Anesthesiology 2009; 110:50–7

Differential Effects of Propofol and Sevoflurane on Ischemia-induced Ventricular Arrhythmias and Phosphorylated Connexin 43 Protein in Rats

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Background: **The effects of anesthetics on ischemia-induced ventricular arrhythmias remain poorly studied. This study investigated the effects of propofol and sevoflurane on the survival rate and morbidity as a result of ventricular arrhythmias, and defined a possible mechanism for the arrhythmogenic properties of anesthetics during acute myocardial ischemia.**

Methods: **Under anesthesia with intraperitoneal sodium pentobarbital, Sprague-Dawley rats underwent 30 min of left anterior descending coronary artery ligation. The rats were divided into a low-dose propofol (Prop-LD) group (39 mg · kg^{-1} · hr^{-1}, n = 18), a high-dose propofol group (78 mg · kg^{-1} · hr^{-1}, n = 18), a sevoflurane group (2.5%, n = 18) and a control group (n = 18). The survival rate and morbidity as a result of ventricular arrhythmias were determined, and the amount of phosphorylated connexin 43 protein was measured 30 min after coronary artery ligation.**

Results: **The survival rate was 83% (15 of 18), 94% (17 of 18), 89% (16 of 18), and 67% (12 of 18, $P = 0.038$ *vs.* Prop-LD) in the control, Prop-LD, high-dose propofol, and sevoflurane groups, respectively. Sustained ventricular tachycardia was observed in 83% (15 of 18), 39% (7 of 18, $P = 0.011$ *vs.* control), 50% (9 of 18, $P = 0.039$ *vs.* control) and 94% (17 of 18, $P < 0.01$ *vs.* Prop-LD) in the control, Prop-LD, high-dose propofol, and sevoflurane groups, respectively. Immunoblotting showed a marked reduction in the amount of phosphorylated connexin 43 in the control and sevoflurane groups, as compared with the Prop-LD and high-dose propofol groups ($P < 0.05$).**

Conclusion: **The authors' results suggest that propofol preserves connexin 43 phosphorylation during acute myocardial ischemia, as compared with sevoflurane, and this might protect the heart from serious ventricular arrhythmias during acute coronary occlusion.**

PROPOFOL and sevoflurane are widely used during anesthesia in patients with ischemic heart disease. These patients are at high risk for perioperative myocardial ischemia (MI), which is a major factor leading to lethal ventricular arrhythmias. Anesthetic agents such as halothane, enflurane, and isoflurane are known to facilitate reentrant excitation in subjects with MI.[1] However, the effects of propofol and sevoflurane on ventricular arrhythmias related to MI remain unknown, even though many studies have suggested that these anesthetics have cardioprotective effects on functional, metabolic, and histologic changes caused by ischemia or reperfusion injury.[2]

In the ischemic myocardium there is a reduction of tissue pH, an increase in interstitial potassium levels and intracellular calcium concentration, and neurohumoral changes that all contribute to the development of electrical instability and lethal cardiac arrhythmias.[3] In particular, cell-to-cell electrical uncoupling of ventricular myocytes plays an important role in arrhythmogenesis during acute MI.[4,5]

Connexin 43 (Cx43), a principal cardiac gap-junction channel protein, has been implicated in the electrical coupling of cardiac muscle cells.[6] Many studies have demonstrated that ischemia and heart failure reduce Cx43 expression,[7,8] and Cx43 undergoes progressive dephosphorylation with a time course similar to that of electrical uncoupling during acute MI.[9] Moreover, transplantation of Cx43-expressing embryonic cardiomyocytes in myocardial infarcts protects against the induction of ventricular tachycardia in mice.[10] These results indicate that Cx43 dysfunction in cardiomyocytes could be an important factor that contributes to the substrate for lethal ventricular arrhythmias.

In this study, we examined the effects of propofol and sevoflurane on ventricular tachyarrhythmias during MI *in vivo*. Furthermore, we clarified whether these anesthetics affect Cx43 dephosphorylation during MI.

Materials and Methods

The experimental protocols used in this study were approved by the Sapporo Medical University Animal Care and Use Committee (Sapporo, Hokkaido, Japan).

Surgical Preparation and Coronary Artery Ligation

Male Sprague-Dawley rats (250-300 g, aged 7–9 weeks) were assigned to the following 6 treatment groups: a sham-operated group (Sham, n = 5), a control group (n = 18), a low-dose propofol (Prop-LD) group (n = 18), a high-dose propofol (Prop-HD) group (n = 18) and a sevoflurane (Sevo) group (n = 18). Moreover, to estimate the effects of propofol on parasympathetic activity, rats with MI were treated with IV atropine before the administration of a low dose of propofol (Prop-Atr, n = 18).

After induction of anesthesia with 50 mg/kg intraperitoneal sodium pentobarbital, the rats were intubated and

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Received from the Department of Anesthesiology, Sapporo Medical University, School of Medicine, Sapporo, Japan. Submitted for publication February 5, 2008. Accepted for publication August 4, 2008. Support was provided solely from institutional and/or departmental sources.

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ventilated artificially with a volume-controlled rodent respirator (Model 683; Harvard Apparatus, Holliston, MA) at 65-80 strokes/min to maintain normal arterial levels of P_{O_2}, partial pressure of carbon dioxide (P_{CO_2}) and pH. A polyethylene catheter was inserted into the femoral artery for direct measurement of blood pressure, and the femoral vein was cannulated for drug administration. A thoracotomy was performed horizontally in the fourth intercostal space, and a suture was loosely tied around the left anterior descending (LAD) coronary artery. Electrodes were placed to allow the measurement of a Lead II electrocardiogram. Mean blood pressure (MBP) and heart rate (HR) were recorded every 5 min throughout the experiment. Body temperature was continuously monitored with a rectal thermometer and maintained at 37°C using a heating pad and overhead lamp.

After preparation for LAD ligation, drugs were administered according to the group assignment. In the Prop-LD group, propofol was continuously infused IV at a sedative dose (39 mg $\cdot$ kg^{-1} $\cdot$ hr^{-1}), which was determined by using the tail-clamp technique.[11] In the Sevo group, rats were exposed to 2.5% sevoflurane that corresponds to a minimum alveolar anesthetic concentration of 1, which was also determined using the tail-clamp technique.[12] In the Sham, Control, and Sevo groups, Intralipid (Pharmacia AB, Stockholm, Sweden) was continuously infused IV (390 mg $\cdot$ kg^{-1} $\cdot$ hr^{-1}) so that the volume of injectate would be the same as in the Prop-LD group. Since the baseline MBP and HR in the Sevo group were obviously lower than in the Control and Prop-LD groups, a twofold higher dose of propofol (78 mg $\cdot$ kg^{-1} $\cdot$ hr^{-1}) was used in another group (Prop-HD group) to match the baseline MBP and HR in the Sevo group. The dose of propofol needed to match the baseline MBP and HR in the Sevo group was selected based on a pilot study (data not shown). In the Prop-Atr group, atropine sulfate (1 mg/kg), which blocks cholinergic receptors in rats,[13] was injected IV just before the infusion of the low dose of propofol.

The rats were allowed to stabilize for 15 min after administration of all drugs, and then MI was induced for 30 min by LAD ligation. In the Sham group, the LAD was isolated but not ligated. MI in the 5 groups with LAD ligation was confirmed visually by the appearance of regional cyanosis and by ST segment changes in the electrocardiogram.

Arrhythmia Study

Each arrhythmia was defined according to the guidelines of the Lambeth Convention.[14] During the 30 min of MI, if ventricular fibrillation (VF) did not spontaneously revert to sinus rhythm within 10 s, precordial taps were used to try to restore sinus rhythm. If resuscitation for 2 min failed to revive the rat, the animal was considered dead. The criteria for scoring arrhythmias were modified from the method described by Leenen *et al.*[15]: 0 for normal sinus rhythm, 1 for premature ventricular contractions, 2 for nonsustained ventricular tachycardia (VT) within 10 beats, 3 for spontaneously reversible VT over 10 beats or reversible VF within 10 s, 4 for sustained VT or reversible VF with precordial taps, and 5 for irreversible VF causing death. The highest arrhythmia score was recorded for each 5-min period during 30 min of LAD ligation.

Determination of Ischemic Areas

At the end of 30 min of LAD ligation or at the time of death as a result of VF, the heart was removed and perfused retrogradely with 10 ml of 0.9% saline to wash out blood from the coronary circulation. Then, 2 ml of 2% Evans blue was injected to confirm the lack of perfusion of the ischemic area. The hearts were excised and frozen and then sliced into 2-mm-thick transverse sections from apex to base. The ischemic area was determined by negative staining with Evans blue. Since myocardial infarction is difficult to measure after only 30 min of coronary ligation, no attempt was made to separate ischemic from infarcted tissue. The ischemic area was measured using NIH image software (version 1.62; National Institutes of Health, Bethesda, MD).

Protein Preparation and Immunoblotting

Because the hearts injected within Evans blue could not be used for immunoblotting, we analyzed another 5 hearts in each group for Cx43 levels.

The ventricular tissues were suspended in 40 ml of ice-cold 10% tricholroacetic acid and homogenized with a tissue homogenizer (2 bursts, 30 s each). Homogenates were centrifuged at 10,000 *g* for 10 min at 4°C, and protein content was determined using detergent-compatible protein assay (Bio-Rad Laboratories Inc., Hercules, CA) with bovine serum albumin. The proteins were then put in a 3× sample buffer consisting of 0.2 M Tris-HCl (pH 6.8), 4% sodium dodecylsulfate, 8 M urea, 0.1 M dithiothreitol, and 0.01% bromophenol blue. Equal amounts of protein per lane were loaded onto a 15% polyacrylamide gel and separated by electrophoresis at 30 mA/gel for 60 min with a running buffer containing 25 mM Tris, 192 mM glycine, and 0.1% sodium dodecylsulfate. Molecular weight markers (Amersham Biosciences, Buckinghamshire, United Kingdom) were used in each gel. Proteins were transferred to a polyvinylidene diflouride membrane (Immobilon-P; Millipore Corp., Bedford, MA) at 36 V for 4 h using a transfer buffer containing 0.01 M 3-[cyclohexylamino]-1-propanesulfonic acid (pH 11)-10% methanol. The blots were incubated with 5% nonfat dry milk in phosphate-buffered saline (PBS; pH 7.4) for 1 h at room temperature to block nonspecific binding of the antibodies. Then the membrane preparation was incubated with a rabbit polyclonal anti-Cx43 antibody (1:1,000 dilution; Zymed Lab-

Table 1. Hemodynamics before and after 30 min of LAD Ligation

		Mean Arterial Pressure (mmHg)		Heart Rate (bpm)	
	n	Before	After 30-min Ligation	Before	After 30-min Ligation
Sham	5	119 ± 14	120 ± 12	398 ± 25	414 ± 27
Control	18	120 ± 15	110 ± 9	405 ± 24	398 ± 43
Propofol low dose	18	117 ± 13	96 ± 15	412 ± 22	387 ± 34
Propofol high dose	18	85 ± 14*	78 ± 24*	353 ± 25*	360 ± 48*
Propofol-atropine	18	110 ± 8	103 ± 10	405 ± 20	389 ± 22
Sevoflurane	18	82 ± 9*	80 ± 22*	325 ± 31*	324 ± 45*

The hemodynamic parameters recorded before ligation were obtained 15 minutes after drug/anesthetic administration. All values are mean ± SD.

* $P < 0.05$ *vs.* control group.

bpm = beat per minute; LAD = left anterior descending coronary artery.

oratories, South San Francisco, CA) in 0.1% bovine serum albumin and placed in PBS overnight at 4°C. The concentration of actin, a housekeeping protein, was also measured using rabbit antiactin antibody (1:1,000; Sigma Chemical Co., St. Louis, MO). The blot was washed for 30 min with 2 changes of 2% nonfat dry milk-0.1% Tween-20 in PBS and then incubated with a goat anti-rabbit immunoglobulin G secondary antibody conjugated to horseradish peroxidase (1:5,000; DAKO, Glostrup, Denmark) in 0.1% bovine serum albumin and PBS for 30 min at room temperature. The membrane was washed 5 times for 30 min with 2% nonfat dry milk-0.1% Tween-20 in PBS. The reaction product was visualized on x-ray film (XAR-5; Kodak, Rochester, NY) using an enhanced chemiluminescence kit (Amersham Biosciences). The amount of protein expression was quantified from the signal intensity of the labeled bands using an image densitometer (NIH Image 1.63; National Institutes of Health).

Data Analysis

The time of death as a result of VF and onset of the first run of VT (arrhythmia score, more than 3) was analyzed by the Kaplan-Meier method, and the groups were compared with the Mantel-Haenzel log-rank test. Arrhythmia scores were presented as the median and interquartile range, and the groups were compared with a Kruskal-Wallis test. Proteins were quantified using a densitometer to give the Cx43/actin ratio. The Cx43/actin ratio was determined for both phosphorylated Cx43 (43kDa) and nonphosphorylated Cx43 (41kDa). Hemodynamics, ischemic areas, and Cx43/actin were presented as the mean ± SD. Differences in ischemic areas and tissue concentration of Cx43 were compared using one-way analysis of variance, followed by a Tukey test. Differences in hemodynamics were compared by 2-way analysis of variance with repeated measures and a Tukey test. A *P* value of less than 0.05 was considered statistically significant.

Results

Hemodynamic Parameters and Ischemic Areas

Table 1 shows hemodynamics changes. In the Prop-HD and Sevo groups, MBP and HR were lower before and during LAD ligation, as compared with the Control group. There were no differences in hemodynamics among the Control, Prop-LD, and Prop-Atr groups.

Table 2 shows arterial levels of P_{O_2}, P_{CO_2}, and pH, and the ischemic area in each group. There were no significant differences in the blood gas analyses during LAD ligation. Ischemic areas were 56 ± 7%, 55 ± 8%, 53 ± 5%, 59 ± 7%, and 53 ± 7% in the Control, Prop-LD, Prop-HD, Prop-Atr, and Sevo groups, respectively. There were no significant differences in ischemic areas among all the groups ($P = 0.88$).

Table 2. Blood Gas Analyses and Ischemic Area

		pH		P_{O_2} (mmHg)		P_{CO_2} (mmHg)		
	n	Before	After 30-min Ligation	Before	After 30-min Ligation	Before	After 30-min, Ligation	Ischemic Area, % LV
Sham	5	7.43 ± 0.02	7.42 ± 0.05	103 ± 7	102 ± 9	39 ± 2	39 ± 5	—
Control	18	7.44 ± 0.02	7.41 ± 0.12	104 ± 5	101 ± 8	38 ± 3	39 ± 5	56 ± 7
Propofol low dose	18	7.43 ± 0.03	7.42 ± 0.08	102 ± 6	102 ± 9	38 ± 2	38 ± 7	55 ± 8
Propofol high dose	18	7.42 ± 0.02	7.41 ± 0.15	103 ± 7	99 ± 8	39 ± 3	39 ± 7	53 ± 5
Propofol-atropine	18	7.44 ± 0.02	7.43 ± 0.10	104 ± 7	101 ± 7	38 ± 2	40 ± 5	53 ± 7
Sevoflurane	18	7.42 ± 0.05	7.41 ± 0.12	100 ± 5	98 ± 9	38 ± 4	39 ± 3	57 ± 7

All values are mean ± SD. There were no significant differences in pH, P_{O_2}, P_{CO_2}, and ischemic area among all the groups.

LV = left ventricle.

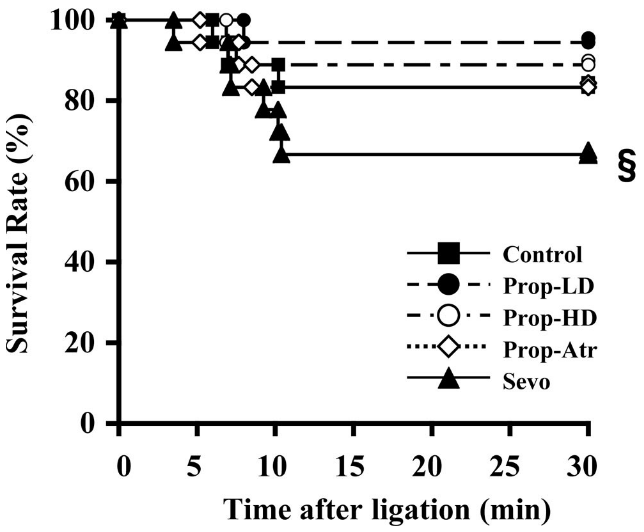

Fig. 1. Survival rate during 30 min of left anterior descending (LAD) coronary artery ligation in all groups. The survival rate was 83% (15 of 18), 94% (17 of 18), 89% (16 of 18), 83% (15 of 18), and 67% (12 of 18) in the Control, low-dose propofol (Prop-LD), high-dose propofol (Prop-HD), propofol with atropine (Prop-Atr), and sevoflurane (Sevo) groups, respectively. A low dose of propofol significantly decreased the mortality rate, as compared with sevoflurane (P = 0.038, Prop-LD *vs.* Sevo group). § P < 0.05 *versus* Prop-LD.

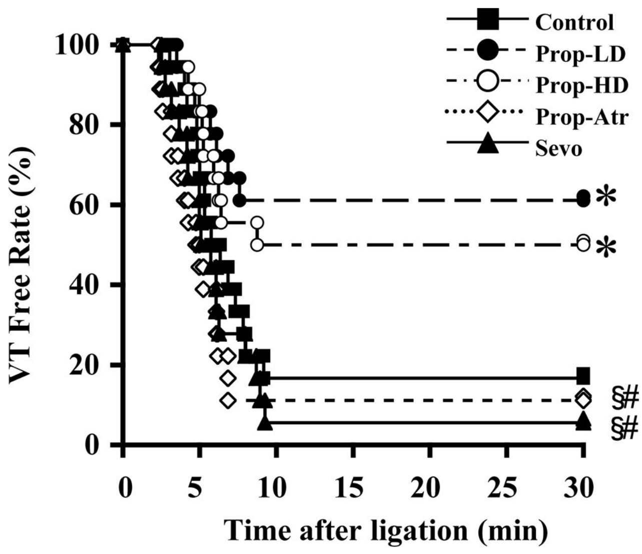

Fig. 2. Ventricular tachycardia (VT)-free rate during 30 min of left anterior descending (LAD) coronary artery ligation. Sustained VT was observed in 83% (15 of 18), 39% (7 of 18), 50% (9 of 18), 89% (16 of 18), and 94% (17 of 18) in the Control, low-dose propofol (Prop-LD), high-dose propofol (Prop-HD), propofol with atropine (Prop-Atr), and sevoflurane (Sevo) groups, respectively. Prop-LD and Prop-HD decreased the incidence of ischemic-induced sustained VT, as compared with the Control (P < 0.05), Prop-Atr, and Sevo groups (P < 0.01). * P < 0.05 *versus* Control. § P < 0.05 *versus* Prop-LD. # P < 0.05 *versus* Prop-HD.

In Vivo *Arrhythmia Study*

Figure 1 shows the survival rates in all experiments. The survival rate during the 30-min LAD ligation was 83% (15 of 18), 94% (17 of 18), 89% (16 of /18), 83% (15 of 18), and 67% (12 of 18) in the Control, Prop-LD, Prop-HD, Prop-Atr, and Sevo groups, respectively. Prop-LD significantly decreased the mortality rate, as compared with to 2.5% sevoflurane (P = 0.038, Prop-LD *vs.* Sevo group). There was no significant difference in the survival rate between the Control group and the other 4 groups.

Figure 2 shows the time of onset and the incidence of the first sustained VT episode (arrhythmia score greater than 3) during the 30-min LAD ligation. In all cases, the first run of VT occurred within 10 min after LAD ligation. Sustained VT was observed in 83% (15 of 18), 39% (7 of 18), 50% (9 of 18), 89% (16 of 18), and 94% (17 of 18) in the Control, Prop-LD, Prop-HD, Prop-Atr, and Sevo groups, respectively. Prop-LD and Prop-HD significantly decreased the incidence of ischemia-induced VT (Prop-LD, $P = 0.011$ *vs.* the Control group and $P < 0.01$ *vs.* the Prop-Atr or Sevo group; Prop-HD, P = 0.039 *vs.* the Control group and $P < 0.01$ *vs.* the Prop-Atr or Sevo group). There were no significant differences in the incidence of sustained VT among the Control, Prop-Atr (P = 0.26 *vs.* Control) and Sevo groups (P = 0.29 *vs.* Control), although sevoflurane slightly increased the incidence of ischemia-induced VT. Figure 3 shows the arrhythmia scores during the 30-min LAD ligation. The peaks of arrhythmia scores were observed from 5 to 10 min after LAD ligation in all groups. The Prop-LD and Prop-HD groups had significantly lower arrhythmia scores ($P < 0.05$ *vs.* the Prop-Atr or Sevo group).

In our study, the Control group was an Intralipid group and not a true control group without any drugs. To evaluate the possibility of modification of ischemia-induced arrhythmias by Intralipid itself, we induced ischemia in a group with normal saline-infused IV (3.9 ml/hr, n = 7) instead of Intralipid in preliminary experiments. The survival rate during the 30-min LAD ligation was 71% (5 of 7), and sustained VT was observed in 86% (6 of 7) of the rats in the normal saline group. There were no significant differences in hemodynamics, arrhythmia scores (data not shown), survival rate, and incidence of sustained VT between the Intralipid control group and the normal saline group.

Effects of Anesthetics on Cx43 Expression

To examine the possibility that the beneficial effect of propofol on ischemia-induced arrhythmias is caused by preventing the loss of phosphorylated Cx43, we measured the tissue concentration of phosphorylated Cx43 after 30 min of LAD ligation. The polyclonal anti-Cx43 antibody in the present study showed closely spaced bands migrating between 43 and 46 kDa, and a faint band migrating at 41 kDa. Previous reports[9] demonstrated that higher and lower molecular weight bands represent phosphorylated (P-Cx43) and nonphosphorylated isoforms of Cx43 (NP-Cx43), respectively. Figure 4A shows a representative immunoblot prepared with

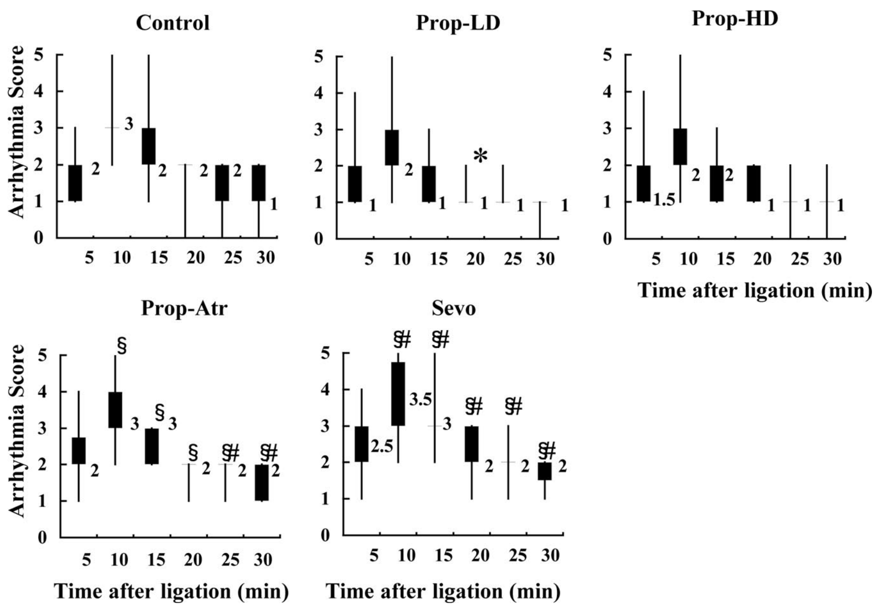

Fig. 3. Median and interquartile range of arrhythmia scores every 5 min during 30 min of left anterior descending (LAD) coronary artery ligation. In all groups, the peaks of arrhythmia scores were observed from 5 to 10 min after LAD ligation. In the low-dose propofol (Prop-LD) and high-dose propofol (Prop-HD) groups, arrhythmia scores were lower than those in the propofol with atropine (Prop-Atr) or sevoflurane (Sevo) groups. * *P* < 0.05 *versus* Control. § *P* < 0.05 *versus* Prop-LD. # *P* < 0.05 *versus* Prop-HD.

polyclonal anti-Cx43 and antiactin antibodies. P-Cx43 (43 kDa band) decreased and NP-Cx43 (41 kDa band) increased after 30 min of LAD ligation in all but the Sham group. The levels of P-Cx43 in the Prop-LD and Prop-HD groups were preserved, as compared with the Control, Prop-Atr, and Sevo groups. The levels of NP-Cx43 (41kDa band) in the Control, Prop-Atr, and Sevo groups increased more than that of the Prop-LD and Prop-HD groups. Optical density analysis of the Cx43/actin ratio revealed that Prop-LD and Prop-HD prevented the loss of P-Cx43 and the increased expression of NP-Cx43, as compared with the Control, Prop-Atr, and Sevo groups. Figure 4B shows that the levels of P-Cx43 and NP-Cx43 were not significantly different among the Control and Prop-Atr and Sevo groups.

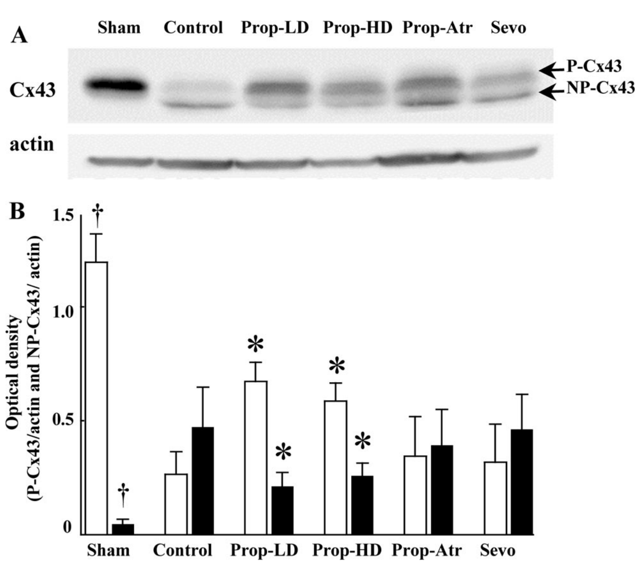

Fig. 4. (*A*) Representative immunoblots of homogenates of left ventricles from the Sham, Control, low-dose propofol (Prop-LD), high-dose propofol (Prop-HD), propofol with atropine (Prop-Atr), and sevoflurane (Sevo) groups, probed with polyclonal Cx43 antibody. Arrows indicate the position of phosphorylated isoform of Cx43 (P-Cx43; 43 kDa) and nonphosphorylated isoform of Cx43 (NP-Cx43; 41kDa) bands, respectively. (*B*) Optical density analysis of P-Cx43:actin (□) and NP-Cx-43: actin (■) ratios in the Control, Prop-LD, Prop-HD, Prop-Atr, and Sevo groups, respectively. Data are expressed as mean ± SD from 5 rats in each group. * *P* < 0.05. † *P* < 0.01 *versus* Cx43 in the Control group.

Discussion

The present study demonstrates that propofol attenuates acute ischemia-induced arrhythmias *via* modulation of a principal cardiac gap-junction protein, Cx43. Continuous infusion of propofol reduced morbidity as a result of acute ischemia-induced ventricular tachyarrhythmias and inhibited loss of the phosphorylated isoform of Cx43. Moreover, these antiarrhythmic effects of propofol were blocked by atropine.

Anesthetics and Arrhythmogenic Properties

In humans, there have been case reports of the antiarrhythmic effects of propofol in patients with VT,[16] atrial fibrillation,[17] and supraventricular tachycardia.[18] These antiarrhythmic effects of propofol are likely explained by increased cardiac parasympathetic tone and reduced cardiac sympathetic tone. Direct effects of propofol on the cardiac conduction system have been demonstrated in various animal studies *in vitro*.[19–21] However, the effects of propofol on ischemia-induced arrhythmias have not been elucidated in previous studies.

Although the manifestation of the ischemia-induced arrhythmias is the result of a variety of interacting factors, sympathetic overactivity is important in the generation of acute[22] and chronic[23] ischemia-induced arrhythmias. MI causes a spatially uneven increase in sympathetic nerve activity in the heart, resulting in regional variation in the release and, consequently, variations in tissue levels of sympathetic neurotransmitters (epinephrine and norepinephrine).[3] Nonuniform elevation of neurotransmitters, through alterations in the expression of L-type Ca^{2+} channels and K^+ channels,[24,25] creates electrophysiological heterogeneity between the ischemic and normal myocardium.[3] Furthermore, locally elevated levels of neurotransmitters increase coronary arterial tone, thereby critically reducing coronary perfusion under conditions of increased oxygen demand (physical and/or emotional stress) and causing regional ischemia, which contributes to the development of arrhythmias.[3] Many studies have demonstrated that sympathetic blockade by β-blockers,[26] left stellate ganglion blockade,[27] and thoracic epidural anesthesia[28] is beneficial in postmyocardial infarction patients. These results suggest an important role of the sympathetic nervous system in the pathogenesis of ischemia-induced arrhythmias.

In the present study, it is noteworthy that propofol's action, including the action on Cx43, was blocked by atropine. It is possible that the antiarrhythmic effect of propofol in our results was because of a reduction in sympathetic tone leading to a dominance of parasympathetic tone. Propofol is also well known to inhibit action potential duration (APD),[29] sarcolemmal L-type Ca^{2+} channels, K^+ channels,[30] and the Ca^{2+} uptake capacity of the sarcoplasmic reticulum,[31]; thus, propofol has multiple sites of action in the cardiac cells that could contribute to antiarrhythmic effects in our study. In the ischemic myocardium, it is commonly believed that an increase in membrane K^+ conductance *via* activation of adenosine triphosphate (ATP)-dependent potassium (K_{ATP}) channels causes APD shortening, which is deeply involved in repolarization and refractoriness to conduction.[32] The dispersion of repolarization and refractoriness to conduction between the ischemic and normal zone has been implicated in the generation of ventricular arrhythmias.[33] Hanouz *et al.*[19] showed that propofol decreased the dispersion of APD between the ischemic and normal zone and decreased the occurrence of arrhythmias. Although the precise mechanisms underlying the effect of propofol on ischemia-induced changes in APD and on potassium channels remain poorly studied, this effect of propofol might be involved in our results.

Intralipid, as a solvent for propofol, has been reported to have no effect on ischemia–reperfusion injury.[34,35] However, the effect of Intralipid on ischemia-induced arrhythmias remains unknown. Our preliminary results demonstrated that Intralipid itself did not affect hemodynamics and ischemia-induced arrhythmias. Based on these results, we believe that the use of Intralipid as a vehicle did influence the effects of propofol during ischemic conditions.

While propofol had beneficial effects on ischemia-induced arrhythmias, sevoflurane lacked this effect. This absence of protection occurred in spite of sevoflurane's well-known cardioprotective effects against myocardial dysfunction and dysrhythmias in ischemic/reperfusion models.[2,36] Sevoflurane is also well known to inhibit L-type Ca^{2+} current,[37] and this might inhibit Ca^{2+} overload and prevent early afterdepolarizations that may contribute to arrhythmias in ischemic myocardium. In contrast, Chae *et al.*[38] showed that sevoflurane prolongs APD, which may lead to fatal ventricular arrhythmias, *via* suppression of transient outward currents (I_{to}) in rat ventricular myocytes. Although it is difficult to determine the effect of sevoflurane on ischemia-induced arrhythmias *via* various ion currents, it is certain that these actions will occur in combination with altered cell-to-cell conduction induced by ischemia. Furthermore, many studies demonstrated that sevoflurane decreases parasympathetic activity,[39–41] resulting in sympathetic dominance that might contribute to ischemia-induced arrhythmias. In our results, it is difficult to determine if sevoflurane decreases parasympathetic more than sympathetic activity, because sevoflurane caused a reduction in heart rate. However, previous studies[40,42] have shown that heart rate and sympathovagal balance were not correlated during anesthesia; thus, to accurately estimate autonomic activity during anesthesia when heart rate is not at a steady state, nonlinear measures such as heart rate entropy may be preferable. Since sevoflurane has both proarrhythmic and antiarrhythmic effects, it is difficult to determine the mechanisms responsible for the proarrhythmic effect of sevoflurane on ischemia-induced arrhythmias in our study, and further investigation is required.

Anesthetics and Connexin 43

Many studies have demonstrated that Cx43 is remarkably reduced in ischemia and heart failure.[9,10] Gene-targeting studies demonstrate that reduced expression of Cx43 increases the incidence of ventricular tachyarrhythmias[43] and causes a significant reduction in conduction velocity in mice during acute MI.[44] Beardslee *et al.*[9] reported that reversible Cx43 dephosphorylation could also contribute to myocardial cellular uncoupling, and thus play a role in arrhythmogenesis during acute ischemia. One potential mechanism of ischemia-induced accumulation of dephosphorylated Cx43 is decreased intracellular ATP concentration and decreased thermodynamic driving force for phosphorylation (the free-energy change of ATP hydrolysis). The decrease in the free-energy change of ATP hydrolysis during ischemia is biphasic, with a moderate immediate decrease and a marked secondary decrease that coincides with cell-to-cell uncoupling.[45] Although these studies demonstrated that Cx43 is an important factor for arrhythmias during MI, the effects of anesthetics on Cx43 remain unknown.

This is the first study showing that anesthetics attenuated a principal cardiac gap-junction protein, Cx43, during MI. In our study, only propofol prevented the loss of

P-Cx43 and the increased expression of NP-Cx43, regardless of the dose, during 30 min of LAD ligation. However, the precise mechanism of the effect of propofol or sevoflurane on modulation of Cx43 remains unknown. Recent studies have demonstrated that vagal nerve stimulation prevented ventricular fibrillation after myocardial infarction in rats[46] and dogs.[47] Ando *et al.*[46] reported that vagal nerve stimulation exerted an antiarrhythmic effect during acute MI by preserving phosphorylated Cx43. Several mechanisms might be involved in the linkage between vagal nerve stimulation and the preservation of protein levels of phosphorylated Cx43 during MI. Vagal nerve stimulation may activate several protein kinases and induce phosphorylation of Cx43 through muscarinic receptors.[48] Our previous study[42] and other studies[39–41] have reported differential effects of propofol and sevoflurane on the autonomic nervous system. Propofol is believed to reduce parasympathetic tone to a lesser degree than sympathetic tone, leading to parasympathetic dominance. In contrast, sevoflurane decreases parasympathetic tone to a larger degree than sympathetic tone, leading to sympathetic dominance. Our present study suggests that these differential effects of propofol and sevoflurane on the autonomic nerve system resulted in attenuation of phosphorylated protein levels of Cx43 from our result that propofol's action, including an action on Cx43, was blocked by atropine.

Limitations

We recognize several limitations of our study. First, although we show a remarkable benefit of propofol during acute MI in the rat model, it should be recognized that there are differences in the timing of ischemia-induced arrhythmias, electrophysiological properties, and functional morphology between rat and human patients or other species. In particular, rat myocytes are known to have a much briefer action potential.[49] Despite species differences, knowledge obtained from the study of anesthetic agents in mouse, rat, guinea pig, or rabbit models, and their underlying electrophysiological and metabolic mechanisms of action, has been instrumental in the development of strategies for anesthesia management in humans. Second, HR and MBP in the Prop-HD and Sevo groups tended to be lower than in the other groups. However, changes in HR and MBP did not appear to significantly influence arrhythmias or the concentration of phosphorylated-Cx43 between the Prop-LD and Prop-HD groups. Therefore, this may indicate that the range of hemodynamic changes barely affected our results. Finally, it is unclear whether the effects of propofol and sevoflurane on the phosphorylated-Cx43 are direct or indirect *via* the autonomic nervous system. Previous studies have demonstrated that halothane might block the gap-junction channels and cause asynchronous beating in cultured neonatal rat ventricular myocytes.[50,51] Based on these findings, further investigation of the direct effects of propofol and sevoflurane on Cx43 is required.

Summary

Propofol and sevoflurane have differential effects on ischemia-induced arrhythmias *via* modulation of the autonomic nervous system and/or a principal gap-junction protein, Cx43. One of the mechanisms of propofol's antiarrhythmic effect during myocardial ischemia might be preservation of phosphorylated-Cx43 protein during myocardial ischemia.

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