

Full Paper

Pharmacological Significance of the Blocking Action of the Intravenous General Anesthetic Propofol on the Slow Component of Cardiac Delayed Rectifier K^+ Current

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Abstract. Propofol is a widely used intravenous general anesthetic. The negative inotropic effect of propofol has been best explained by inhibition of the L-type Ca^{2+} current (I_{Ca}). Using guinea-pig cardiac preparations, however, we found that the propofol concentration producing a 50% decrease in force of contraction was more than 10 times higher than that producing a 50% inhibition of I_{Ca} , implying that a compensatory mechanism may be present to counteract the negative inotropic effect associated with the I_{Ca} inhibition. Consistent with I_{Ca} inhibition, propofol produced a shortening of action potential duration (APD) in single cardiomyocytes. Yet, the concentrations necessary to shorten APD were greater than that for 50% inhibition of I_{Ca} . This was associated with the potent and effective inhibition of the slowly activating component of the delayed rectifier K^+ current (I_{Ks}). Thus, the I_{Ks} blockade with propofol may counterbalance the APD shortening evoked by its I_{Ca} inhibition. Taken together, the negative inotropic effect of propofol is detectable only at supratherapeutic concentrations. At clinically relevant concentrations, the action potential prolongation mechanism due to I_{Ks} inhibition appears to alleviate the reduction in transsarcolemmal Ca^{2+} influx through L-type Ca^{2+} channels, which may help to counteract the net negative inotropism of propofol.

Keywords: propofol, cardiac contractility, L-type Ca^{2+} current, delayed rectifier K^+ current, cardiac action potential

Introduction

Propofol (2,6-diisopropylphenol) is a widely used intravenous anesthetic with advantageous properties such as a rapid onset, short duration of action, and rapid elimination (1). Therefore, this anesthetic agent is used for induction and maintenance of general anesthesia and for sedation in intensive care units. However, undesirable cardiovascular side effects associated with

its use have been reported, including bradycardia, decreased vascular resistance, hypotension, and decreased cardiac output (1). The pronounced drop in blood pressure caused by propofol appears to be due to peripheral vasodilation (2, 3), but in turn, it may result from a direct negative effect on myocardial contractility. In patients, cardiac depression has been observed during propofol infusion (4, 5). Furthermore, the negative inotropic effect of propofol has been demonstrated in different animal models and isolated heart preparations (6–13), although it should be noted that others have not observed this negative inotropic effect or found it only in supraclinical concentrations (14–16).

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The mechanism of the negative inotropic effect of propofol is suggested to be a decreased transsarcolemmal Ca^{2+} influx. Propofol has been found to induce a dose-dependent lowering of free cytosolic Ca^{2+} concentration in fura-2-loaded rat myocardial cells (17). Studies on single cardiomyocytes of guinea pigs and rats using the voltage clamp technique have shown that propofol decreases the L-type Ca^{2+} current (I_{Ca}) (18–20). The decrease in I_{Ca} is likely to be ascribed to the altered kinetics of opening and closing of Ca^{2+} channels to favor shut states (21). Contrarily, possible effects of propofol on sarcoplasmic reticulum function and on myofilament Ca^{2+} sensitivity have been proposed for the regulatory mechanisms of its inotropism (16, 22, 23). Moreover, propofol is known to modulate several ionic currents other than I_{Ca} (24–28). The inhibitory effects of propofol on cardiac Na^+ current (I_{Na}), transient outward K^+ current (I_{to}), and delayed rectifier K^+ current (I_K) have been demonstrated (24, 25, 28). Propofol also inhibits the current generated by hyperpolarization-activated cyclic nucleotide-gated channels at clinically relevant concentrations (27). Moreover, propofol has been shown to inhibit ATP-sensitive K^+ (K_{ATP})-channel activity in rat ventricular myocytes, although very high, nonclinical concentrations were needed (26).

Some of changes in those currents may affect myocardial contractility, albeit less directly than does I_{Ca} . Therefore, the L-type Ca^{2+} channel-blocking property of propofol could largely explain its negative inotropic effect in myocardium, but the change in myocardial contractility caused by propofol may involve other potentially decisive factors. Yet, systematic comparison of the effects of propofol on force of contraction, myofilament Ca^{2+} sensitivity, ionic membrane currents, and action potential configurations in cardiac preparations from the same species is currently unavailable. Thus, the present study sought to systemically evaluate the net negative inotropic effect of propofol using isolated right ventricular papillary muscles, chemically skinned trabecular muscles, and single ventricular myocytes from guinea pigs.

Materials and Methods

Measurement of isometric tension

All experiments were performed in accordance with the approved institutional animal care guidelines. Male Hartley guinea pigs (250–500 g) were killed by an overdose of pentobarbital. Hearts were quickly excised and placed in a dissection bath containing oxygenated Krebs-Henseleit solution. The composition of the solution was 119 mM NaCl, 4.8 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4 , 2.5 mM $CaCl_2$, 24.9 mM

$NaHCO_3$, and 10.0 mM glucose. The right ventricular papillary muscle was carefully dissected free from the ventricular wall. The muscle was mounted under 0.5 g of resting tension in a water-jacketed organ bath containing 10 ml of Krebs-Henseleit solution. The solution in the bath was bubbled with 95% O_2 and 5% CO_2 , and its temperature was kept at 35°C. The muscle was stimulated by rectangular pulses of 1-Hz frequency, 5-ms duration, and 1.5 times the threshold voltage delivered by a pair of spiral platinum electrodes connected to a stimulator via an isolation unit (29). Changes in isometric tension were measured by a force displacement transducer (29). The preparations were allowed to equilibrate for at least 90 min before the experiments commenced.

Experiments on skinned cardiac muscle

Fiber bundles less than 200 μm in diameter were prepared by blunt dissection from guinea-pig ventricular trabecular muscles. These strips were chemically skinned as previously described (30). In brief, the small bundles were treated with the relaxing solution containing 50 μM β -escin (Sigma-Aldrich, St. Louis, MO, USA) for 30 min. The relaxing solution contained 87 mM potassium methanesulfonate, 20 mM piperazine-*N,N*-bis-(2-ethanesulfonic acid) (PIPES), 5.1 mM Mg (methanesulfate) $_2$, 4.2 mM ATP, 10 mM phosphocreatine, 0.5 mg/ml creatine phosphokinase, and 10 mM EGTA (pH 7.0). The skinned fibers were connected to a strain gauge transducer for measurement of isometric tension. Various Ca^{2+} concentrations were prepared by adding the appropriate amount of Ca (methanesulfate) $_2$ to the relaxing solution. The pH of the solution was adjusted to 7.0 with KOH and the ionic strength was kept constant at 0.2 M by changing the amount of potassium methanesulfonate added. To determine the relation between the Ca^{2+} concentration and force development of the muscle fibers, the bundles were successively immersed in activating solution containing increasing concentrations of Ca^{2+} until the force had reached a stable plateau at each Ca^{2+} concentration. Force was expressed as a percentage of the maximal force obtained at 30 μM Ca^{2+} in the same preparation. Experiments were carried out at room temperature (22°C–25°C).

Measurements of action potentials and membrane currents

Single ventricular myocytes of the guinea pig were obtained essentially by the same techniques as described previously (31). Briefly, collagenase (0.01% w/v; Wako Pure Chemical, Osaka) in a nominally Ca^{2+} -free Tyrode solution was perfused for 30 min through the coronary artery using a Langendorff apparatus. The collagenase

solution was washed out by KB solution of the following composition: 70 mM KOH, 50 mM L-glutamic acid, 40 mM KCl, 20 mM taurine, 20 mM KH_2PO_4 , 2 mM MgCl_2 , 10 mM glucose, 2 mM EGTA, and 10 mM Hepes (pH 7.2). The ventricular tissue was cut into small pieces, agitated gently in a small beaker with KB solution, and then filtered through 100- μm stainless steel mesh. The cell suspension was stored in a refrigerator (4°C) for later use.

Electrophysiological recordings were performed by the whole-cell patch-clamp technique, using glass patch electrodes with a tip diameter 1 μm and a resistance of 3–5 M Ω (32–35). In order to record membrane currents under whole-cell voltage clamp and action potentials under the current-clamp version of patch clamping, Axopatch 200B and pCLAMP version 8 (Axon Instruments, Foster City, CA, USA) were used. In general, I_{Ca} was elicited by a 200-ms depolarizing test pulse to +10 mV from a holding potential (V_{H}) of –40 mV in order to avoid I_{Na} and the T-type Ca^{2+} current. The compositions of the external and internal solutions were formulated to eliminate the involvement of K^+ currents in the whole-cell membrane currents. Thus, the composition of the internal solution (pH 7.2) was 130 mM CsCl, 3 mM MgATP, 20 mM BAPTA, 10 mM Hepes, and 3 mM MgCl_2 , while that of the external solution (pH 7.4) was 140 mM NaCl, 5.4 mM CsCl, 0.33 mM NaH_2PO_4 , 5 mM Hepes, 1.0 mM MgCl_2 , 5.5 mM glucose, and 1.5 mM CaCl_2 . Myocytes that exhibited a clear run-down with the first 10 min were discarded. To minimize the influence of the rundown, the time window between 10 and 30 min after the initial recording was chosen to measure I_{Ca} with respect to drug effects (31). The amplitude of I_{Ca} was measured as the difference between the peak of the inward current and the current at the end of the test pulse. Meanwhile, I_{K} was elicited by a 3000-ms depolarizing test pulse to +30 mV from a V_{H} of –30 mV. When I_{K} was measured, the pipette solution contained 120 mM KOH, 120 mM L-aspartic acid, 30 mM KCl, 4 mM $\text{Na}_2\text{-ATP}$, 10 mM EGTA, 5 mM Hepes, 1 mM MgCl_2 , and 1.0 mM CaCl_2 (pH 7.2). The composition of the external solution was 140 mM *N*-methyl-D-glucamine chloride, 5.4 mM KCl, 0.33 mM NaH_2PO_4 , 5 mM Hepes, 1.0 mM MgCl_2 , 5.5 mM glucose, 1.8 mM CaCl_2 , and 0.1 mM CdCl_2 (pH 7.4). The temperature of the perfusate was kept constant at $36 \pm 1^\circ\text{C}$.

Drugs

Propofol at 10 mg/ml (Diprivan®) was purchased from AstraZeneca (Osaka). Propofol was formulated in 10% of intralipid emulsion, and dilutions to the desired concentrations were made with a suitable buffer solu-

tion. This was used as the clinically available preparation. Control experiments for the solvent were performed using Intralipid® 10%, which was purchased from Fresenius Kabi (Stockholm, Sweden). Intralipid contained 10 g purified soybean oil, 1.2 g purified egg phospholipids, 2.25 g glycerol USP, and appropriate quantities of NaOH in 100 ml. A suitable buffer solution was used for dilutions of the final concentration of Intralipid, which resulted in no significant changes in cardiac contractions, ionic currents, or action potentials. We also used propofol obtained from Sigma-Aldrich. It was first dissolved in dimethyl sulfoxide and then diluted in suitable buffer solution. We confirmed that application of propofols obtained from different sources substantially produced the same effects for any cardiac variables. The experiments with nifedipine and Bay K 8644 were carried out in the dark and the solution bottles and tubing were covered with aluminium foil for further security against degradation.

Statistics

The data are expressed as means \pm S.E.M. Statistical assessment of the data was made by Student's *t*-test or one-way ANOVA followed by Bonferroni's multiple comparison test, where appropriate. Differences were considered to be statistically significant when $P < 0.05$.

Results

Negative inotropic effect of propofol

In guinea-pig right ventricular papillary muscles electrically paced at 1 Hz, propofol caused a concentration-dependent negative inotropic effect (Fig. 1). A significant decrease in force of contraction was observed at concentrations of 10 μM and above. The IC_{50} value (i.e., the drug concentration required to decrease force of contraction to half of the basal value) for propofol was $124 \pm 28 \mu\text{M}$ ($n = 7$). This value was much higher than that for the Ca^{2+} -channel antagonist nifedipine ($22 \pm 3 \text{ nM}$, $n = 7$).

The influences of propofol and nifedipine at concentrations close to their IC_{50} on the positive inotropic effects of Bay K 8644, a Ca^{2+} -channel agonist, and EMD 57033, a Ca^{2+} sensitizer, were examined. As expected, the concentration–response curve for the positive inotropic effect of Bay K 8644 was significantly shifted to the right by pretreatment with 30 nM nifedipine (Fig. 2A). The same effect on Bay K 8644 was observed when the muscles were treated with 100 μM propofol. However, neither propofol nor nifedipine caused any significant inhibition of the positive inotropic effect of EMD 57033 (Fig. 2B).

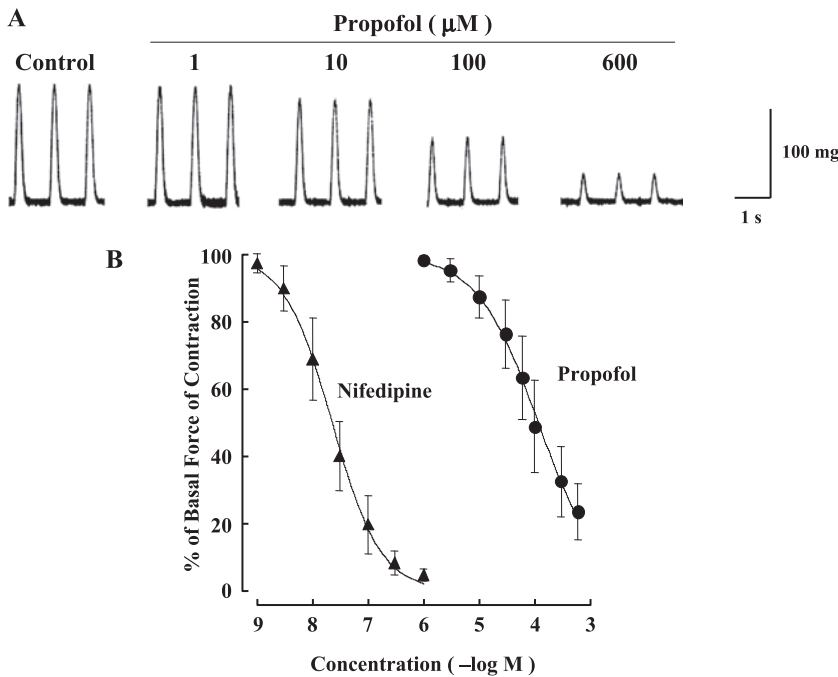


Fig. 1. Effects of propofol and nifedipine on force of contraction in guinea-pig right ventricular papillary muscles electrically paced at 1 Hz. A: Representative traces showing the isometric curves obtained in papillary muscles before and after the addition of propofol at different concentrations. B: Concentration-response curves for the negative inotropic effect of propofol and nifedipine. The drugs were added to the bathing solution in a cumulative manner. Each point is the mean \pm S.E.M. of seven different preparations. The basal values of force of contraction were not significantly different between the groups for propofol and nifedipine.

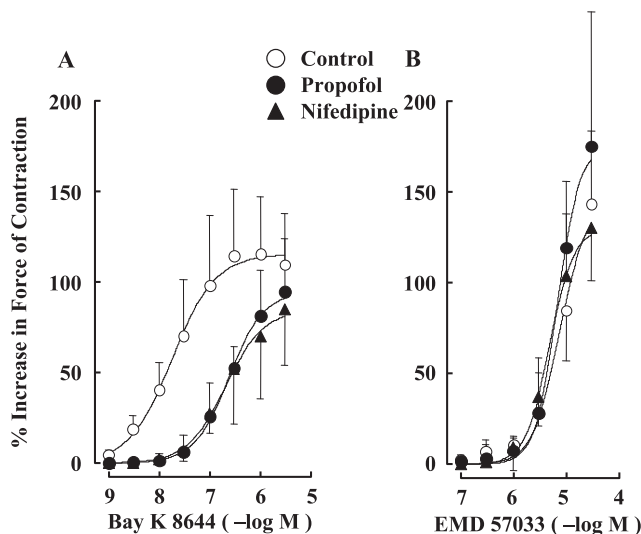


Fig. 2. Influences of propofol and nifedipine on the concentration-response curves for the positive inotropic effects of Bay K 8644 (A) and EMD 57033 (B) in guinea-pig right ventricular papillary muscles electrically paced at 1 Hz. Each agonist was added to the bathing solution in a cumulative manner 30 min after vehicle, propofol, or nifedipine was given. Propofol and nifedipine were used at concentrations of 100 μM and 30 nM, respectively, which are close to their respective IC_{50} . Each point is the mean \pm S.E.M. of six to seven different preparations.

Lack of effect of propofol on myofilament Ca^{2+} sensitivity

The pCa-tension relationships for β -escin-skinned guinea-pig trabecular muscles in the absence and presence of 100 μM propofol are shown in Fig. 3A. The pCa₅₀ values (i.e., the pCa of half-maximum tension

generation) were 5.56 ± 0.04 ($n=9$) for the control and 5.57 ± 0.04 ($n=9$) for 100 μM propofol, and no difference was found between these values. Even though the propofol concentration was increased to 200 μM , the relationship between relative tension and pCa remained unchanged (pCa₅₀ = 5.50 ± 0.04 , $n=8$). Representative recordings indicating that the addition of 200 μM propofol had no effect on Ca^{2+} -induced contractions in β -escin-skinned muscles are depicted in Fig. 3B. In the same preparations, we confirmed that the myofilament Ca^{2+} -sensitizer pimobendan (50–100 μM) evidently enhanced Ca^{2+} -induced contractions (36).

Inhibitory effect of propofol on I_{Ca}

Typical tracings of the concentration-dependent effect of propofol on I_{Ca} elicited by a depolarizing pulse from a V_H of -40 mV to $+10$ mV in ventricular myocytes are shown in Fig. 4A. The addition of propofol (1–100 μM) caused a decrease in I_{Ca} in a concentration-dependent manner. Statistical significance was achieved from a concentration of 1 μM . At a concentration of 100 μM , propofol decreased I_{Ca} by $78 \pm 6\%$ ($n=5$). The calculated IC_{50} value was 9.8 μM (Fig. 4B).

Inhibitory effect of propofol on I_K

Figure 5A illustrates typical tracings showing the effect of propofol on I_K under the condition where the influences of I_{Na} and I_{Ca} were excluded. Both the activating current and tail current of I_K were concentration-dependently reduced by propofol. In six different cells, a significant inhibition of the amplitude of the

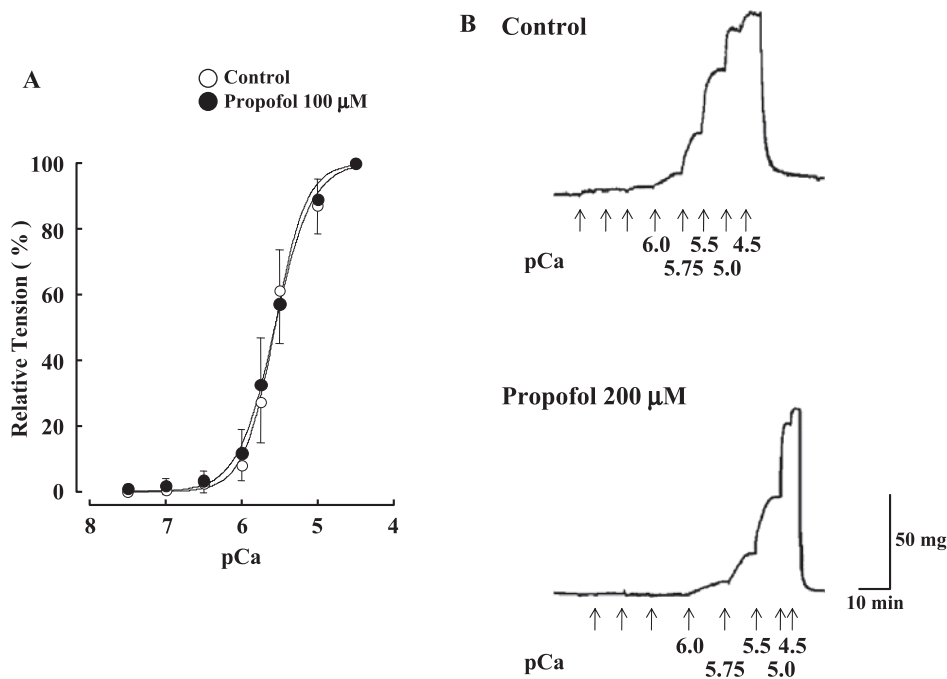


Fig. 3. Effect of propofol on myofilament Ca^{2+} sensitivity using β -escin-skinned trabecular muscles from guinea pigs. A: pCa-tension relationships in the absence (open circle) or presence (closed circle) of 100 μ M propofol. Each point is the mean \pm S.E.M. of nine different preparations. B: Representative recordings showing no effect of 200 μ M propofol on Ca^{2+} -induced contractions.

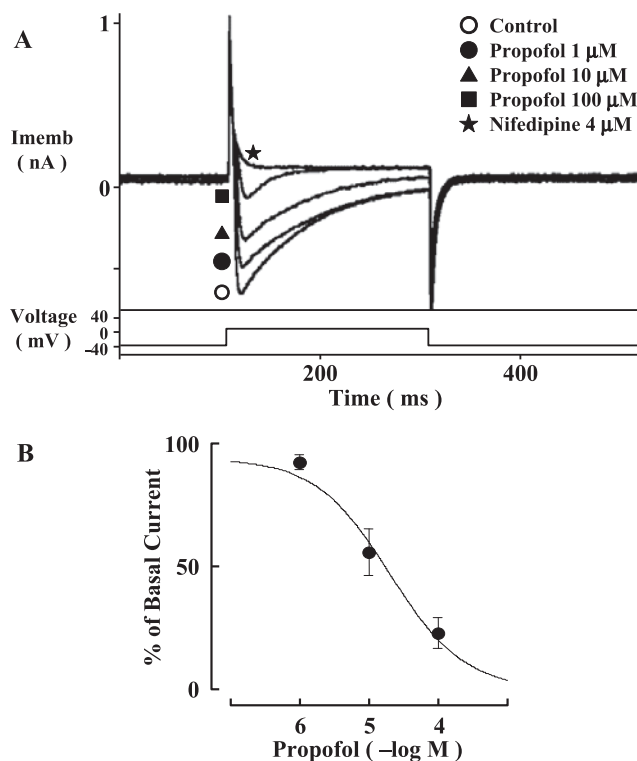


Fig. 4. Effect of propofol on I_{Ca} in guinea-pig ventricular myocytes. A: Current traces after cumulative application of propofol at concentrations of 1, 10, and 100 μ M. The addition of propofol decreased I_{Ca} about 2 min after the addition, which attained a steady level within 4–5 min. I_{Ca} was completely blocked by 4 μ M nifedipine. B: Concentration–response curve for the inhibitory effect of propofol on I_{Ca} . Each point is the mean \pm S.E.M. of five cells from at least three guinea pigs.

tail current, defined as the difference between the peak current of the tail and the holding current, occurred at a concentration of 1 μ M, and 100 μ M propofol inhibited it by $84.6 \pm 5.2\%$. The estimated IC_{50} was 23 μ M (Fig. 5B).

Even after the rapid component of I_{K} (I_{Kr}) was blocked by its specific blocker E-4031 (5 μ M), propofol (100 μ M) strikingly reduced the amplitude of the tail current (Fig. 6A). The residual amount of the tail current was eliminated by further administration of 30 μ M 293B, an inhibitor of the slow component of I_{K} (I_{Ks}) (Fig. 6B). Conversely, in the presence of chromanol 293B, propofol had little effect on the tail current of I_{K} (Fig. 6C). The tail current in the presence of chromanol 293B and propofol was marginally affected by further addition of E-4031 (Fig. 6C).

Shortening effect of propofol on action potentials

When action potentials in ventricular myocytes were elicited by current injection at a rate of 0.1 Hz, propofol concentration-dependently shortened action potential duration (APD) (Fig. 7A, Table 1). However, propofol concentrations of 100 μ M and above were required to reduce APDs by over 50% (Table 1). Neither resting membrane potential nor action potential amplitude was significantly affected by propofol (Table 1). In the presence of 4 μ M nifedipine, 100 μ M propofol prolonged rather than shortened APD (Fig. 7B). Furthermore, application of the I_{Ks} inhibitor chromanol 293B (30 μ M) to myocytes led to an evident increase in APD (Fig. 7C).

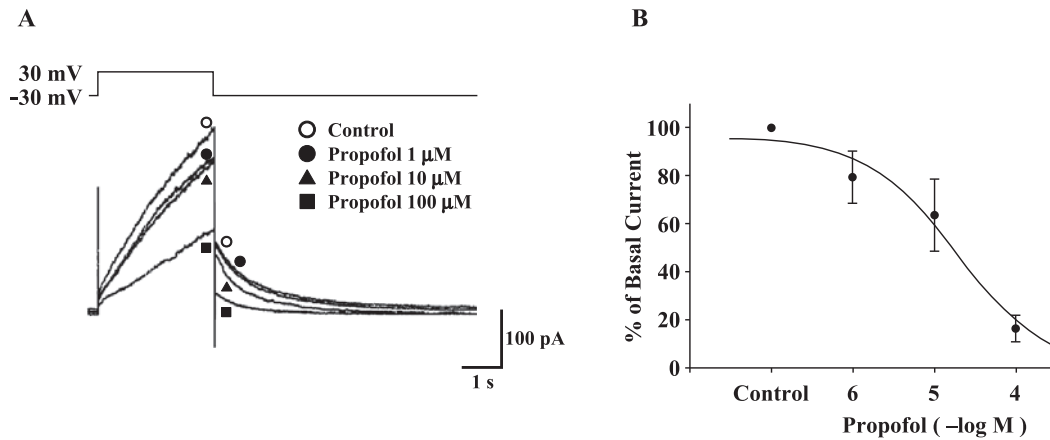
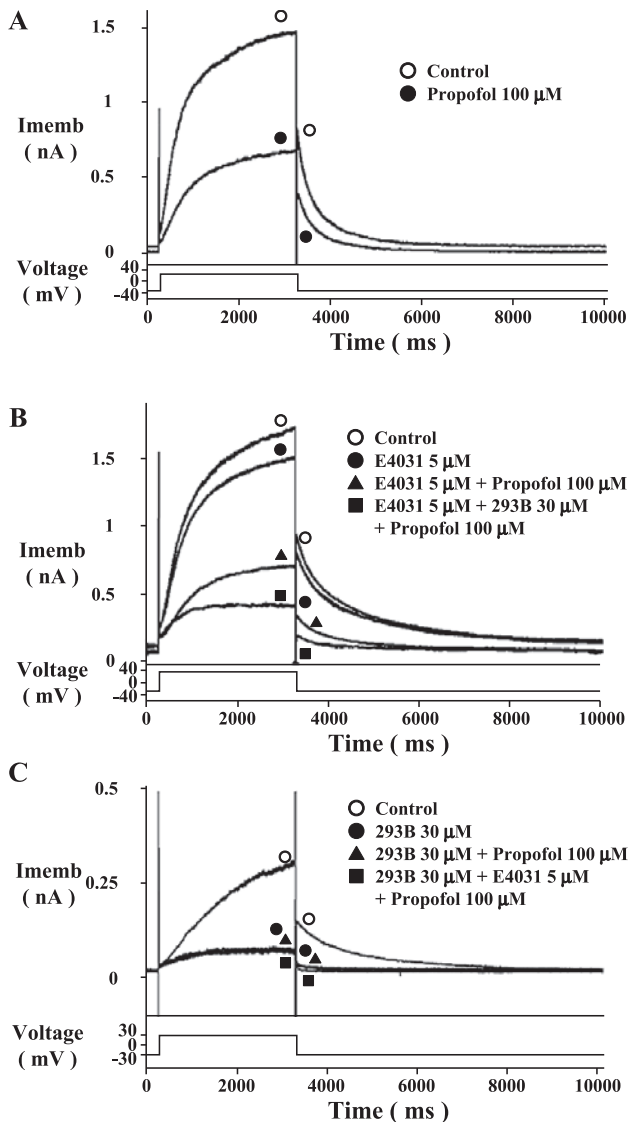


Fig. 5. Effect of propofol on I_K in guinea-pig ventricular myocytes. A: Current traces after cumulative application of propofol at concentrations of 1, 10, and 100 μ M. The addition of propofol inhibited I_K about 2 min after the addition, which attained a steady level within 4–5 min. B: Concentration–response curve for the inhibitory effect of propofol on I_K . Each point is the mean \pm S.E.M. of six cells from at least three guinea pigs.



Discussion

In accordance with many previous reports (see Introduction), this study demonstrated a clear negative inotropic effect of propofol in isolated guinea-pig right ventricular papillary muscles. The inhibitory potency of propofol ($IC_{50} = 124 \mu$ M) is in agreement with those obtained in left atrial muscles (96.6 μ M, ref. 12) and ventricular muscles (approximately 100 μ M, ref. 9) of the same species. Interestingly, when atrial and ventricular trabecular muscles were obtained from the failing human hearts of transplant patients or atrial muscles were from the nonfailing hearts of patients undergoing coronary artery bypass surgery, propofol produced a significant decrease in developed tension in all muscles at concentrations exceeding 56 μ M (37). Furthermore, another study has shown that the IC_{50} value for propofol in human atrial tissue contractility is 235–246 μ M (15). Clinically, the relevant blood concentrations during intravenous infusion of propofol are less than 23 μ M (38, 39), and free fractions of propofol in plasma, which are active, appear to be much lower because of its high protein-binding property, which exceeds 95% (40). Thus, the concentration range of in vitro cardiac depression observed in our and other studies is far from that used in clinical practice. Therefore, it would be reasonable to conclude that the direct decrease in cardiac

Fig. 6. Effects of propofol on I_K in the presence of the I_{Kr} inhibitor E-4031 or the I_{Ks} inhibitor chromanol 293B in guinea-pig ventricular myocytes. A: Propofol at the concentration of 100 μ M was given alone. B and C: Cells were pretreated with 5 μ M E-4031 and 30 μ M chromanol 293B, followed by addition of propofol at 100 μ M, and then chromanol 293B and E-4031 were added, respectively.

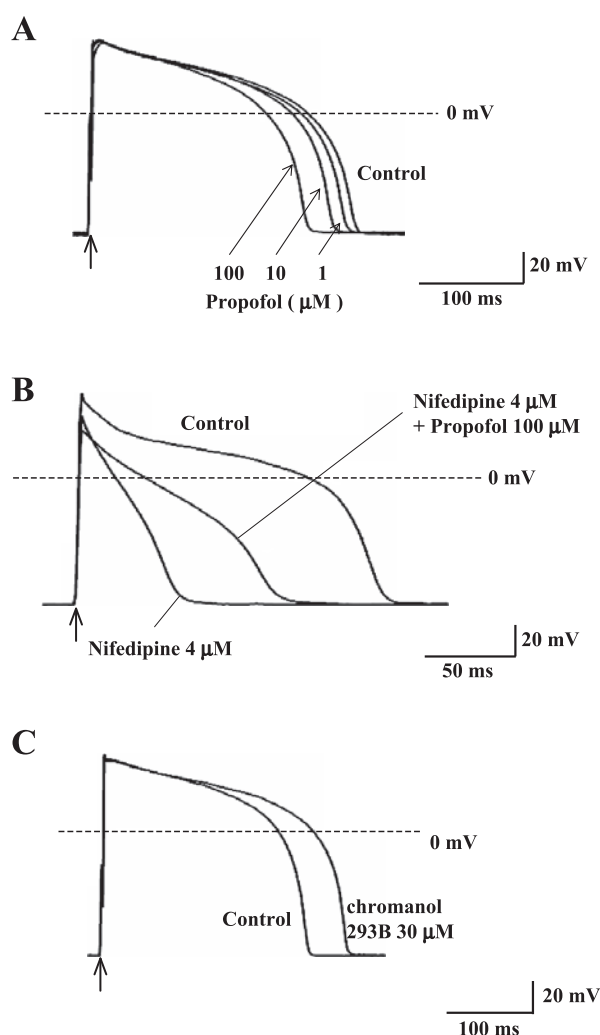


Fig. 7. Effects of propofol and 293B on action potentials in guinea-pig ventricular myocytes. A: Propofol was added to the organ bath in a cumulative manner. Traces of action potentials before and after application of 1, 10, and 100 μM propofol are superimposed. APD shortening appeared about 2 min after the addition, reaching a steady level within 4–5 min. B: The myocytes were treated for 2 min with 4 μM nifedipine, which remained in the bath solution during exposure to 100 μM propofol. C: Superimposed records before and after application of 30 μM chromanol 293B are shown.

contractility to a lesser extent contributes to the cardiovascular depression of propofol in vivo.

The negative inotropic effect of propofol may arise largely from a reduction in transsarcolemmal Ca^{2+} influx. Suppression of I_{Ca} would be expected to lower the cytosolic Ca^{2+} transient because of decreases in both direct Ca^{2+} entering and indirect Ca^{2+} -induced Ca^{2+} release from the sarcoplasmic reticulum, thereby reducing cardiac contraction. Indeed, previous studies have shown that propofol exerts a reduction in I_{Ca} in isolated single cardiomyocytes under voltage clamp conditions (18–20). In this experiment using guinea-pig ventricular myocytes, we found that propofol had a calculated IC_{50} of 9.8 μM to reduce I_{Ca} . This is identical to the 50% inhibition of I_{Ca} caused by 8.3 μM propofol in rabbit ventricular myocytes (25). Thus, our present study showed that propofol was much more potent in inhibiting I_{Ca} than in decreasing force of contraction in myocardium. This suggests that there is a compensatory mechanism, which actually offsets the reduced amount of Ca^{2+} through L-type Ca^{2+} channels. Consequently, such a mechanism may help to counteract the net negative inotropic effect of propofol.

Nakane et al. (23) have reported that propofol decreases available intracellular Ca^{2+} without decreasing contraction in intact beating guinea-pig hearts, and they proposed that this anesthetic can enhance myofilament Ca^{2+} sensitivity at clinical concentrations. Sprung et al. (37) have also shown that the Ca^{2+} -activated actomyosin ATPase activity of human nonfailing atrial trabecular muscles is shifted significantly leftward in the presence of propofol, implying an increase in myofilament sensitivity to Ca^{2+} . In contrast, the findings of other groups that the reduction in the Ca^{2+} transient is proportionately less than the reduction in contraction when propofol is applied to fura-2-loaded rat ventricular myocytes has suggested that propofol may lead to a modest decrease in myofilament Ca^{2+} sensitivity (41). Moreover, no influence of propofol on myofilament sensitivity to Ca^{2+} has been demonstrated by measuring isometric force in Triton-skinned trabeculae from rats and guinea pigs at

Table 1. Effect of propofol on action potential characteristics of guinea-pig ventricular myocytes

	RMP (mV)	APA (mV)	APD ₂₀ (ms)	APD ₅₀ (ms)	APD ₉₀ (ms)	n
Control	-72.9 ± 0.8	157.6 ± 5.7	36.2 ± 18.1	244.7 ± 23.8	273.3 ± 24.5	10
Propofol (10 μM)	-73.2 ± 0.8	152.5 ± 5.1	30.8 ± 17.3 (85%)	$181.0 \pm 24.2^*$ (74%)	$206.6 \pm 20.9^*$ (76%)	5
Propofol (100 μM)	-73.1 ± 2.0	151.1 ± 7.1	$11.8 \pm 6.4^*$ (33%)	$113.3 \pm 13.6^*$ (46%)	$143.9 \pm 18.2^{**}$ (53%)	9
Propofol (300 μM)	-71.8 ± 2.7	147.5 ± 8.5	$11.0 \pm 3.2^*$ (31%)	$62.8 \pm 13.4^{**}$ (26%)	$94.0 \pm 5.0^{**}$ (34%)	4

Each value is a mean \pm S.E.M. Percentages relative to the respective control parameter are shown in parentheses. RMP: resting membrane potential; APA: action potential amplitude; APD₂₀, APD₅₀, and APD₉₀: action potential duration at 20%, 50%, and 90% repolarization, respectively; n: number of experiments. * $P < 0.01$, ** $P < 0.001$ vs. control.

different Ca^{2+} concentrations (42). Thus, the evaluation of the propofol effect on myofilament Ca^{2+} sensitivity does not reach general agreement. The reasons for this controversy are not apparent, but are generally attributed to the wide variations in the experimental conditions used. In the present study, the influence of propofol on the contractile protein was assessed in β -escin-skinned trabeculae from guinea pigs. The pCa-tension curve was not changed by propofol even at higher concentrations. This suggests that propofol did not alter the contractile properties of the myofilaments nor did it affect Ca^{2+} sensitivity of force production. In support of this, we found that propofol marginally affected the positive inotropic response of guinea-pig papillary muscles to the Ca^{2+} -sensitizer EMD 57033. On the other hand, propofol had the same inhibitory effect on the positive inotropic response to the Ca^{2+} -channel agonist Bay K 8644 as nifedipine. We interpret these results to indicate that an effect on Ca^{2+} sensitivity of the contractile myofilaments cannot provide an explanation for the intrinsic mechanism to potentially counteract the negative inotropy of propofol.

In the heart, I_K is important in initiating repolarization and therefore plays a key role in controlling the duration of cardiac action potentials. In this study, propofol caused a concentration-dependent inhibition of I_K in single guinea-pig ventricular myocytes. Its estimated IC_{50} value of 23 μ M represents a relatively high potency for blocking I_K . It is generally accepted that I_K is composed of two components: a rapidly activating component, I_{Kr} , and a slowly activating component, I_{Ks} (43). Our experiments with the use of E-4031 and chromanol 293B, which selectively block I_{Kr} and I_{Ks} , respectively, suggest that propofol served as a selective blocker of I_{Ks} . In line with this, our recent study has shown that propofol has a much less pronounced effect on the HERG channel that encodes I_{Kr} (28). Furthermore, Heath and Terrar (24) have indicated that plasma levels of propofol during induction and maintenance of anesthesia are in the range observed to reduce cardiac I_{Ks} .

Although I_{Kr} is considered to be a major repolarizing current responsible for governing terminal repolarization of the action potential, several lines of evidence now show that I_{Ks} also plays a crucial role in cardiac repolarization (44, 45). Actually, we found that the I_{Ks} -blocking agent chromanol 293B led to action potential prolongation in guinea-pig ventricular myocytes. As previously demonstrated in multicellular cardiac preparations (46), propofol produced a concentration-dependent APD shortening in single ventricular myocytes. It is most likely that the APD shortening induced by propofol results from a reduction in I_{Ca} since it disappeared when I_{Ca} was nearly completely blocked by nifedipine.

Instead, the APD prolonging effect of propofol was observed in the presence of nifedipine, which is thought to be attributable to the inhibition of I_{Ks} . Thus, the actual values of changes in APD would represent the sum of the inhibitory effects of propofol on I_{Ca} and I_{Ks} . Alternatively, the ability of propofol to reduce Ca^{2+} influx through L-type Ca^{2+} channels may be counteracted to some extent by its I_{Ks} inhibition responsible for the APD prolongation. Support for such ideas is provided by the present findings of this study that the propofol concentrations required to cause a significant shortening of APD were much larger than the IC_{50} value for its effect on I_{Ca} .

In conclusion, we confirmed the negative inotropic effect of propofol observed by many other investigators. Although the negative inotropic effect is associated with the reduction in transsarcolemmal Ca^{2+} influx via L-type Ca^{2+} channels, the inhibitory effect of propofol on the Ca^{2+} channel-mediated Ca^{2+} influx appears to be limited as a consequence of the action potential elongation mechanism due to inhibition of I_{Ks} . Thus, the net negative inotropic effect of propofol may be the result of its counteracting action on L-type Ca^{2+} channels and I_{Ks} channels. In terms of results, propofol seems to cause no significant negative inotropic action in the heart at clinically relevant concentrations. Therefore, the present results strongly support the view that the risk of direct myocardial depression after anesthetic induction with propofol would be minimal, although the presence of other hemodynamic compromises by propofol cannot be ruled out.

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