Cardioprotective effects of propofol in isolated ischemia-reperfused guinea pig hearts: role of K_{ATP} channels and GSK-3β

[Effets cardioprotecteurs du propofol dans des cœurs ischémiques puis reperfusés isolés chez le cobaye : rôle des canaux K_{ATP} et du GSK-3β]

Noriko Kamada MD, Noriaki Kanaya MD PHD, Naoyuki Hirata MD, Saori Kimura MD PHD, Akiyoshi Namiki MD PHD

Purpose: Propofol exerts cardioprotective effects, but the involved mechanisms remain obscure. The present study examines the cardioprotective effects of propofol and its role in cardiac function, including its effect on K_{ATP} channel opening and the inhibition of GSK-3β activity in ischemia-reperfused hearts.

Methods: Ischemia-reperfusion (I/R) was produced in isolated guinea pig hearts by stopping coronary perfusion for 25 min, followed by reperfusion. The hearts were incubated for ten minutes, with or without propofol (25 or 50 μM), or for five minutes with 500 μM 5-hydroxydecanoate (a mitochondrial K_{ATP} channel blocker) or 30 μM HMR1098 (sarcolemmal K_{ATP} channel blocker), followed by five minutes with 50 μM propofol before ischemia. Action potentials on the anterior epicardial surface of the ventricle were monitored using a high-resolution charge-coupled device camera system, and at five minutes after reperfusion, GSK-3β phosphorylation at the serine residue, Ser9, was examined.

Results: After 35 min of reperfusion, propofol (25 and 50 μM) blunted the adverse effects of I/R and reduced infarct size ($P < 0.05$). In addition, prior incubation with 5-hydroxydecanoate or HMR1098 had no effect on functional recovery improved by 50 μM propofol. At five minutes after reperfusion, propofol (25 and 50 μM) shortened the duration of the action potential and increased the levels of phospho-GSK-3β ($P < 0.05$).

Conclusions: Propofol enhanced mechanical cardiac recovery and reduced infarct size. The data further suggest that GSK-3β play an important role in propofol cardioprotective actions during coronary reperfusion, but mitochondrial K_{ATP} channels do not.

CAN J ANESTH 2008 / 55: 9 / pp 595–605

Objectif *: Le propofol exerce des effets cardioprotecteurs, mais les mécanismes sous-jacents demeurent obscurs. Cette étude examine les effets cardioprotecteurs du propofol et son rôle dans la fonction cardiaque, notamment son effet sur l'ouverture du canal K_{ATP} et l'inhibition de l'activité du GSK-3β dans des cœurs ischémiques puis reperfusés.*

Méthode *: L'ischémie reperfusion (I/R) a été provoquée dans des cœurs isolés de cobayes en interrompant la per fusion coronarienne pendant 25 min, puis en les reperfusant. Les cœurs ont été incubés pendant dix minutes, avec ou sans propofol (25 ou 50 μM), ou pendant cinq minutes avec 500 μM de 5-hydroxydecanoate (un bloqueur du canal K_{ATP} mitochondrial) ou 30 μM de HMR1098 (un bloqueur du canal K_{ATP} sarcolemmal), suivi par cinq minutes avec 50 μM de propofol avant l'ischémie. Les potentiels d'action sur la surface épicardique antérieure du ventricule ont été surveillés à l'aide d'un système de caméra à dispositif à transfert de charge et, cinq minutes après la reperfusion, la phosphorylation du GSK-3β au résidu de sérine, Ser9, a été examiné.*

Résultats *: Après 35 min de reperfusion, le propofol (25 et 50 μM) a émoussé les effets négatifs de l'I/R et réduit la taille de l'infarctus ($P < 0,05$). De plus, l'incubation antérieure avec le 5-hydroxydecanoate ou l'HMR1098 n'a pas eu d'effet sur la récupération fonctionnelle améliorée par 50 μM de propofol. Cinq minutes après la reperfusion, le propofol (25 et 50 μM) a abrégé la durée*

From the Department of Anesthesiology, Sapporo Medical University School of Medicine, Sapporo, Japan.

Address correspondence to: Dr. Noriaki Kanaya, Department of Anesthesiology, Sapporo Medical University School of Medicine, S-1, W-6, Chuo-ku, Sapporo 060-8543, Japan. Phone: +81-11-611-2111; Fax: +81-11-631-9683; E-mail: kanaya@sapmed.ac.jp

This study was supported in part by grants-in-Aid (No. C-12671487 and B-15390477) from the Japan Society for the Promotion of Science, Tokyo, Japan.

Competing interests: None declared.

Accepted for publication February 7, 2008.

Revision accepted May 22, 2008.

Final revision accepted June 23, 2008.

du potentiel d'action et augmenté les niveaux de phospho-GSK-3β (P < 0,05).

***Conclusion** : Le propofol a amélioré la récupération cardiaque mécanique et réduit la taille de l'infarctus. Les données suggèrent aussi que le GSK-3β joue un rôle important dans les actions cardioprotectrices du propofol pendant la reperfusion coronarienne, mais pas les canaux K_{ATP} mitochondriaux.*

PROPOFOL is an intravenous hypnotic agent which is widely used for the induction and maintenance of anesthesia in patients scheduled for cardiac surgery. However, the cardioprotective effects of propofol are less recognized than those of the volatile anesthetics.[1] In studies involving isolated rat heart preparations, there have been reports of absent cardiac protective effects[2] as well as concentration-dependent, cardiac protective effects[3,4] of propofol against ischemia-reperfusion injury. Several mechanisms for cardioprotection have been proposed, the primary consideration being that propofol, by its ability to block plasma membrane Ca^{2+} channels,[5] may attenuate the Ca^{2+} overload during ischemia-reperfusion. Propofol is known to act as an antioxidant reacting with free radicals to form a phenoxy radical[6] with the ability to attenuate ischemia-reperfusion injury in the isolated rat heart.[3,4] Propofol also activates protein kinase C (PKC) in rat cardiomyocytes, resulting in phosphorylation of several myofibrillar proteins such as myosin light chain-2 and troponin I, and an increase in myofilament Ca^{2+} sensitivity.[7] Propofol increased myofilament Ca^{2+} sensitivity, at least in part, by increasing *p*Hi via PKC-dependent activation of Na^+-H^+ exchange.[8] Therefore, propofol may have a cardioprotective effect via cellular mechanisms triggered by PKC activation.[9]

Ischemic preconditioning (IPC) is a powerful endogenous form of cardioprotection mediated by a repetitive brief period of ischemia and reperfusion. Several studies have demonstrated that activation of PKC and mitochondrial K_{ATP} (mK_{ATP}) channels is the crucial component of IPC.[10,11] Moreover, glycogen synthase kinase (GSK) is an important regulator of cellular function.[12] The selective inhibition of GSK-3β is similar to the effects of IPC in isolated rat hearts, and IPC reduces GSK-3β activity by phosphorylation at the N-terminal serine residue, Ser^9.[13] Recently, Gross *et al.*[14] reported that both the sarcolemmal K_{ATP} (sK_{ATP}) and the mK_{ATP} channels are involved in acute opioid-induced cardioprotection, and the GSK signalling axis regulates cardioprotection via K_{ATP} channel opening. Another study demonstrated that GSK-3β phosphorylation and inactivation play an important role in volatile anesthetics-induced cardioprotection by preventing the opening of mitochondrial permeability transition pores (mPTP).[15] However, it is unknown whether propofol produces cardioprotective effect via K_{ATP} channel opening. It is also unknown whether GSK-3β plays a role in propofol-induced myocardial protection during ischemia and reperfusion.

The aim of the current study was to test the hypothesis that propofol exerts myocardial protection during ischemia and reperfusion via GSK-3β inhibition, by activation of the K_{ATP} channel. We specifically sought to evaluate the importance of K_{ATP} channels in propofol-induced cardioprotection during ischemia and reperfusion and attempted to determine whether direct sK_{ATP} and/ or mK_{ATP} channel openers can reduce infarct size. We also examined whether propofol-induced cardioprotection was mediated via GSK-3β inhibition by measuring phosphorylation of GSK-3β at Ser^9.

Methods

This investigation conformed to the guidelines for care and use of laboratory animals published by the National Institutes of Health, and our institutional Animal Care Committee approved the experimental protocols.

Isolated Langendorff and working-heart preparation

As previously described,[16,17] male English short-haired guinea pigs (250–300 g) were intraperitoneally injected with 20 mg of ketamine and 1000 U of heparin and, after becoming unresponsive to noxious stimulation, were decapitated. After thoracotomy, the hearts were rapidly excised, and the aorta was connected to the isolated perfusion heart system (model IPH-W2, Primetech Co., Japan) using a cannula. Retrograde (Langendorff) perfusion was instantly started with modified Krebs-Ringer solution (in mM: 137 Na^+, 5 K^+, 1.2 Mg^+, 2.5 Ca^{2+}, 134 Cl^-, 15.5 HCO_3^-, 1.2 $H_2PO_4^-$, 11.5 glucose, 2 pyruvate, 16 mannitol, and 0.05 ethylenediamine tetraacetic acid) that was equilibrated with 95% oxygen and 5% carbon dioxide. The temperature was maintained at 37°C throughout the experiment with a thermostatically-controlled, circulating water bath. During this retrograde perfusion, the left atrium was connected to an angled steel cannula via a pulmonary vein to provide a constant filling pressure (preload of 10 mmHg). The pressure chamber that was connected to the aortic outflow provided an afterload pressure of 60 mmHg. Subsequently, this preparation was converted to a working-heart model

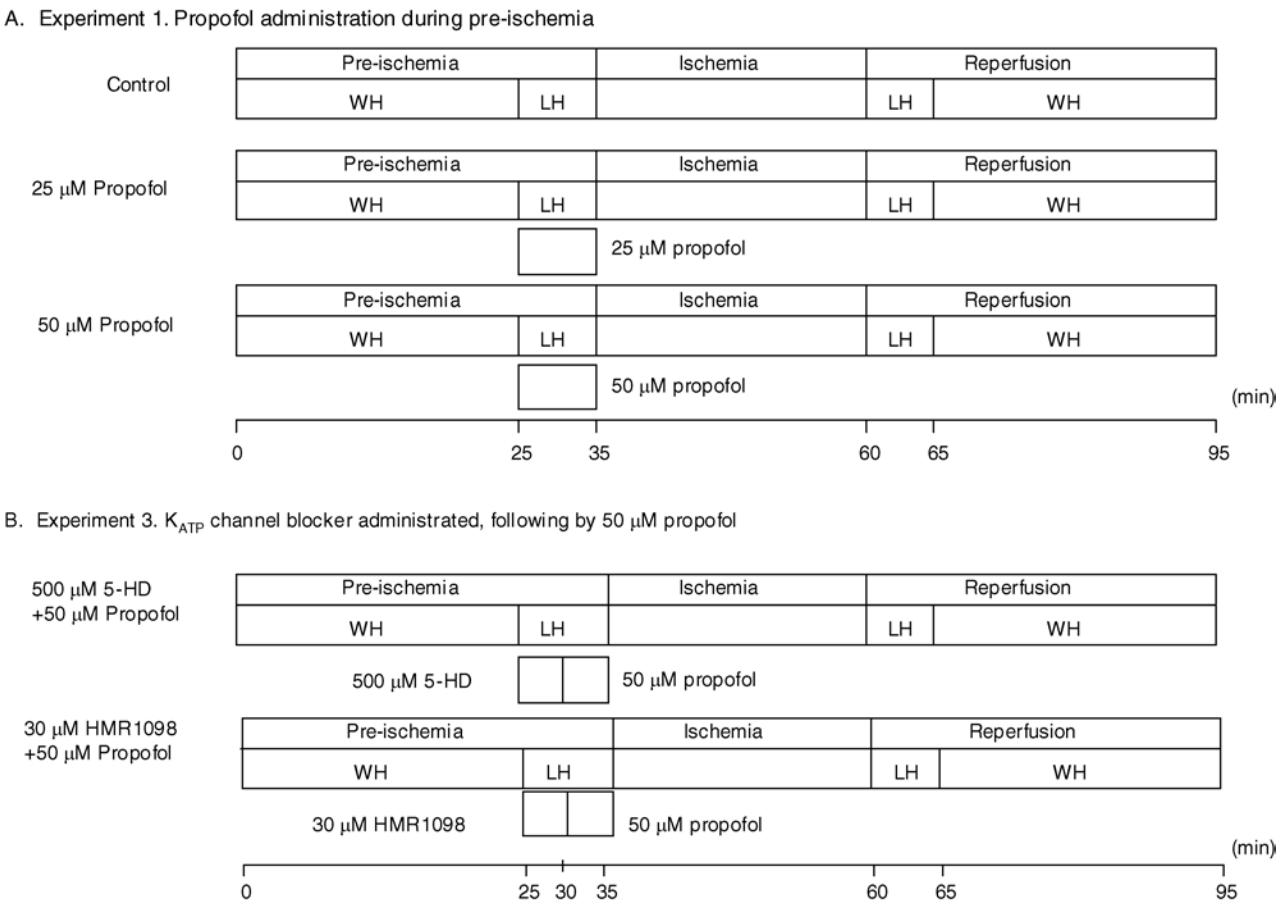

FIGURE 1 Scheme of experimental protocols
After preliminary perfusion (working heart perfusion for 25 min and Langendorff perfusion for ten minutes), 25-min ischemia was followed by 35-min reperfusion (Langendorff perfusion for five minutes and working heart perfusion for 25 min). A. In Experiment 1, the hearts were randomly divided into three groups; control, 25 μM propofol, and 50 μM propofol. Propofol administered for ten minutes before ischemia (n = 6 hearts per group). B. In Experiment 2, the hearts were randomly divided into four groups; control, 50 μM propofol, 500 μM 5-HD + 50 μM propofol, and 30 μM HMR1098 + 50 μM propofol. 5-HD and HMR 1098 administered for five minutes, followed by 50 μM propofol for five minutes (n = 6 hearts per group). WH = working heart perfusion; LH = Langendorff perfusion; 5-HD = 5-hydroxydecanoate.

by switching the system mode, perfusing the left atrium, and releasing the aortic outflow.[4] Left ventricular pressure (LVP), LV developed pressure (LVDP; calculated as [LV systolic – LV end-diastolic pressure]), LV end-diastolic pressure (LVEDP), heart rate (HR), coronary flow (COF), and maximal left ventricular pressure increase and decrease rate (± dP/dt_{max}) were continuously measured using a polygraph (PEG-1000, Nihon Kohden Co., Japan).

Experimental protocol

EXPERIMENT 1: DOES PROPOFOL EXERT CARDIOPROTECTIVE EFFECTS BY REDUCING CARDIAC WORK?

Figure 1A shows the experimental protocol. Hearts were stabilized by perfusion with Krebs-Ringer solution for 25 min; cardiac function was measured, and then the hearts (n = 6 per group) were perfused with Krebs-Ringer solution containing 0 (control), 25, and 50 μM propofol (donated by Maruishi Pharmaceutical Co. Ltd., Osaka, Japan) for ten minutes. Perfusion was stopped to induce global ischemic cardiac arrest for 25 min at 37°C. Finally, the hearts were reperfused for 35 min (37°C), and postischemic function was measured. To assess the role of bradycardia induced by propofol, HR was maintained by pacing the heart at 300 beats·min^{-1} for ten minutes using electronic stimuli (3F46, San-Ei Instruments, Tokyo, Japan), before ischemia. After stabilization for 25 min, the hearts were paced from the right ventricle at a rate of 300 beats·min^{-1} using a silver bipolar electrode (4V, two-time threshold; 2 msec dura-

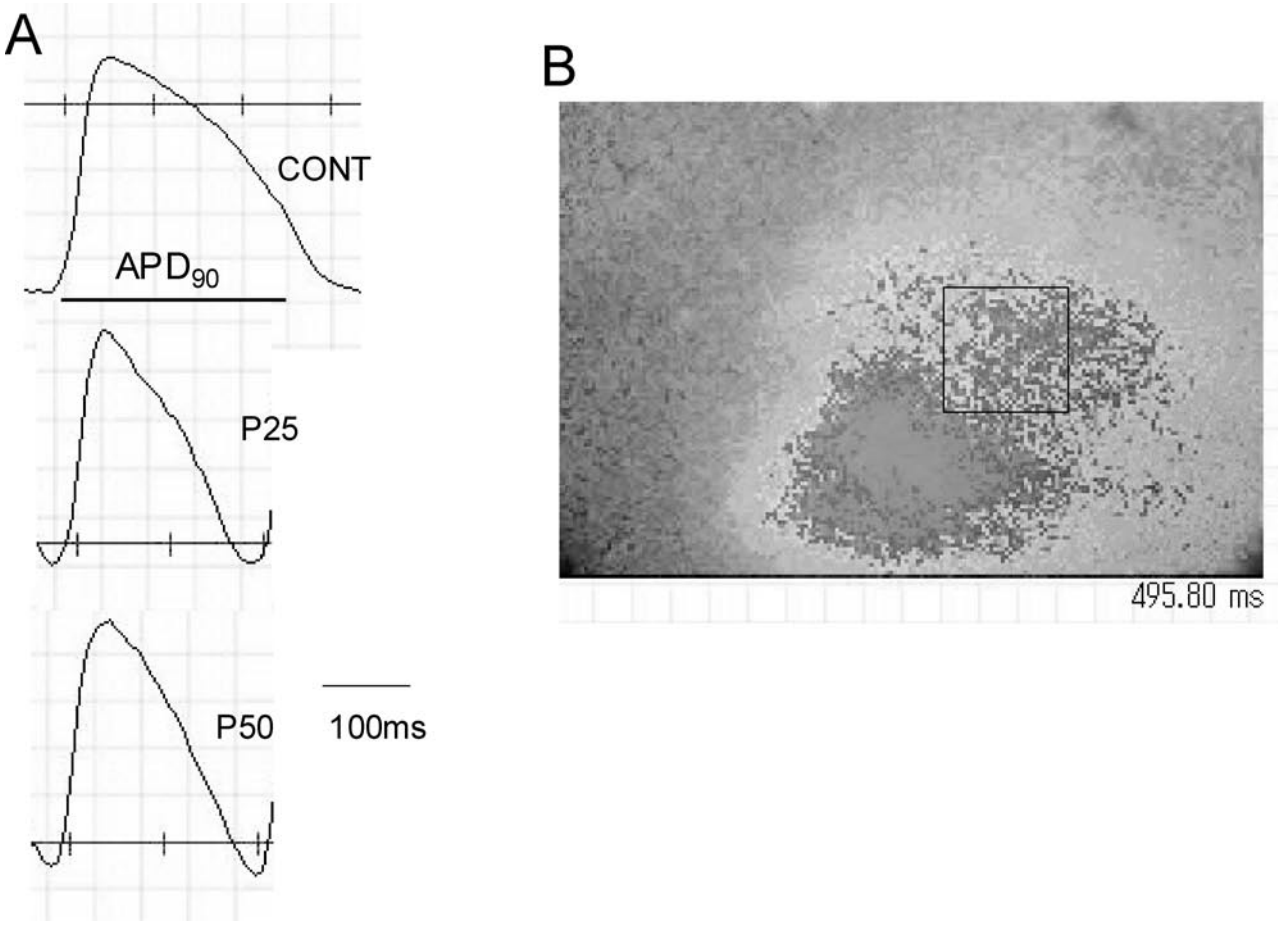

FIGURE 2 Optical recording of ventricular action potential durations in isolated guinea pig heart
A. Representative trace of optical action potential records from one pixel at five minutes of reperfusion. Propofol shortened APD at five minutes after reperfusion. B. Colour map shows APD_{90} distribution across preparation. CONT = control group; P25 = 25 μM propofol group; P50 = 50 μM propofol group; APD_{90} = action potential duration at 90% repolarization.

tion) throughout the administration of Krebs-Ringer solution containing 0 (control group), 25, and 50 μM propofol (n = 6 per group) for ten minutes. After 25-min of global ischemia, the hearts were reperfused for 35 min without pacing.

EXPERIMENT 2: DOES PROPOFOL EXERT CARDIOPROTECTIVE EFFECTS VIA CHANGES IN ACTION POTENTIALS?

The hearts (n = 6 per group) were stained with the voltage-sensitive dye 4-{β-[2(di-n-butylamino)-6-naphthylvinylpyridinium (Di-4-ANEPPS,1 μM; Sigma-Aldrich, Tokyo, Japan) during the 25-min stabilization period. Di-4-ANEPPS was prepared as a 10 mM stock solution in DMSO (stored frozen) and added to standard Krebs-Ringer solution (final DMSO concentration, 0.01%).[18] The hearts were perfused with Krebs-Ringer solution, plus 20 mM 2,3-butanedione monoxime (Sigma-Aldrich, St. Louis, MO, USA) to eliminate motion artifacts,[18] and were paced at a rate of 300 beats·min^{-1} from the right ventricle. Before 25-min of global ischemic arrest, the hearts were incubated with propofol (25 and 50 μM) or with Krebs-Ringer solution alone (control group) for ten minutes. Action potentials on the anterior epicardial surface of the ventricle were monitored through a high resolution, charge-coupled device camera system (MiCAM02, 192 × 128 points, 3.7 msec time resolution, Brain Vision, Japan) at emission and excitation wavelengths of > 7 00 and 550 nm, respectively (Figure 2). Data were processed using BV analyzer software (Brain Vision, Japan). Action potential duration at 90% repolarization (APD_{90}) was measured.

EXPERIMENT 3: DOES PROPOFOL EXERT CARDIOPROTECTIVE EFFECT VIA K_{ATP} CHANNEL OPENING?

We investigated the effect of propofol on K_{ATP} chan-

nel in hearts exposed to ischemia-reperfusion. Figure 1B shows the experimental protocol. After 25 min of stabilization, the hearts (n = 6 per group) were incubated with 500 μM 5-hydroxydecanoate (5-HD, a mitochondrial K_{ATP} blocker; Sigma-Aldrich, Tokyo, Japan) or with 30 μM HMR1098 (a sarcolemmal K_{ATP} blocker; Aventis Pharma Deutschland GmbH, Frankfurt, Germany) for five minutes, and then with 50 μM propofol for five minutes. Global ischemia was induced for 25 min, and then the hearts were reperfused for 35 min.

EXPERIMENT 4: DOES PROPOFOL EXERT CARDIOPROTECTIVE EFFECT VIA INHIBITION OF GSK-3B ACTIVITY?

The hearts were stabilized by perfusion with Krebs-Ringer solution for 25 min, were rendered globally ischemic for 25 min, and were then reperfused with Krebs-Ringer solution. The hearts were assigned to control and propofol (25 and 50 μM) groups (n = 6 per group). At five minutes of reperfusion, following 25 min ischemia, the hearts were freeze-clamped. Frozen, left ventricular myocardial samples were disrupted in 9 volumes of homogenization buffer containing 250 mM sucrose, 1 mM EDTA, 50 mM Tris, and protease inhibitor cocktail. Next, the homogenate was centrifuged at 13,000 x g for 15 min, and then the protein concentration was determined in the supernatant using a Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, CA, USA). Protein samples (40 μg of total protein/ lane) were resolved by SDS-polyacrylamide gel electrophoresis on 10 to 20% gradient slab gels. Proteins were transferred to polyvinylidene difluoride membranes (Immobilon-P, Millipore, Bedford, MA, USA) probed with 1,000-fold diluted antibodies against total GSK-3β or ^{9}Ser-phospho-GSK-3β (Cell Signaling Technology, Beverly, MA, USA) and visualized using the chemiluminescent ECL plus kit (Amersham Life Science, Buckinghamshire, UK). The signal intensity of the bands was quantified by optical densitometry.

Determination of infarct size

At the end of each experiment, the hearts were frozen, cut into transverse slices 2 mm thick, and incubated in 1% triphenyltetrazolium chloride (TTC; Sigma-Aldrich, St. Louis, MO, USA) buffer (pH 7.4) for 15 min. Reduced nicotinamide adenine dinucleotide reacts with TTC in the presence of dehydrogenase enzymes, and living tissues become stained a deep red. Infarct size was calculated using NIH Image software.[19]

Statistical analysis

To determine differences in functional recovery and APD_{90} between the groups, data were compared by using a two-way (for time and treatment) analysis of variance with repeated measures and, if significant F-ratios were obtained, Fisher's protected least significant difference test, with 95% confidence limits. Differences among groups in infarct size and the ratio of phosphor-GSK-3β / total GSK-3β were compared by using one-way analysis of variance followed by Fisher's protected least significant difference, with 95% confidence limits. Results are expressed as means ± SD. A P value < 0.05 was considered statistically significant. Statistical analyses were performed using Stat View software version 5.0 (SAS Institute. Cary, NC, USA).

Results

Propofol diminishes the ischemia reperfusion injury-related reduction in cardiac function

Before ischemia, the values of LVDP, LVEDP, and –dP/dt did not significantly differ among the control, 25, and 50 μM propofol groups. (Table I) After 35 min of reperfusion, LVDP was restored to 55 ± 11% of the baseline value in the control group. In hearts pretreated with 25 and 50 μM propofol, LVDP was restored to 73 ± 11% and 75 ± 13% of the baseline value, respectively. Propofol (25 and 50 μM) significantly attenuated the decrease of LVDP at 15, 25, and 35 min of reperfusion (Re-15, Re-25, and Re-35) (P = 0.012, P = 0.0069, and P = 0.018, respectively). The magnitude of recovery in LVDP was similar between the two propofol concentrations. Increases in LVEDP indicated isolated heart contracture (ventricular stiffness) during ischemia (ischemic contracture). During reperfusion, LVEDP in the control group increased and peaked at five minutes of reperfusion (Re-5). At both concentrations, propofol similarly attenuated the increase of LVEDP at Re-5, Re-25, and Re-35 (P = 0.0010, P = 0.0027, and P = 0.011, respectively). After 35 min of reperfusion, –dP/dt in the control group was 62 ± 12% of the baseline value. Prior exposure to 25 and 50 μM propofol restored –dP/dt to 86 ± 14% and 86 ± 12% of the baseline value, respectively. At both concentrations, propofol similarly attenuated the increase of –dP/dt at Re-15, Re-25, and Re-35 (P = 0.0030, P = 0.015, and P = 0.0006, respectively) and caused a significant decrease in HR at ten minutes after administration (P = 0.037 and P = 0.047, respectively). Also, at both concentrations, propofol significantly reduced HR at Re-25, and Re-35 (P = 0.031 and P = 0.037, respectively). After 35 min of reperfusion, COF was restored to 257 ± 145% of the baseline value in the control group. In hearts pretreated with 25 and 50 μM propofol, COF was

TABLE I Summary of cardiac function induced by propofol in Experiment 1

		Baseline	Treatment	Re-5	Re-15	Re-25	Re-35
LVDP (mmHg)	CONT	76 ± 13	79 ± 7	45 ± 9	37 ± 9	40 ± 3	40 ± 6
	P25	77 ± 9	76 ± 9	51 ± 12	56 ± 13#	58 ± 12#	56 ± 10#
	P50	81 ± 7	78 ± 7	63 ± 12#	55 ± 9#	62 ± 14#	60 ± 9#
LVEDP (mmHg)	CONT	4 ± 3	1 ± 4	18 ± 11	7 ± 5	6 ± 2	5 ± 1
	P25	3 ± 1	1 ± 1	1 ± 1#	3 ± 1#	2 ± 1#	3 ± 1#
	P50	1 ± 1	1 ± 2	3 ± 1#	5 ± 3	4 ± 2#	3 ± 2
HR (beats·min^{-1})	CONT	220 ± 30	290 ± 24	240 ± 53	230 ± 45	250 ± 24	250 ± 24
	P25	230 ±45	200 ± 31#	260 ± 31	210 ± 50	200 ± 31#	210 ± 33#
	P50	230 ± 24	210 ± 33#	220 ± 31	220 ± 49	200 ± 48#	210 ± 32#
-dP/dt (mmHg·sec^{-1})	CONT	852 ± 160	1285 ± 212	779 ± 181	399 ± 95	533 ± 122	510 ± 91
	P25	862 ± 88	1188 ± 153	737 ± 338	737 ± 153#	750 ± 181#	735 ± 129#
	P50	972 ± 135	1115 ± 140	828 ± 182	676 ± 85 #	774 ± 91#	818 ± 102#
+dP/dt (mmHg·sec^{-1})	CONT	1165 ± 203	1327 ± 183	968 ± 328	606 ± 131	673 ± 145	643 ± 160
	P25	1193 ± 310	1313 ± 194	987 ± 419	902 ± 253	935 ± 204	875 ± 172
	P50	1288 ± 273	1338 ± 198	1033 ± 199	820 ± 106	924 ± 146	918 ± 163
COF (mL·min^{-1})	CONT	11.5 ± 4.0	10.4 ± 2.0	25.5 ± 10.0	22.4 ± 11.6	25.4 ± 7.5	26.2 ± 8.1
	P25	13.4 ± 2.4	12.5 ± 5.6	16.2 ± 7.5	16.3 ± 9.5	14.8 ± 8.5	16.6 ± 8.1
	P50	10.1 ± 2.1	14.1 ± 4.0	18.4 ± 4.0	13.3 ± 4.0	13.0 ± 5.7#	12.6 ± 6.0#

Values are means ± SD (n = 6 per group). Re-5 = five minutes after reperfusion; Re-15 = 15 min after reperfusion; Re-25 = 25 min after reperfusion; Re-35 = 35 min after reperfusion. CONT = control; P25 = 25 μM propofol; P50 = 50 μM propofol; #$P < 0.05$ *vs* CONT. LVDP = left ventricular developed pressure; LVEDP = left ventricular end-diastolic pressure; HR = heart rate; COF = coronary flow.

TABLE II Summary of cardiac function induced by pacing in Experiment 1

		Baseline	Treatment	Re-5	Re-15	Re-25	Re-35
LVDP (mmHg)	P-CONT	73 ± 13	77 ± 6	77 ± 4	52 ± 14	49.± 14	47 ± 13
	P-P25	77 ± 10	72 ± 7	74 ± 11#	72 ± 5#	72 ± 6#	70 ± 6#
	P-P50	79 ± 7	75 ± 5	81 ± 3#	70 ± 8#	70 ± 10#	72 ± 7#
LVEDP (mmHg)	P-CONT	4 ± 4	5 ± 2	5 ± 2	8 ± 2	8 ± 4	8 ± 2
	P-P25	3 ± 3	4 ± 2	4 ± 2	3 ± 2#	4 ± 2#	4 ± 3
	P-P50	2 ± 3	3 ± 3	4 ± 3	2 ± 4#	2 ± 3#	3 ± 3#
HR (beats·min^{-1})	P-CONT	240 ± 37	300 ± 0	260 ± 31	220 ± 31	240 ± 33	220 ± 31
	P-P25	230 ± 31	300 ± 0	230 ± 24	210 ± 33	210 ± 33	240 ± 42
	P-P50	210 ± 33	300 ± 0	250 ± 24	210 ± 30	210 ± 31	220 ± 44
-dP/dt (mmHg·sec^{-1})	P-CONT	823 ± 119	1275 ± 190	1109 ± 204	574 ± 237	537 ± 197	532 ± 173
	P-P25	882 ± 140	1218 ± 114	1171 ± 177	969 ± 61#	993 ± 83#	944 ± 129#
	P-P50	944 ± 149	1100 ± 136	1140 ± 127	976 ± 137#	1027 ± 225#	924 ± 163#
+dP/dt (mmHg·sec^{-1})	P-CONT	1460 ± 295	1673 ± 211	1440 ± 154	937 ± 180	870 ± 202	853 ± 178
	P-P25	1416 ± 334	1418 ± 178	1320 ± 172	1210 ± 192#	1342 ± 138#	1356 ± 197#
	P-P50	1465 ± 301	1588 ± 158	1445 ± 130	1273 ± 166#	1293 ± 168#	1385 ±142#
COF (mL·min^{-1})	P-CONT	16.4 ± 2.8	16.7 ± 2.9	20.6 ± 4.3	16.7± 4.4	18.5 ± 6.0	19.1 ± 7.9
	P-P25	16.3 ± 1.6	23.6 ± 2.9#	22.4 ± 1.9	13.0 ± 3.0	14.0 ± 1.8	13.9 ± 3.0
	P-P50	17.9 ± 4.3	28.3 ± 3.5#	22.0 ± 3.9	14.9 ± 3.8	13.7 ± 5.3	16.5 ± 4.7

Values are means ± SD (n = 6 per group). Re-5 = five minutes after reperfusion; Re-15 = 15 min after reperfusion; Re-25 = 25 min after reperfusion; Re-35 = 35 min after reperfusion. P-CONT = control with pacing; P-P25 = 25 μM propofol with pacing; P-P50 = 50 μM propofol with pacing; #$P < 0.05$ *vs* P-CONT. LVDP = left ventricular developed pressure; LVEDP = left ventricular end-diastolic pressure; HR = heart rate; COF = coronary flow.

restored to 116 ± 44% and 127 ± 58% of the baseline value, respectively.

In another set of experiments, hearts (n = 6 per group) were paced at 300 beats·min^{-1} for ten minutes, before ischemia, to assess the role of the direct bradycardia induced by propofol during administration (Table II). Propofol, at both concentrations, attenuated the increase of LVEDP at Re-15, and Re-25 (P = 0.0014 and P = 0.022, respectively); attenuated the increase of –dP/dt at Re-15, Re-25, and Re-35 (P = 0.0016, P = 0.0029, and P = 0.0006, respectively); and attenuated the increase of +dP/dt at Re-25 and Re-35 (P = 0.0028 and P = 0.0014, respectively). After 35 min of reperfusion, LVDP was restored to 65 ± 16% of the baseline value in the control group and to 94 ± 8% and 91 ± 8% of baseline value in hearts preincubated with 25 and 50 μM propofol, respectively. Propofol (25 and 50 μM) significantly attenuated the decrease of LVDP at Re-15, Re-25, and Re-35 (P = 0.0044, P = 0.0027, and P < 0.001, respectively). After 35 min of reperfusion, COF was restored to 122 ± 62% of the baseline value in the control group. In hearts pretreated with 25 and 50 μM propofol, COF was restored to 85.5 ± 18% and 92.3 ± 16% of the baseline value, respectively.

Propofol shortens APD during early reperfusion (Figure 2)

Before ischemia, the APD_{90} among control, 25, and 50 μM propofol groups did not significantly differ (P = 0.31). The APD_{90} of the control group was similar to the baseline value at five minutes of reperfusion. At five minutes of reperfusion, propofol (25 and 50 μM) significantly shortened APD_{90} (P = 0.033 and P = 0.014, respectively). At 35 min of reperfusion, APD_{90} was similar to the baseline value in both propofol groups and did not significantly differ among all groups (P = 0.18) (Table III).

TABLE III Summary of action potential duration

	Treatment	Re-5	Re-35
APD90 (msec)			
CONT	152 ± 7	145 ± 13	145 ± 7
P25	145 ± 11	125 ± 21#	153 ± 13
P50	144 ± 12	121 ± 13#	143 ± 7

Values are mean ± SD (n = 6 per group). Re-5 = five minutes after reperfusion; Re-35 = 35 min after reperfusion. CONT = control group; P-25 = 25 μM propofol; P-50 = 50μM propofol; APD_{90} = action potential duration at 90% repolarization. #P < 0.05 *vs* CONT.

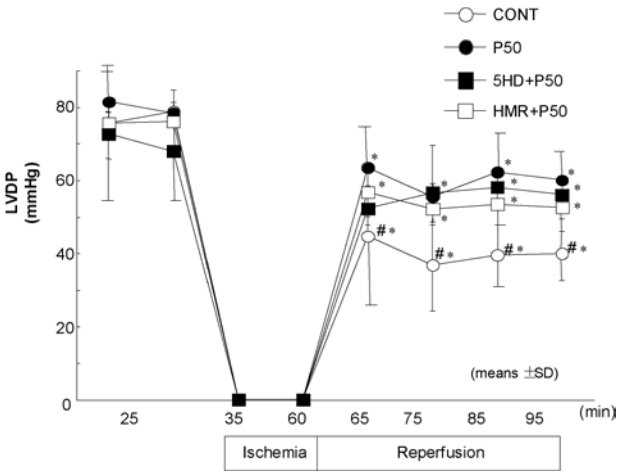

FIGURE 3 Effects of K_{ATP} channels on functional recovery induced by propofol

Hearts were stabilized by perfusion with Krebs-Ringer solution for 25-min, incubated with 500 μM 5-HD or 30 μM HMR1098 for five minutes, 50 μM propofol for five minutes and then global ischemic arrest was applied for 25 min. Hearts were finally reperfused for 35 min to measure postischemic function. CONT = control group; P50 = 50 μM propofol group; 5HD = 5-hydroxydecanoate; HMR = HMR1098. Values are means ± SD (n = 6 per group). *, P < 0.05 *vs* corresponding baseline value; #P < 0.05 *vs* P50 group.

Propofol does not exert cardioprotective effects by opening K_{ATP} channels

Since a shortened APD may be caused by opening of sarcolemmal K^+ channels, we examined whether propofol exerts cardioprotective effect via K_{ATP} channel opening. Our preliminary study showed that neither 500 μM 5-HD nor 30 μM HMR1098, alone, affected LVDP recovery (P = 0.18, data not shown). The changes of LVDP, by prior incubation with 500 μM 5-HD and 30 μM HMR1098, are shown in Figure 3. Prior incubation with 500 μM 5-HD or 30 μM HMR1098 did not affect the beneficial effect of 50 μM propofol on LVDP, LVEDP, +dP/dt, and –dP/dt.

Prior incubation with 25 and 50 μM propofol significantly reduced infarct size as compared with control hearts (P < 0.0001). Such protection induced by 50 μM propofol was not blocked by either 500 μM 5-HD or 30 μM HMR1098. When HR was maintained at a constant rate by right ventricular pacing (Table IV), infarct size was likewise significantly lower in the propofol group, compared to the control group (P < 0.0001).

Propofol exerts cardioprotective effects by increasing phosphorylation of GSK-3β Ser9 residue

At five minutes of reperfusion, propofol (25 and 50 μM) significantly increased levels of phosphor-GSK-3β compared to the control group (P = 0.038 and P = 0.019, respectively) (Figure 4). The ratios of phospho-GSK-3β/ total GSK-3β were 0.27 ± 0.17 in the control group and 0.72 ± 0.32 and 0.79 ± 0.47 in the

TABLE IV Summary of infarct size

Infarction (%)	
CONT	74 ± 3
P25	48 ± 9 #
P50	47 ± 9 #
HMR+P50	45 ± 9 #
5-HD+P50	51 ± 8 #
P-CONT	74 ± 2
P-P25	43 ± 4 #
P-P50	44 ± 4 #

Values are mean ± SD (n = 6 per group). CONT = control; P-25 = 25 μM propofol group; P-50 = 50 μM propofol; P-CONT = control with pacing; P-P25 = 25 μM propofol with pacing; P-P50 = 50 μM propofol with pacing; 5-HD = 5-hydroxydecanoic acid; HMR = HMR1098. #$P < 0.05$ *vs* CONT.

groups pre-incubated with 25 and 50 μM propofol, respectively.

Discussion

We found that 25 and 50 μM propofol, applied before ischemia, enhanced the recovery of mechanical function and reduced infarct size during postischemic reperfusion. This cardioprotective effect was not prevented by prior incubation with the K_{ATP} channel blocker, the 5-HD (a selective mK_{ATP} channel blocker), or the HMR1098 (a sK_{ATP} channel blocker). Furthermore, this is the first study to assess the effects of propofol on GSK-3β inhibition in isolated guinea pig hearts. However, these findings did not support our hypothesis that propofol protected the heart during ischemia and reperfusion via GSK-3β inhibition by activation of the K_{ATP} channel.

In the current study, propofol (25 and 50 μM) improved the recovery of mechanical function induced by ischemia/reperfusion injury in isolated guinea pig hearts, and this finding is similar to previous studies using rat hearts.[3,4] Ko *et al.*[3] demonstrated that propofol (30 and 100 μM) improved the recovery of LVDP and dP/dt and significantly lowered LVEDP in isolated rat hearts. Kokita *et al.*[4] reported that propofol (25 and 50 μM) improved the recovery of mechanical function in isolated ischemia-reperfusion rat hearts. In our current study, propofol caused a significant decrease in HR, not only during administration, but also after reperfusion. Such pre-ischemic energy-sparing effects, due to the depression of cardiodynamic function, might be one possible mechanism for the cardioprotective effect of propofol. However, the beneficial effect of propofol was not affected in our paced-heart study. Therefore, our present results indicate that propofol exerts anti-ischemic effects independent of its effect on reducing cardiac work.

Activation of mitochondrial K_{ATP} channels is obviously important in various cardioprotective mechanisms, for example, preconditioning,[10,11] and K_{ATP} channel activation might protect mitochondria against mPTP opening. Volatile anesthetics, including isoflurane, desflurane, and sevoflurane, also seem to protect the myocardium against stunning and infarction by activating K_{ATP} channels.[20–22] Vincent *et al.*[23] reported that desflurane-induced preconditioning improves the resistance of mPTP to ischemia-reperfusion, and they suggested a link between mitochondrial K_{ATP} and mPTP. There are several studies evaluating the effect of propofol on K_{ATP} channel. Kalkan *et al.*[24] reported that the activation of K_{ATP} channels seemed to be one of the mechanisms by which propofol induced beneficial effect on contractility of myocardium in hypercholesterolemic rabbit hearts. Kawano *et al.*[25] reported that

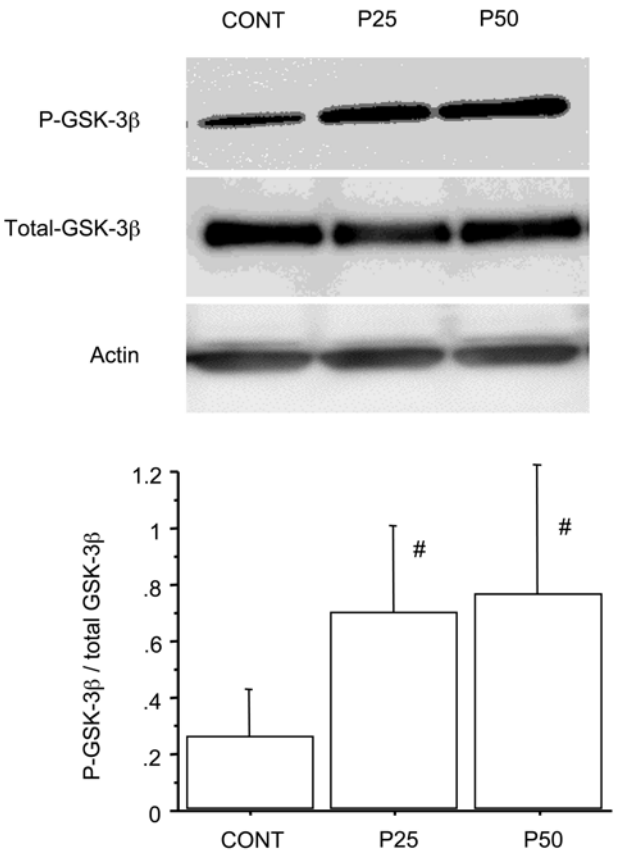

FIGURE 4 Effects of propofol on phosphorylation of Ser^9 residue on GSK-3β in ischemia-reperfused hearts
Upper and lower panels show representative immunoblots and summarized phospho-GSK-3β (p-GSK-3β) levels, respectively. Levels of GSK-3β phosphorylation in samples are expressed as ratios of densitometric values of p-GSK-3β to that of total GSK-3β. CONT = control; P25 = 25 μM propofol; P50 = 50 μM propofol; GSK-3β = glycogen synthase kinase 3β. Values are means ± SD (n = 6 per group). #$P < 0.05$ *vs* control group.

propofol significantly inhibited K_{ATP} channel activities at high concentrations and suggested that the major effect of propofol on K_{ATP} channel activity was mediated via the Kir6.2 subunit. In contrast, Mathur *et al.*[26] reported that propofol did not provide cardioprotection via the K_{ATP} channel._Here, we investigated the effect of propofol on mitochondrial K_{ATP} channel in hearts exposed to ischemia-reperfusion. Prior incubation with 5-HD or HMR1098 did not affect the ability of 50 μM propofol to improve functional recovery and reduce infarct size. Therefore, these findings indicate that propofol exerts anti-ischemic effects independent of its effect on K_{ATP} channel activities and shortening of the APD.

During acute ischemia, sarcolemmal K_{ATP} channels open early in response to inhibited cell metabolism. This should significantly shorten the APD. Several studies using optical mapping have demonstrated that APD is shortened during low flow ischemia.[27–29] However, in the current study, we induced global ischemia. Under these experimental conditions, our high resolution imaging device detected only weak change in fluorescence, therefore, we could not record the APD during ischemia. We found that 25 and 50 μM propofol significantly shortened APD_{90} at five minutes of reperfusion. Prior incubation with HMR1098 did not affect the ability of 50 μM propofol to improve functional recovery and reduce infarct size. These results indicate that propofol shortened APD during the early phase of reperfusion and not via sarcolemmal K_{ATP} channels. One possible mechanism of propofol action would be to reduce the calcium overload that occurs during ischemia-reperfusion through inhibitory effects on calcium channels.[5,30] This might also account for the decrease in LV relaxation (–dP/dt) as well as the lower LVEDP during reperfusion.

Propofol increases PKC activity in rat ventricular myocytes.[7–9] Wickley *et al.*[9] recently found that propofol stimulates PKC-α, PKC-δ, PKC-ε, and PKC-ζ translocation to distinct intracellular locations. Protein kinase C is an important signal transducer that regulates many cardiac functions, including ion channels, contractility, gene expression, and hypertrophy. The PKC-dependent production of nitric oxide, the activation of K_{ATP} channels, and the prevention of Ca^{2+} overload are several key factors involved in conferring myocardial protection through medication. Fang *et al.*[31] reported that PKC activation induces GSK-3β phosphorylation, and that PKC-δ interacts with GSK-3β in several cell lines.[31,32] In contrast, PKCε might indirectly regulate GSK through phosphatidylinositol-3-kinase (PI3K), because the induction of a dominant-negative PKCε blocks the activity of PI3K induced by phorbol ester and insulin.[33] Therefore, the selective PKC isoform responsible for modulating GSK-3β remains an area of interest for future studies.

A recent study has shown that GSK-3β mediates the convergence of myocyte protection signalling through inhibiting the opening of mPTP.[34] Reperfusion injury salvage kinases afford protection against ischemia and phosphorylate GSK-3β during reperfusion.[13,35] Pharmacological GSK-3β inhibition reduces infarct size and improves postischemic function.[13] Opioids induce cardioprotection by inactivating GSK-3β at Ser^9,[36] and GSK inhibition enhances the protective effect of isoflurane-induced post-conditioning via an mPTP-dependent mechanism *in vivo*.[37] The present study found that propofol (25 and 50 μM) reduced infarct size and increased levels of p-GSK-3β. Thus, GSK-3β inhibition by phosphorylation at the N-terminal serine residue, Ser^9, might act as a central mediator of the cardioprotective effects of propofol. Sztark *et al.*[38] reported that propofol might directly inhibit mPTP at concentrations above those applied clinically. Javadov *et al.*[39] reported that propofol is associated with a significant reduction in mPTP opening, probably as a result of diminished oxidative stress. The link between mitochondrial K_{ATP} channels and mPTP opening and whether mitochondrial K_{ATP} channels directly, or indirectly, interact with GSK-3β, due to the mitochondrial localization of GSK-3β, remain unknown. However, our data showed that propofol enhances GSK-3β phosphorylation upon reperfusion, suggesting that propofol inactivates GSK-3β during this process. These data also suggest that GSK-3β, and not mitochondrial K_{ATP} channels, plays an important role in the action of propofol on mPTP opening at reperfusion.

There is no direct evidence that the phosphorylation of GSK-3β by propofol is cardioprotective, unless demonstrating that a GSK inhibitor such as SB216763 can attenuate the effect of propofol in further myocardial recovery after ischemia-reperfusion. However, it is now believed that inhibition of GSK-3β plays a critical role in myocardial protection against ischemia-reperfusion injury. Selective inhibition of GSK-3β mimicked the beneficial actions of ischemic preconditioning[13] and opioid-induced myocardial protection during reperfusion.[36] Further study is required to clarify the relationships between the cardioprotective effects of propofol and GSK-3β phosphorylation. However, we believe that we are the first to assess the effects of propofol on GSK-3β inhibition in isolated guinea pig hearts.

Extrapolating our data to a clinical setting must be done with caution, because of possible species differences and differences between *in vivo* and *in vitro* conditions. It is also well established that the response to

propofol is widely variable among patients receiving the same dose. The drug's binding to serum proteins exceeds 98%,[40] so small changes in protein concentrations can be amplified in the unbound fraction of the drug and in its effect. It is likely that that part of the variability in the response among patients is due to differences in protein levels among individuals and, in particular, among those with pathologies such as liver disease. The clinical relevance of *in vitro* studies using propofol is often questioned because the concentrations of propofol that cause changes in cell, tissue, or organ function are typically outside of our estimations of what we perceive as a clinically relevant concentration. However, it is difficult, for several reasons, to estimate the clinically relevant plasma concentrations of propofol *in vivo,* as well as to relate these clinical plasma concentrations to free aqueous drug concentrations *in vitro*. First, the rate of exchange among propofol-containing liposomes, the aqueous phase, serum proteins, and cellular constituents is not precisely known, which could significantly affect plasma concentrations *in vivo*. In addition, protein binding *in vivo* is unlikely to be instantaneous, so free drug concentrations with a bolus injection would probably be higher than the steady state value. Peak plasma levels, after a bolus injection, have been estimated at 50 μM and stable levels at approximately 10–25 μM during maintenance infusion.[41] Additional factors such as speed of injection, volume of distribution, and *p*H can affect plasma concentrations of anesthetics. Given the difficulty and uncertainty in estimating the *in vivo* concentrations and the likelihood that these estimations may be different in pathologic conditions (hemodilution, liver disease, diabetes), we believe that the concentration of propofol used in this study is likely to be similar to that encountered in clinical practice.

In summary, the current investigation confirmed that propofol enhances the recovery of mechanical function and reduces infarct size when administered before ischemia. In contrast to our hypothesis, prior incubation with K_{ATP} channel blockers did not interfere with these benefits. Furthermore, we demonstrated that a propofol-induced cardioprotective effect is involved in the inhibition of GSK-3β.

References

1 *De Hert SG, Turani F, Mathur S, Stowe DF.* Cardioprotection with volatile anesthetics: mechanisms and clinical implications. Anesth Analg 2005; 100: 1584–93.

2 *Ebel D, Schlack W, Comfere T, Preckel B, Thamer V.* Effect of propofol on reperfusion injury after regional ischaemia in the isolated rat heart. Br J Anaesth 1999; 83: 903–8.

3 *Ko SH, Yu CW, Lee SK, et al.* Propofol attenuates ischemia-reperfusion injury in the isolated rat heart. Anesth Analg 1997; 85: 719–24.

4 *Kokita N, Hara A, Abiko Y, Arakawa J, Hashizume H, Namiki A.* Propofol improves functional and metabolic recovery in ischemic reperfused isolated rat hearts. Anesth Analg 1998; 86: 252–8.

5 *Buljubasic N, Marijic J, Berczi V, Supan DF, Kampine JP, Bosnjak ZJ.* Differential effects of etomidate, propofol, and midazolam on calcium and potassium channel currents in canine myocardial cells. Anesthesiology 1996; 85: 1092–9.

6 *Murphy PG, Myers DS, Davies MJ, Webster NR, Jones JG.* The antioxidant potential of propofol (2,6-diisopropylphenol). Br J Anaesth 1992; 68: 613–8.

7 *Kanaya N, Gable B, Murray PA, Damron DS.* Propofol increases phosphorylation of troponin I and myosin light chain 2 via protein kinase C activation in cardiomyocytes. Anesthesiology 2003; 98: 1363–71.

8 *Kanaya N, Murray PA, Damron DS.* Propofol increases myofilament Ca2+ sensitivity and intracellular pH via activation of Na+-H+ exchange in rat ventricular myocytes. Anesthesiology 2001; 94: 1096–104.

9 *Wickley PJ, Ding X, Murray PA, Damron DS.* Propofol-induced activation of protein kinase C isoforms in adult rat ventricular myocytes. Anesthesiology 2006; 104: 970–7.

10 *Liang BT.* Protein kinase C-mediated preconditioning of cardiac myocytes: role of adenosine receptor and KATP channel. Am J Physiol 1997; 273: H847–53.

11 *Wang Y, Ashraf M.* Role of protein kinase C in mitochondrial KATP channel-mediated protection against Ca2+ overload injury in rat myocardium. Circ Res 1999; 84: 1156–65.

12 *Frame S, Cohen P.* GSK3 takes centre stage more than 20 years after its discovery. Biochem J 2001; 359: 1-16.

13 *Tong H, Imahashi K, Streenbergen C, Murphy E.* Phosphorylation of glycogen synthase kinase-3β during preconditioning through a phosphatidylinositol-3-kinase-dependent pathway is cardioprotective. Cir Res 2002; 90: 377–9.

14 *Gross ER, Hsu AK, Gross GJ.* GSK3β inhibition and K(ATP) channel opening mediate acute opioid-induced cardioprotection at reperfusion. Basic Res Cardiol 2007; 102: 341–9.

15 *Feng J, Lucchinetti E, Ahuja P, Pasch T, Perriard JC, Zaugg M.* Isoflurane postconditioning prevents opening of the mitochondrial permeability transition pore through inhibition of glycogen synthase kinase 3β. Anesthesiology 2005; 103: 987–95.

16 *Kurosawa S, Kanaya N, Niiyama Y, Nakayama M, Fujita S, Namiki A.* Landiolol, esmolol and propranolol protect from ischemia/reperfusion injury in

isolated guinea pig hearts. Can J Anesth 2003; 50: 489–94.

17 *Kimura-Kurosawa S, Kanaya N, Kamada N, Hirata N, Nakayama M, Namiki A.* Cardioprotective effect and mechanism of action of landiolol on the ischemic reperfused heart. J Anesth 2007; 21: 480–9.

18 *Laurita KR, Singal A.* Mapping action potentials and calcium transients simultaneously from the infarct heart. Am J Physiol Heart Circ Physiol 2001; 280: H2053–60.

19 *Lennard PR.* Image analysis for all. Nature 1990; 347: 103–4.

20 *Kersten JR, Schmeling T, Tessmer J, Hettrick DA, Pagel PS, Warltier DC.* Sevoflurane selectively increases coronary collateral blood flow independent of KATP channels in vivo. Anesthesiology 1999; 90: 246–56.

21 *Cason BA, Shubayev I, Hickey RF.* Blockade of adenosine triphosphate–sensitive potassium channels eliminates isoflurane-induced coronary artery vasodilation. Anesthesiology 1994; 81: 1245–55.

22 *Toller WG, Gross ER, Kersten JR, Pagel PS, Gross GJ, Warltier DC.* Sarcolemmal and mitochondrial adenosine triphosphate–dependent potassium channels: mechanism of desflurane-induced cardioprotection. Anesthesiology 2000; 92: 1731–9.

23 *Piriou V, Chiari P, Gateau-Roesch O, et al.* Desflurane-induced preconditioning alters calcium-induced mitochondrial permeability transition. Anesthesiology 2004; 100: 581–8.

24 *Kalkan S, Eminoglu O, Akugun A, Guven H, Tuncok Y.* The role of adenosine triphosphate-regulated potassium channels in propofol-induced beneficial effect on contractile function of hypercholesterolemic isolated rabbit hearts. Saudi Med J 2007; 28: 701–6.

25 *Kawano T, Oshita S, Takahashi A, et al.* Molecular mechanisms of the inhibitory effects of propofol and tiamylal on sarcolemmmal adenosine triphosphate-sensitive potassium channels. Anesthesiology 2004; 100: 338–46.

26 *Mathur S, Farhangkhgoee P, Karmazyn M.* Cardioprotective effects of propofol and sevoflurane in ischemic and reperfused rat hearts: role of K(ATP) channels and interaction with the sodium-hydrogen exchange inhibitor HOE 642 (cariporide). Anesthesiology 1999; 91: 1349–60.

27 *Liu YB, Pak HN, Lamp ST, et al.* Coexistence of two types of ventricular fibrillation during acute regional ischemia in rabbit ventricle. J Cardiovasc Electrophysiol 2004; 15: 1433–40.

28 *Cheng Y, Mowrey KA, Nikolski V, Tchou PJ, Efimov IR.* Mechanisms of shock-induced arrhythmogenesis during acute global ischemia. Am J Physiol 2002; 282: H2141–51.

29 *Nygren A, Baczko I, Giles WR.* Measurements of electrophysiological effects of components of acute ischemia in Langendorff-perfused rat hearts using voltage-sensitive dye mapping. J Cardiovasc Electrophysiol 2006; 17: S113–23.

30 *Kanaya N, Murray PA, Damron DS.* Propofol and ketamine only inhibit intracellular Ca2+ transients and contraction in rat ventricular myocytes at supraclinical concentrations. Anesthesiology 1998; 88: 781–91.

31 *Fang X, Yu S, Tanyi JL, Lu Y, Woodgett JR, Mills GB.* Convergence of multiple signaling cascades at glycogen synthase kinase 3: Edg receptor-mediated-phosphorylation and inactivation by lysophosphatidic acid through a protein kinase C-dependent intracellular pathway. Mol Cell Biol 2002; 22: 2099–110.

32 *Kostyak JC, Hunter JC, Korzick DH.* Acute PKCdelta inhibition limits ischaemia-reperfusion injury in the aged rat heart: role of GSK-3beta. Cardiovasc Res 2006; 70: 325–34.

33 *Huang C, Ma WY, Dong Z.* Potentiation of insulin-induced phosphatidylinositol-3 kinase activity by phorbol ester is mediated by protein kinase Cε. Cell Signal 1998; 10: 185–90.

34 *Juhaszova M, Zorov DB, Kim SH, et al.* Glycogen synthase kinase-3β mediates convergence of protection signaling to inhibit the mitochondrial permeability transition pore. J Clin Invest 2004; 113: 1535–49.

35 *Matsui T, Rosenzweig A.* Convergent signal transduction pathways controlling cardiomyocyte survival and function: the role of PI 3-kinase and Akt. J Mol Cell Cardiol 2005; 38: 63–71.

36 *Gross ER, Hsu AK, Gross GJ.* Opioid-induced cardioprotection occurs via glycogen synthase kinase β inhibition during reperfusion in intact rat hearts. Circ Res 2004; 94: 960–6.

37 *Pagel PS, Krolikowski JG, Neff DA, et al.* Inhibition of glycogen synthase kinase enhances isoflurane-induced protection against myocardial infarction during early reperfusion in vivo. Anesth Analg 2006; 102: 1348–54.

38 *Sztark F, Ichas F, Ouhabi R, Dabadie P, Mazat JP.* Effects of the anaesthetic propofol on the calcium-induced permeability transition of rat heart mitochondria: direct pore inhibition and shift of the gating potential. FEBS Lett 1995; 368: 101–4.

39 *Javadov SA, Lim KH, Kerr PM, Suleiman MS, Angelini GD, Halestrap AP.* Protection of hearts from reperfusion injury by propofol is associated with inhibition of the mitochondrial permeability transition. Cardiovasc Res 2000; 45: 360–9.

40 *Morgan DJ, Campbell GA, Crankshaw DP.* Pharmacokinetics of propofol when given by intravenous infusion. Br J Clin Pharmacol 1990; 30: 144–8.

41 *Cockshott ID.* Propofol (Diprivan) pharmacokinetics and metabolism: an overwiew. Postgrad Med J 1985; 61(Suppl 3): 45–50.