propofol. Among the arrhythmias induced by propofol, bradycardia (<50 beats/min) was the most frequent (4.8% of patients with propofol).9 Experimental studies demonstrated that blockade of cardiac L-type calcium current ($I_{Ca,L}$),10–12 sodium current ($I_{Na}$),13,14 and/or pacemaker current15,16 likely contributes to the proarrhythmia observed in patients receiving propofol.

What We Already Know about This Topic
- Propofol has potential proarrhythmic and antiarrhythmic effects on our patients
- The mechanisms involved with the effects of propofol on heart rhythms are not fully understood, and there is currently no information on the effects of propofol on the important ultra-rapid delayed rectifier potassium current ($I_{Kur}$) or action potential in human atria

What This Article Tells Us That Is New
- Propofol blocks several important potassium currents including $I_{to}$ in human atrial myocytes or stably expressing cell lines and prolongs the action potential in human atria
- The findings have particular relevance to the understanding of the potential antitachycardia and antiatrial fibrillation effects of propofol on our patients

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been found to be effective in the conversion of supraventricular tachycardia\textsuperscript{21,22} or atrial fibrillation\textsuperscript{23} to sinus rhythm. Nonetheless, the ionic mechanism underlying the atrial tachycardia or atrial fibrillation effects of propofol is not fully understood. The ultrarapid delayed rectifier potassium current $I_{\text{Kur}}$ (encoded by $h$Kv1.5) is present in the atria, but not in the ventricles of the human heart,\textsuperscript{24} and is therefore believed to be a target for atrial fibrillation therapy.\textsuperscript{25} No information is available in the literature regarding the effect of propofol on human atrial $I_{\text{Kur}}$ and action potential. We hypothesized that propofol would inhibit multiple human cardiac potassium channels including $I_{\text{Kur}}$. The current study was therefore designed to determine the potential effect of propofol on $I_{\text{Kur}}$, transient outward potassium current ($I_{\text{to}}$), and action potential in human atrial myocytes using the whole cell patch clamp technique. We also investigated the effects of propofol on $h$Kv1.5, human ether-à-go-go-related gene ($h$ERG or KCNH2, coding for the $\alpha$ subunit of the rapid delayed rectifier potassium current $I_{\text{Kr}}$) channels, and $h$KCNQ1/hKCNE1 (coding for the slow delayed rectifier potassium current $I_{\text{Ks}}$) channels stably expressed in HEK 293 cells. Our results demonstrated that propofol inhibited multiple human cardiac ion channels including $I_{\text{to}}$, $I_{\text{Kur}}$, $h$ERG, and $h$KCNQ1/hKCNE1 channels, and slightly prolonged human atrial action potential duration.

Materials and Methods

Human Atrial Myocytes Preparation

Atrial myocytes were isolated from right atrial appendage specimens obtained from 15 patients (46 to 71 yr old) undergoing coronary artery bypass. The procedure for obtaining the tissue was approved (reference No. UW-10–174) by the institutional review board of the University of Hong Kong/ Hospital Authority Hong Kong West Cluster (Hong Kong, China) and a written consent was obtained from each patient. All atrial specimens were grossly normal at the time of cardiac surgery, and all patients were free of supraventricular tachyarrhythmias and symptomatic congestive heart failure. The patients were administered angiotensin-converting enzyme inhibitors, $\beta$-blockers, or calcium channel blockers before surgery. After excision, the samples were quickly immersed in an oxygenated calcium-free cardioplegic solution and immediately transported to the laboratory. Atrial myocytes were enzymatically dissociated with the procedure described previously.\textsuperscript{25,26} The isolated myocytes were kept in a high potassium medium\textsuperscript{26,27} at room temperature for at least 1 h before experimental recording. No randomization or blind methods were used in the present electrophysiological study.

Cell Culture

The HEK 293 cell line stably expressing $h$Kv1.5 (KCNA5, coding $I_{\text{Kur}}$),\textsuperscript{28} $h$KCNQ1/hKCNE1 ($I_{\text{Ks}}$),\textsuperscript{29} or $h$ERG ($I_{\text{Kr}}$)\textsuperscript{30} was maintained in Dulbecco modified Eagle medium (DMEM; Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum and 400 $\mu$g/ml G418 (Sigma-Aldrich, St. Louis, MO) for the cell line expressing $h$Kv1.5 and $h$ERG, or 100 $\mu$g/ml hygromycin (Invitrogen) for the cell line expressing $h$KCNQ1/hKCNE1. Cells used for electrophysiology were seeded on a glass cover slip.

Whole Cell Patch Clamp Recordings

Membrane currents were recorded with the whole cell patch clamp technique as described previously.\textsuperscript{26,28,31} In brief, a small aliquot of the solution containing the isolated myocytes was placed in an open perfusion chamber (1 ml) mounted on the stage of an inverted microscope (IX50; Olympus, Tokyo, Japan). Myocytes were allowed to adhere to the bottom of the chamber for 10 to 20 min and then superfused at 2 to 3 ml/min with Tyrode solution. For current recording in cell lines, the glass coverslips with HEK 293 cells expressing different ion channels were transferred into the cell chamber and superfused with Tyrode solution.

Borosilicate glass electrodes (1.2-mm outside diameter) were pulled with a Brown-Flaming puller (model P-97; Sutter Instrument Co., Novato, CA) and had tip resistances of 2 to 3 $M\Omega$ when filled with pipette solution. Membrane currents were recorded in voltage clamp mode, and action potentials were recorded in current clamp mode using an EPC-9 amplifier and Pulse software (HEKA, Lambrecht, Germany). After a giga-ohm seal was obtained, the cell membrane was ruptured by gentle suction to establish whole cell configuration. The cell membrane capacitance ($C_{\text{m}}$) was directly measured using the lock-in module of the Pulse software and used for normalizing the current in individual cells. The series resistance (3 to 5 $M\Omega$) was compensated by 50 to 80% to minimize voltage errors. Electrical signals were recorded with a sampling rate of 5 kHz and filtered at 2 kHz.

Pulse Protocols and Current Measurements

Voltage-dependent $I_{\text{to}}$ traces were recorded using a protocol with 300-ms voltage steps in 10-mV increments between −40 and +60 mV from a holding potential of −50 mV at 0.2 Hz. The current was measured from the peak to “quasi”-steady-state level. $I_{\text{Kur}}$ was determined using a 100-ms prepulse to +40 mV (to inactivate $I_{\text{to}}$) followed by a 10-ms interval before 200-ms test potentials between −40 to +60 mV from a holding potential of −50 mV, then to −30 mV (pulse interval of 10 s). The current was measured from zero current level to the current at the end of the voltage step. Voltage-dependent $h$Kv1.5 current was elicited by 5-s voltage pulses to potentials between −40 mV and +60 mV from a holding potential of −80 mV (pulse interval, 20 s). The current amplitude was measured at the end of the 5-s depolarizing pulse. Voltage-dependent $h$ERG current was recorded with 3-s voltage steps from −80 mV to potentials between −60 to +60 mV, then to −50 mV every 15 s. The step current was measured as the difference between zero current and the level at the end of voltage step. The tail current was measured at its peak. Voltage-dependent $h$KCNQ1/hKCNE1...
current traces were recorded with 3-s voltage steps from −80 mV to potentials between −40 to +60 mV, then to −50 mV (pulse interval of 15 s). The voltage step-activated current was measured from the beginning of the significant activation of time-dependent current to the level at the end of the depolarization step.

To obtain IC$_{50}$ values, the fractional blocks obtained at different drug concentrations were fitted with the Hill equation: $E = \frac{E_{\text{max}}}{1 + ([C]/IC_{50})^h}$, where $E$ is the inhibition of currents in percentage at concentration $C$, $E_{\text{max}}$ is the maximum inhibition, IC$_{50}$ is the concentration for 50% inhibition of maximum effect, and $h$ is the Hill coefficient. The activation or inactivation conductance variables of $I_{\text{to}}$, hKv1.5, hERG, or hKCNQ1/hKCNE1 were determined with normalized currents. Current activation and inactivation were fitted by the Boltzmann distribution: $y = \frac{1}{1 + \exp[(V_m - V_{0.5})/S]}$, where $V_m$ is the membrane potential, $V_{0.5}$ is the midpoint, and $S$ is the slope factor. The relation of $1/\tau_{\text{block}}$ against the concentration is described by the linear function: $1/\tau_{\text{block}} = k[D] + I$, where $1/\tau_{\text{block}}$ is the time constant of development of block, and $k$ and $I$ are the apparent rate constants for association and dissociation of the drug.

### Solutions and Chemicals

Tyrode solution contained 140 mM NaCl, 5.4 mM KCl, 1 mM MgCl$_2$, 1 mM CaCl$_2$, 0.33 mM Na$_2$PO$_4$, 5 mM HEPES, and 10 mM glucose; pH was adjusted to 7.4 with NaOH. The pipette solution for current recording contained 20 mM KCl, 110 mM K-aspartate, 1 mM MgCl$_2$, 10 mM HEPES, 5 mM EGTA, 0.1 mM guanosine-5'-triphosphate, 5 mM Na$_2$-phosphocreatine, and 5 mM adenosine triphosphate; pH was adjusted to 7.2 with KOH. The pipette solution for action potential recording contained 20 mM KCl, 110 mM K-aspartate, 1 mM MgCl$_2$, 10 mM HEPES, 0.05 mM EGTA, 0.1 mM GTP, 5 mM Na$_2$-phosphocreatine, and 5 mM adenosine triphosphate; pH was adjusted to 7.2 with KOH. For $I_{\text{to}}$ and $I_{\text{k}}$ recording, BaCl$_2$ (200 μM) and CdCl$_2$ (200 μM) were added to the bath solution to block $I_{\text{Ki}}$ and $I_{\text{Ca-L}}$. Atropine (1.0 μM) was used to minimize possible $I_{\text{KACa}}$ contamination during the current recording. Diphenyl phosphoryl oxide-1 (DPO-1; 2 μM) was added to inhibit $I_{\text{Kur}}$ for determining $I_{\text{to}}$. A stock solution of propofol (Sigma-Aldrich) at 200 mM was made in dimethyl sulfoxide.

### Statistical Analysis

A group size of number of 5 or more was determined based on previous experience. Nonlinear curve fitting was performed using PulseFit (HEKA) and Sigmaplot (SPSS, Chicago, IL). Paired and/or unpaired Student two-tailed $t$ test was used as appropriate to evaluate the statistical significance of differences between two group means. Two-way ANOVA followed by the Newman–Keuls test was used for multiple comparisons, which is referred to as "ANOVA" in the results, unless otherwise noted. Data are presented as mean ± SEM. Values of $P$ less than 0.05 were considered to indicate statistical significance.

### Results

#### Inhibition of Cardiac $I_{\text{to}}$ by Propofol in Human Atrial Myocytes

It is well established that both $I_{\text{to}}$ and $I_{\text{k}}$ are present in human atrial myocytes. Voltage-dependent membrane currents were elicited in a representative human atrial myocyte with a voltage protocol as shown in the inset in the absence and the presence of propofol (fig. 1A). Propofol at 50 μM inhibited both $I_{\text{to}}$ peak and the sustained $I_{\text{k}}$. To accurately evaluate the propofol effect on $I_{\text{to}}$, the selective $I_{\text{Kur}}/Kv1.5$ blocker DPO-1 was used to separate $I_{\text{to}}$ as in a previous study. Figure 1B shows the voltage-dependent $I_{\text{to}}$ traces during control, in the presence of 2 μM DPO-1, co-presence of DPO-1 and 30 μM propofol, and washout of propofol. DPO-1 (2 μM) induced a slight reduction of peak current amplitude and almost a full inhibition of $I_{\text{k}}$. Propofol at 30 μM remarkably suppressed $I_{\text{to}}$ and the effect was partially reversed by washout. Figure 1C displays the time course of $I_{\text{to}}$ recorded with a protocol as shown in the inset in the absence or presence of 30 μM propofol in a typical experiment with DPO-1 treatment. $I_{\text{to}}$ was gradually decreased by 30 μM propofol, and the inhibition reached a steady-state level within 4 min and was partially reversed upon washout. Experiments to determine the blocking properties of $I_{\text{to}}$ by propofol were conducted in the presence of 2 μM DPO-1.

The effect of propofol on current–voltage ($I–V$) relations of $I_{\text{to}}$ was determined at 3, 10, 30, and 100 μM (fig. 2A). Propofol inhibited $I_{\text{to}}$ in a concentration-dependent manner. Propofol at 10 to 100 μM suppressed $I_{\text{to}}$ at test potentials of 0 mV to +60 mV ($n = 6$, $P < 0.05$ or $P < 0.01$ vs. control, ANOVA). No significant change in voltage dependence was observed at any concentration of propofol (fig. 2B). The concentration–response relation for inhibiting the peak current of $I_{\text{to}}$ by propofol was evaluated at +50 mV and fitted with a Hill equation (fig. 2C). The IC$_{50}$ for inhibiting peak current of $I_{\text{to}}$ by propofol was 33.5 ± 2.0 μM (Hill coefficient 1.8 ± 0.2, $n = 6$). Propofol decreased the peak current and increased the inactivation of $I_{\text{to}}$, which implies open-channel blockade. Therefore, the inhibition was also estimated from the integral of the total current charge crossing the membrane at +50 mV, and propofol inhibited the $I_{\text{to}}$ charge with an IC$_{50}$ of 20.9 ± 5.0 μM (Hill coefficient 2.1 ± 0.4, $n = 6$; fig. 2C).

To analyze the open-channel blocking property of $I_{\text{to}}$ by propofol, the time to peak and the inactivation time constant were determined in human atrial myocytes in the absence and the presence of propofol. Figure 3A illustrates the expanded $I_{\text{to}}$ traces recorded at +40 mV in the absence and the presence of 30 μM propofol. The time to peak of $I_{\text{to}}$ was clearly reduced by propofol. The mean value of the time to peak of the current at 0 to +60 mV was significantly reduced by 30 μM propofol (fig. 3B, $n = 6$, ...
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P < 0.01 vs. control, ANOVA). $I_{to}$ traces (+40 mV) were fitted by a mono-exponential function with the time constants shown before and after the application of 30 μM propofol. The mean value of the time constant of $I_{to}$ inactivation was reduced by 30 μM propofol. The mean value of the time constant of $I_{to}$ inactivation at 0 to +60 mV is shown in figure 3D. The time constant was significantly reduced by 10 or 30 μM propofol at all test potentials (n = 6, *P < 0.05 or **P < 0.01 vs. control, ANOVA).

The onset of the open-channel blockade was further analyzed with the method described by Slawsky and Castle35 using the current traces recorded at +50 mV with 10, 30, and 100 μM propofol. Acceleration of $I_{to}$ inactivation by 10, 30, and 100 μM propofol suggested an open-channel blocking effect. The drug-sensitive current expressed as a proportion of the current in the absence of the drug \(\left[\frac{I_{control} - I_{propofol}}{I_{control}}\right]\), where $I_{control}$ and $I_{propofol}$ are the current in the absence and the presence of propofol. The drug-induced block was then plotted as a function of time. The blockade developed in a time-dependent manner with an exponential onset as shown by the curve fits in figure 3E. The rate of blocking development was enhanced as the concentration increased; the time constants averaged 26.3 ± 2.9 ms, 19.1 ± 1.1 ms, and 7.6 ± 0.6 ms, at 10, 30, and 100 μM propofol, respectively (n = 6). The $1/\tau_{block}$ was plotted as a function of propofol concentrations as shown in figure 3F. The straight line is a regression fit of the equation $1/\tau_{block} = k[D] + l$, and the apparent rate constants for association ($k$) and dissociation ($l$) were (1.1 ± 0.2) $\times 10^6$ M$^{-1}$ s$^{-1}$ and 25.2 ± 5.8 s$^{-1}$, respectively. The apparent $K_d$ ($K_d = l/k$) derived from this relation for $I_{to}$ current block by propofol was 22.9 μM, which is close to the IC$_{50}$ of 20.9 μM obtained from the concentration–response curve determined by the integral of current charge.

Fig. 1. Inhibition of $I_{to}$ by propofol. (A) Membrane currents were recorded with a voltage protocol shown in the inset in a representative human atrial myocyte in the absence (control) and the presence of 50 μM propofol. (B) Membrane currents recorded in another myocyte in the absence and the presence of 2 μM diphenyl phosphate oxide-1 (DPO-1; to inhibit $I_{Kur}$). DPO-1 plus 30 μM propofol, and washout of propofol. (C) Time course of $I_{to}$ recorded in a representative cell pre-treated with 2 μM DPO-1. Propofol at 30 μM gradually inhibited $I_{to}$, and the inhibition was reversed by washout.

Fig. 2. Concentration-dependent effects of propofol on $I_{to}$. (A) Current–voltage relations of $I_{to}$ density in cells pretreated with 2 μM diphenyl phosphate oxide-1 (control) and in the co-presence of 3, 10, 30, and 100 μM propofol. Propofol significantly inhibited $I_{to}$ at concentrations from 10 to 30 and 100 μM (n = 6, *P < 0.05 or **P < 0.01 vs. control, ANOVA). (B) Mean percent inhibition of $I_{to}$ from 0 to +60 mV by propofol at 3 to 100 μM. (C) Concentration–response relation for reducing total $I_{to}$ charge (n = 6) and peak current (n = 6) at +50 mV. The data were fitted with a Hill equation.
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Figure 4A illustrates the current and protocol (1-s conditioning pulses from −100 to +30 mV at a holding potential of −80 mV, followed by a 300-ms test pulse to +50 mV after a 30-ms interval at −50 mV) used for determining the steady-state inactivation (availability, \( I_{\max} / I_{\text{to}} \)) of \( I_{\text{to}} \), while figure 4B shows the tail current recorded by a voltage protocol (8-ms voltage steps from −50 mV to potentials between −40 to +50 mV, then to −40 mV) for determining the steady-state activation \( (g / g_{\text{max}})_{\text{to}} \). The normalized variable of \( I_{\max} / I_{\text{to}} \) or \( g / g_{\text{max}} \) was calculated and fitted with a Boltzmann function in individual cells to obtain the half potential \( V_{0.5} \) of \( I_{\text{to}} \) availability or activation and slope factor. Figure 4C displays the mean values of \( I_{\max} / I_{\text{to}} \) fitted by a Boltzmann distribution in the absence or the presence of 30 μM propofol. The \( V_{0.5} \) of \( I_{\text{to}} \) availability was −22.0 ± 1.3 mV in the control and −24.6 ± 2.1 mV in the presence of propofol \((n = 8, P = 0.168, \text{pared Student } t \text{ test})\). The fractional blockade was plotted as a function of the voltage of the preceding pulse as described by Caballero et al., demonstrating that the blockade remained unchanged at potentials between −100 mV (48.1 ± 3.0%) and −50 mV (48.3 ± 3.1%, \( n = 8, P > 0.05 \), ANOVA). At more positive potentials, it tended to increase as the amount of inactivated channels increased, reaching a maximum at −20 mV (57.7 ± 6.4%), but the difference was not statistically significant \((n = 8, P > 0.05 \text{ vs. blockade at −100 mV, ANOVA})\).

Figure 4D displays the mean values of \( g / g_{\text{max}} \) fitted by a Boltzmann distribution in the absence or the presence of 30 μM propofol. The \( V_{0.5} \) of \( I_{\text{to}} \) activation was 21.9 ± 1.5 mV in the control and 15.9 ± 1.6 mV in the presence of propofol \((n = 7, P = 0.055, \text{pared Student } t \text{ test})\). No difference was observed for the slope factor of \( I_{\text{to}} \) availability or activation before and after the application of propofol.

Time-dependent recovery of \( I_{\text{to}} \) from inactivation was determined with a paired-pulse protocol as shown in the legend of Figure 3. Effects of propofol on time-dependent kinetics of \( I_{\text{to}} \). (A) Expanded current traces of \( I_{\text{to}} \) at +40 mV before and after 30 μM propofol in a representative recording, showing the measurement of the time to peak of \( I_{\text{to}} \). (B) Mean values of the time to the peak of \( I_{\text{to}} \) activation at 0 mV to +60 mV under control conditions and in the presence of 10 and 30 μM propofol \((n = 6, P < 0.01, 30 \text{ μM vs. control, ANOVA})\). (C) Inactivation raw data (points) of \( I_{\text{to}} \) at +40 mV before and after 30 μM propofol fitted to a mono-exponential function (solid lines) with time constants shown. (D) Mean values of time constant of \( I_{\text{to}} \) inactivation at 0 mV to +60 mV and before and after the application of 10 and 30 μM propofol \((n = 6, P < 0.05 \text{ or } P < 0.01 \text{ vs. control, ANOVA})\). (E) Development of \( I_{\text{to}} \) inhibition by propofol after channel activation. The solid lines represent that the mono-exponential function is fitted to the onset blocking data (points) by propofol at 10, 30, and 100 μM. (F) Rate constants for the block of \( I_{\text{to}} \) by propofol. \( 1 / \tau_{\text{block}} \) is plotted against the concentration of propofol. The line is a regression fit of the equation, \( 1 / \tau_{\text{block}} = k[D] + I(n = 6) \).
Figure 5A shows the time course of $I_{Kur}$ recorded in a representative cell with a voltage protocol: as shown in the inset, a 100-ms prepulse to +40 mV to inactivate $I_{to}$, followed by a 200-ms test pulse to +50 mV, then to −30 mV every 15 s.26,27 The current was slowly reduced by 30 μM propofol and reached a steady state in 10 min, and the inhibitory effect was partially reversed by washout. The voltage-dependent $I_{Kur}$ (fig. 5B) was recorded in a representative cell with a voltage protocol shown in the inset. Propofol at 30 μM substantially inhibited both $I_{Kur}$ and tail current, and the effect partially recovered on washout. At test potential of +50 mV, $I_{Kur}$ was reduced by 57.4 ± 5.2% with 30 μM propofol ($n = 7$, $P = 0.002$ vs. control, paired Student $t$ test).

$I-V$ relations of $I_{Kur}$ are illustrated in figure 5C in the absence and the presence of 3, 10, 30, and 100 μM propofol in a total of six myocytes. Propofol inhibited $I_{Kur}$ in a concentration-dependent manner. The inhibition fraction of the current was more at potentials positive to +20 mV than that at 0 mV with 10 to 100 μM propofol (fig. 5D, $n = 6$, $P < 0.05$ or $P < 0.01$ vs. 0 mV, ANOVA). Figure 5E shows the concentration–response relation of propofol, which was fitted by a Hill equation. The $IC_{50}$ (at +50 mV) of propofol for inhibiting $I_{Kur}$ was 35.3 ± 1.9 μM (Hill coefficient 1.1 ± 0.05, $n = 6$).

$I_{Kur}$/Kv1.5 channel inhibitors usually block the open state of the channel and induce an increased inactivation of the current, that is, blocking increase during depolarization.33,34,37,38 However, propofol inhibited $I_{Kur}$ without showing the increased inactivation in human atrial myocytes (fig. 5), suggesting that the open-channel blockade may not be involved in the current inhibition. Nevertheless, $I_{Kur}$ was recorded in human atrial myocytes using short-duration test pulses with a prepulse, which might cover the open-channel blocking property of propofol. We therefore determined the effect of propofol on hKv1.5 channels expressed in HEK 293 cells using longer-duration pulses.

Figure 6A illustrates hKv1.5 current elicited by 5-s voltage pulses between −40 mV and +60 mV from a holding potential of −80 mV with a pulse interval of 20 s. Propofol at 50 μM significantly inhibited hKv1.5 current, and the effect partially recovered on washout. Figure 6B shows the $I-V$ relations of hKv1.5 current measured at the end of the depolarization step before and after the application of 10, 30, 100 μM propofol. The effect of propofol was concentration-dependent, with IC50 values of 35.3 ± 1.9 μM (Hill coefficient 1.1 ± 0.05, $n = 6$).
30, 50, and 100 μM propofol. The current was inhibited by propofol in a concentration-dependent manner (n = 9, 
\( P < 0.05 \) or \( P < 0.01 \) vs. control at 0 mV to +60 mV, ANOVA). Concentration–response relation for inhibiting the peak current, the “quasi”–steady-state current measured at the end of the voltage pulse, and the current charge crossing the membrane by propofol at 10 to 300 μM were evaluated at +50 mV and fitted with a Hill equation (fig. 6C). The IC\textsubscript{50} values were 132.2 ± 6.9 μM (Hill coefficient 1.4 ± 0.1, n = 7), 58.1 ± 2.7 μM (Hill coefficient: 1.7 ± 0.1, 
\( n = 7 \)), and 67.0 ± 2.8 μM (Hill coefficient 1.6 ± 0.1, n = 7), respectively.

Although the IC\textsubscript{50} values are similar for inhibiting the ‘quasi’–steady-state current measured at the end of the voltage pulse and the current charge, the IC\textsubscript{50} for inhibiting the peak current is greater, suggesting that propofol enhanced the inactivation of hKv1.5 current elicited by 5-s pulses, and open-channel blockade is involved in the current inhibition. The open-channel blocking properties were analyzed as for \( I_{\text{to}} \) (fig. 3).36,39 The current traces (at +50 mV) were fitted by

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**Fig. 5.** Inhibition of \( I_{\text{kur}} \) by propofol. (A) Time course of \( I_{\text{kur}} \) recorded in a typical experiment in the absence and the presence of 30 μM propofol with original \( I_{\text{kur}} \) traces at corresponding time points shown. (B) Voltage-dependent \( I_{\text{kur}} \) traces recorded in a representative cell with the voltage protocol shown in the inset. \( I_{\text{kur}} \) was reversibly inhibited by 30 μM propofol. (C) Current–voltage relations of \( I_{\text{kur}} \) in the absence and the presence of 3, 10, 30, and 100 μM propofol. Propofol significantly inhibited \( I_{\text{kur}} \) at concentrations from 10 to 30 and 100 μM (n = 6, *\( P < 0.05 \) or **\( P < 0.01 \) vs. control, ANOVA). (D) Mean percentage value of \( I_{\text{kur}} \) at 0 to +60 mV in the presence of 3 to 100 μM propofol. Significant voltage dependence was observed for the drug effect at 10 to 100 μM, and a stronger effect was observed at potentials positive to between +20 and +60 mV (\( P < 0.05 \) or \( P < 0.01 \) vs. 0 mV, ANOVA). (E) Concentration–response relation for inhibiting \( I_{\text{kur}} \) (at +50 mV) by propofol fitted with a Hill equation (n = 6).
a mono-exponential function with time constants shown in the absence and the presence of 50 μM propofol (fig. 7A). The mean values of the time constant (fig. 7B) were significantly reduced by 50 μM propofol at potentials of +10 to +60 mV (n = 11, *P < 0.05 or **P < 0.01 vs. control, paired Student t test). Figure 7C illustrates the current traces at +50 mV in the absence and the presence of 30, 50, and 100 μM propofol. The drug-sensitive hKv1.5 current expressed as a proportion of the current in the absence of the drug [(I_{control} - I_{propofol})/I_{control}], 1/τ_{block} as a function of the propofol concentration for data obtained at 30, 50, and 100 μM is shown in figure 7D. The line is a regression fit of the equation, 1/τ_{block} = k[D] + l (n = 6). The apparent rate constants for association (k) and dissociation (l) were (1.5 ± 0.1) × 10^4 M⁻¹ s⁻¹ and 0.88 ± 0.09 s⁻¹, respectively. The apparent K_{D} (K_{D} = k/l) derived from this relation for hKv1.5 blocking by propofol was 58.7 μM, which is close to the IC_{50} of 57.3 μM obtained from the concentration–response curve.

To obtain the steady-state inactivation of hKv1.5 channels, a protocol with 10-s conditioning pulses from −80 to +50 mV and then a 1-s test pulse to +50 mV was used with 20-s pulse interval. Figure 7E shows the mean variables of I_{V,50} of hKv1.5 current in the absence and the presence of 50 μM propofol. The variables were fitted with a Boltzmann distribution in individual cells to obtain the V_{0.5} of hKv1.5 inactivation. The inactivation V_{0.5} was −9.5 ± 0.7 mV in control and −8.7 ± 0.9 mV in 50 μM propofol (n = 9, P = 0.433, paired Student t test). Fractional block by propofol showed that current blockade did not change significantly even in the voltage range of channel inactivation.

Recovery of hKv1.5 current from inactivation was determined with a paired-pulse protocol (a 5,000-ms step to +50 mV from a holding potential of −80 mV, followed by a 300-ms step to +50 mV with variable P1–P2 interval between 2 and 9,000 ms, pulse interval of 25 s). Both of the recovery curves in the absence and the presence of 50 μM propofol were well fitted with a bi-exponential function (fig. 7F). Propofol slightly slowed the recovery of hKv1.5 current from inactivation, but the difference was not significant (fast time constant: 285 ± 15 ms in the control, 469 ± 86 ms in the presence of propofol, n = 8, P = 0.125, paired Student t test; slow time constant: 3,956 ± 399 ms in the control, 4,075 ± 437 ms in the presence of propofol, n = 8, P = 0.873, paired Student t test).

**Effects of Propofol on Cardiac hERG and hKCNQ1/hKCNE1**

It has been demonstrated that both I_{Kr} and I_{Ks} are present in human cardiac myocytes and play an important role in cardiac repolarization in human heart. It is interesting to investigate whether the inhibition of human cardiac I_{Kr} and/ or I_{Ks} contributes to the antitachycardia/fibrillation of propofol. It has been difficult to record these two currents in cardiac myocytes from the chunk dissociation method with a small atrial specimen from patients undergoing coronary bypass surgery. Therefore, HEK 293 cells stably expressing hERG (coding for I_{Kr}) or hKCNQ1/hKCNE1 (coding for I_{Ks}) were used here to determine the effects of propofol on these two types of currents.

Figure 8A shows the time course of hERG tail current recorded with a voltage step as shown in the inset in the absence and the presence of 30 and 100 μM propofol. The current was inhibited by 30 and 100 μM propofol, and the inhibition was reversed by washout. Similar results were observed in voltage-dependent hERG current. Both hERG tail current and step current were clearly decreased by propofol (fig. 8B). Figure 8, C and D, illustrates the I–V relations of mean values of hERG tail and step current in the absence and the presence of 30, 100, and 300 μM propofol. Propofol...
at 30 μM inhibited hERG tail and step current from +20 mV to +60 mV (n = 10, \( P < 0.05 \) vs. control, ANOVA), while at 100 and 300 μM, propofol significantly decreased the current from 0 mV to +60 mV (\( P < 0.05 \) or \( P < 0.01 \) vs. control, ANOVA). The concentration–response curves (fig. 8E) of propofol for inhibiting hERG tail current and step current at +40 mV were fitted with a Hill equation. The IC\(_{50}\) of propofol was 84.4 ± 15.5 μM for inhibiting hERG tail current and 73.2 ± 16.6 μM (n = 7) for inhibiting hERG step current.

The steady-state activation (g/g\(_{\text{max}}\)) of hERG channels was determined by normalized tail current in the absence and presence of 30 and 100 μM propofol (fig. 8F). The activation curves were fitted with a Boltzmann function. The \( V_{0.5} \) of hERG channel activation was 4.1 ± 1.6 mV in control, 0.5 ± 2.1 mV in 30 μM propofol (n = 7, \( P > 0.05 \) vs. control), and −10.1 ± 2.2 mV in 100 μM propofol (n = 7, \( P < 0.05 \) vs. control, one-way ANOVA followed by the Newman–Keuls test). The activation of hERG channels was significantly shifted to the negative potential by propofol.

Figure 9A shows the time course of human cardiac hKCNQ1/hKCNE1 channels expressed in HEK 293 cells in a typical experiment with the voltage step as shown in the inset. Propofol at 30 and 100 μM significantly inhibited hKCNQ1/hKCNE1 step current, and the inhibition was partially reversed by washout. The voltage-dependent hKCNQ1/hKCNE1 current was also suppressed by propofol (fig. 9B). Figure 9C illustrates the I–V relations of mean values of hKCNQ1/hKCNE1 current density in the absence and presence of 10, 30, and 100 μM propofol. Propofol significantly inhibited hKCNQ1/
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hKCNE1 current at test potential of 0 to +60 mV (n = 6, P < 0.05 or P < 0.01 vs. control, ANOVA). The concentration–response curves (fig. 9D) of propofol for decreasing hKCNQ1/hKCNE1 current at +40 mV were fitted with a Hill equation. The IC$_{50}$ of propofol was 32.4 ± 6.0 μM (n = 6) for inhibiting hKCNQ1/hKCNE1 channels.

The steady-state activation (g/g$_{max}$) of hKCNQ1/hKCNE1 channels was determined by normalized tail current in the absence and the presence of 30 μM propofol (fig. 9E). The activation curves were fitted with the Boltzmann function. The V$_{1/2}$ of hKCNQ1/hKCNE1 current activation was 16.3 ± 4.5 mV in control and 20.1 ± 4.1 mV in 30 μM propofol (n = 6, P = 0.088 vs. control, paired Student t test). The activation conductance of hKCNQ1/hKCNE1 channels was not significantly affected by propofol.
Effects of Propofol on Human Atrial Action Potential

The effect of propofol on cardiac action potential was determined in human atrial myocytes at 36°C. Figure 10A displays the action potential traces recorded in a representative cell at 2 Hz. Propofol at 30 μM slightly prolonged action potential duration. Action potential duration at 90% repolarization was significantly increased by the application of propofol (fig. 10B, n = 6, P = 0.024 vs. control, paired Student t test). The measured dV/dT of action potential phase 0 was reduced from 78.2 ± 5.7 V/s to 72.7 ± 5.9 V/s (n = 6, P = 0.034 vs. control, paired Student t test), which may be related to the inhibition of I_Na as reported previously.13,14 However, resting membrane potential was not affected by propofol (−73.0 ± 1.7 mV vs. −72.4 ± 1.1 mV, n = 6, P = 0.528, paired Student t test).

Discussion

The current study demonstrates that the intravenous anesthetic propofol blocks human atrial native I_w, I_Kur, hERG, and hKCNQ1/hKCNE1 channels stably expressed in HEK 293 cells with concentrations ranging from 3 to 100 μM and it slightly prolongs human atrial action potential duration.

Previous reports demonstrated that propofol inhibits several cardiac ion channels.1 Propofol at 1 to 100 μM decreases the cardiac depolarization currents including I_cal in cardiac ventricular myocytes from rat12 and guinea pig,11 rabbit,14 and human atrial myocytes,10 I_Na in rat and rabbit ventricular myocytes,13,14 and pacemaker current (i.e., hyperpolarization-activated cyclic nucleotide–regulated channels) expressed in HEK 293 cells.16,41 These effects may count for the ionic mechanisms of sinus arrest, bradycardia, and/or atrioventricular blockade observed in patients with propofol as an intravenous anesthetic.4,5,9

For cardiac repolarization currents, the reports for cardiac inward rectifier potassium current (I_K1) are controversial. One study reported that propofol at 3 to 30 μM decreased I_K1 in rabbit ventricular myocytes,14 whereas other reports demonstrated that propofol (28 to 60 μM) had no effect on I_K1 in ventricular myocytes from guinea pig12 and canine17 hearts. Our results support the notion that propofol has no significant effect on cardiac I_K1 because propofol at 30 μM only slightly inhibited human cardiac Kir2.1 channels expressed in HEK 293 cells without statistical significance (Supplemental Digital Content 1, http://links.lww.com/ALN/B107, figure 1, n = 8, inhibited by approximately 3.3% at −120 mV, P = 0.124 vs. control, paired Student t test). This may explain why no change is observed in the resting membrane potential of human atrial myocytes with application of propofol.

The transient outward potassium current I_to plays a role in the early rapid repolarization of cardiac action potential in mammalian heart, including that of humans. Propofol (3 to 60 μM) inhibited I_to in ventricular myocytes from rat,13 rabbit,14 and canine17 hearts. The current study demonstrated the novel information that propofol decreased I_to by binding to the open channels in human atrial myocytes. However, propofol showed no use- or rate-dependent blockade of I_to, which is similar to the effect of propafenone35 and allitridi33 on cardiac I_to.

The ultrarapid delayed rectifier potassium current I_Kur is present in human atria but not in the ventricles of the human heart.24 It is believed that I_Kur is a target for developing atrial-selective antiatrial fibrillation drugs.24,44,45 No information is available in the literature regarding the effect of propofol on I_Kur/Kv1.5. In this study, we provide the novel information that propofol inhibits human atrial I_Kur with an IC₅₀ of 35.3 μM. We found that the blocking fraction of propofol for I_Kur was slightly but significantly higher at potentials positive to +20 mV than that at 0 mV, which suggests a stronger inhibition of the current at positive potential of action potential. Because I_to and I_Kur play a crucial role in the repolarization of human atrial myocytes,25,32,45,46 blockade of I_to and I_Kur would significantly prolong atrial action potential duration and therefore would exert the antiatrial arrhythmic effect.25,45 Therefore, the antiatrial tachycardia and antiatrial fibrillation observed in patients with propofol21–23 is likely related to the inhibition of I_to and I_Kur.

Although propofol did not show the development blockade of I_Kur using short depolarization pulses with a prepulse.
in human atrial myocytes, the development blockade was observed in HEK 293 cells expressing hKv1.5 channels with a longer duration of depolarization pulses without prepulse. Propofol enhanced the inactivation of hKv1.5 current elicited by 5-s pulses, suggesting that propofol inhibits hKv1.5 by preferentially binding to the open channels. It is interesting that propofol is more sensitive to inhibit native \( I_{\text{Kur}} \) than hKv1.5 expressed in HEK 293 cells.

The delayed rectifier potassium currents \( I_{\text{Kr}} \) and \( I_{\text{Ks}} \) are also important repolarization currents in human heart.\(^34,40\) Blockade of \( I_{\text{Kr}} \) may be antiarrhythmic or proarrhythmic.\(^47-49\) The proarrhythmic effect of \( I_{\text{Kr}}/\text{hERG} \) blockade is mainly related to induction of prolonged QT interval of electrocardiography or Torsades de Pointes.\(^47,48\) An earlier report did not find the inhibition of \( I_{\text{Kr}} \) in guinea pig cardiac myocytes,\(^50\) while in \textit{Xenopus} oocytes expressing hERG channels, propofol significantly inhibited hERG current at a high concentration of 100 \( \mu \)M.\(^51\) The current study showed that significant inhibition of hERG channels expressed in HEK 293 cells was observed at 30 \( \mu \)M. The IC\(_{50}\) of propofol was 73.2 \( \mu \)M for hERG step current and 84.4 \( \mu \)M for hERG tail current, which is much greater than potassium concentrations in patients.\(^52,53\) Experimental study did not find the prolonged QT interval in guinea pigs.\(^51\) Clinical reports demonstrated that QTc interval was unaffected\(^45,55\) or shortened by propofol.\(^56\) Interestingly, propofol reversed the QT prolongation induced by sevoflurane\(^56\) and reduced the QT dispersion.\(^57\) These clinical reports suggest that the slight hERG/\( I_{\text{Kr}} \) inhibition would not induce a prolonged QT interval.

In \textit{Xenopus} oocytes expressing the cardiac \( I_{\text{Kr}} \) gene \( I_{\text{sK}} \), propofol showed less sensitivity for the current inhibition with an estimated IC\(_{50}\) value of 250 \( \mu \)M,\(^58\) while in guinea pig cardiac myocytes propofol at 300 \( \mu \)M fully inhibited \( I_{\text{Kr}} \).\(^50\) However, a recent report demonstrated that propofol inhibited \( I_{\text{Kr}} \) in guinea pig ventricular myocytes with an IC\(_{50}\) of 23 \( \mu \)M,\(^15\) which is close to that observed in the current study. We found that propofol inhibited human cardiac hKCNQ1/hKCNE1 channels expressed in HEK 293 cells with an IC\(_{50}\) of 32.4 \( \mu \)M. Reduction of human cardiac \( I_{\text{Ks}} \) may also contribute to the suppression of supraventricular tachycardia/fibrillation in patients with propofol.\(^21-23\)

Pharmacokinetic studies have shown that an intravenous dose of propofol for anesthesia may reach a peak level at 44 \( \mu \)M in blood plasma and generally 10 to 20 \( \mu \)M for anesthetic maintenance.\(^32,53\) In the current study, we found that significant inhibition of \( I_{\text{CaL}} \), \( I_{\text{Kur}} \) and hKCNQ1/hKCNE1 current by propofol was observed at 3 to 10 \( \mu \)M, which is in the range of anesthetic maintenance. Therefore, the conversion of supraventricular tachycardia/fibrillation to sinus rhythm in patients with propofol\(^21-23\) may be related to its inhibition of \( I_{\text{CaL}} \), \( I_{\text{Kur}} \) and \( I_{\text{Kr}} \). It should be noted that the current study demonstrated that significant inhibition of \( I_{\text{CaL}} \), \( I_{\text{Kur}} \) and hKCNQ1/hKCNE1 current was observed with 30 \( \mu \)M propofol. The effect was supposed to significantly prolong action potential duration. However, we found that action potential duration was slightly prolonged by propofol in human atrial myocytes. It is well recognized that for the effect of a drug on action potential duration, \( I_{\text{CaL}} \) blockade has a counter effect with potassium current inhibition. The slight increase in action potential duration with propofol is most likely due to the \( I_{\text{CaL}} \) blockade observed previously in human atrial myocytes\(^10\) and other species.\(^12\) This phenomenon is similar to that of \( \text{Ca}^{2+} \) channel blocker diltiazem on potassium currents and cardiac action potential.\(^36\) The conversion of supraventricular tachycardia/fibrillation to sinus rhythm in patients with propofol\(^21-23\) may be related to inhibiting more for potassium currents than for \( I_{\text{CaL}} \).

The limitation of the current study was that we were unable to record \( I_{\text{Kr}} \) and \( I_{\text{Ks}} \) in human atrial myocytes isolated from small atrial specimens with the tissue chunk method. It is unclear whether the channels are digested by enzymes. If so, it may lead to an alteration of the action potential waveform and action potential duration prolongation and induce an underestimation of propofol effect on the action potential. The observed slight prolongation of action potential duration by propofol may be related partially to the loss of these two channels.

In summary, the current study reported for the first time that propofol inhibited human cardiac atrial repolarization potassium currents including human atrial \( I_{\text{Kr}} \) and \( I_{\text{Ks}} \), and slightly prolonged human atrial action potential duration. Propofol also inhibited hERG and hKCNQ1/hKCNE1 channels expressed in HEK 293 cells. Reduction of \( I_{\text{CaL}}, I_{\text{Kur}}, \) and \( I_{\text{Kr}} \) likely contributes to the suppression of supraventricular tachycardia and atrial fibrillation observed in patients given propofol.

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**Competing Interests**

The authors declare no competing interests.

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