

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

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

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**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-3 $\beta$  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  $\mu$ M), or for five minutes with 500  $\mu$ M 5-hydroxydecanoate (a mitochondrial  $K_{ATP}$  channel blocker) or 30  $\mu$ M HMR1098 (sarcolemmal  $K_{ATP}$  channel blocker), followed by five minutes with 50  $\mu$ M 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-3 $\beta$  phosphorylation at the serine residue, Ser9, was examined.

**Results:** After 35 min of reperfusion, propofol (25 and 50  $\mu$ M) 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  $\mu$ M propofol. At five minutes after reperfusion, propofol (25 and 50  $\mu$ M) shortened the duration of the action potential and increased the levels of phospho-GSK-3 $\beta$  ( $P < 0.05$ ).

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

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**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-3 $\beta$  dans des cœurs ischémiques puis reperfusés.

**Méthode :** L'ischémie reperfusion (I/R) a été provoquée dans des cœurs isolés de cobayes en interrompant la perfusion coronarienne pendant 25 min, puis en les reperfusant. Les cœurs ont été incubés pendant dix minutes, avec ou sans propofol (25 ou 50  $\mu$ M), ou pendant cinq minutes avec 500  $\mu$ M de 5-hydroxydecanoate (un bloqueur du canal  $K_{ATP}$  mitochondrial) ou 30  $\mu$ M de HMR1098 (un bloqueur du canal  $K_{ATP}$  sarcolemmal), suivi par cinq minutes avec 50  $\mu$ M de propofol avant l'ischémie. Les potentiels d'action sur la surface épicaudique 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-3 $\beta$  au résidu de sérine, Ser9, a été examinée.

**Résultats :** Après 35 min de reperfusion, le propofol (25 et 50  $\mu$ M) 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  $\mu$ M de propofol. Cinq minutes après la reperfusion, le propofol (25 et 50  $\mu$ M) a abrégé la durée

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

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

**P**ROPOFOL 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.<sup>1</sup> In studies involving isolated rat heart preparations, there have been reports of absent cardiac protective effects<sup>2</sup> as well as concentration-dependent, cardiac protective effects<sup>3,4</sup> 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,<sup>5</sup> 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<sup>6</sup> with the ability to attenuate ischemia-reperfusion injury in the isolated rat heart.<sup>3,4</sup> 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.<sup>7</sup> Propofol increased myofilament  $Ca^{2+}$  sensitivity, at least in part, by increasing  $pHi$  via PKC-dependent activation of  $Na^+H^+$  exchange.<sup>8</sup> Therefore, propofol may have a cardioprotective effect via cellular mechanisms triggered by PKC activation.<sup>9</sup>

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.<sup>10,11</sup> Moreover, glycogen synthase kinase (GSK) is an important regulator of cellular function.<sup>12</sup> The selective inhibition of GSK-3 $\beta$  is similar to the effects of IPC in isolated rat hearts, and IPC reduces GSK-3 $\beta$  activity by phosphorylation at the N-terminal serine residue, Ser<sup>9</sup>.<sup>13</sup> Recently, Gross *et al.*<sup>14</sup> reported that both the sarcolemmal  $K_{ATP}$  ( $sK_{ATP}$ ) and the  $mK_{ATP}$  channels are involved in acute opioid-induced cardioprotection, and the GSK signaling axis regulates cardioprotection via  $K_{ATP}$  channel

opening. Another study demonstrated that GSK-3 $\beta$  phosphorylation and inactivation play an important role in volatile anesthetics-induced cardioprotection by preventing the opening of mitochondrial permeability transition pores (mPTP).<sup>15</sup> However, it is unknown whether propofol produces cardioprotective effect via  $K_{ATP}$  channel opening. It is also unknown whether GSK-3 $\beta$  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-3 $\beta$  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-3 $\beta$  inhibition by measuring phosphorylation of GSK-3 $\beta$  at Ser<sup>9</sup>.

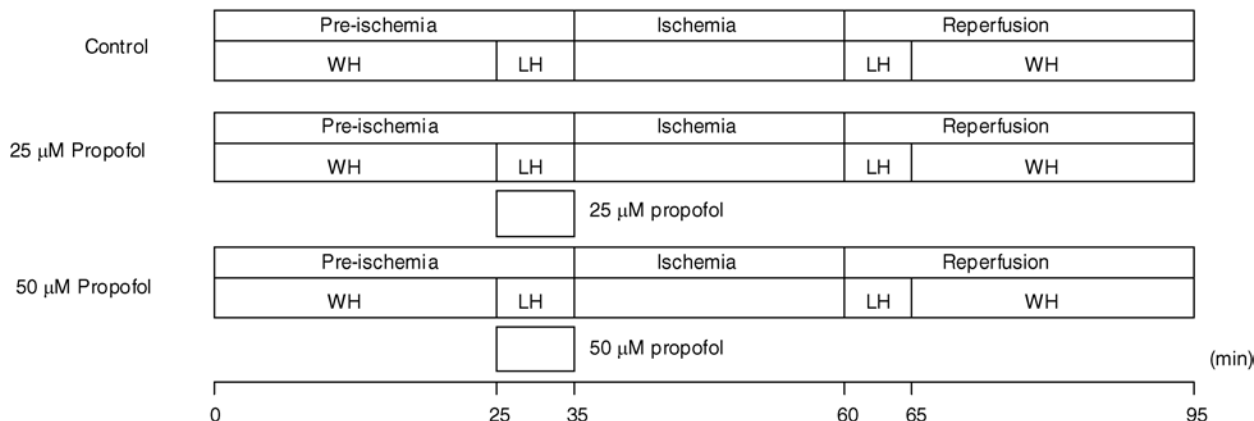
## Methods

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,<sup>16,17</sup> 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  $Mg^{2+}$ , 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

A. Experiment 1. Propofol administration during pre-ischemia



B. Experiment 3. K<sub>ATP</sub> channel blocker administrated, following by 50 μM propofol

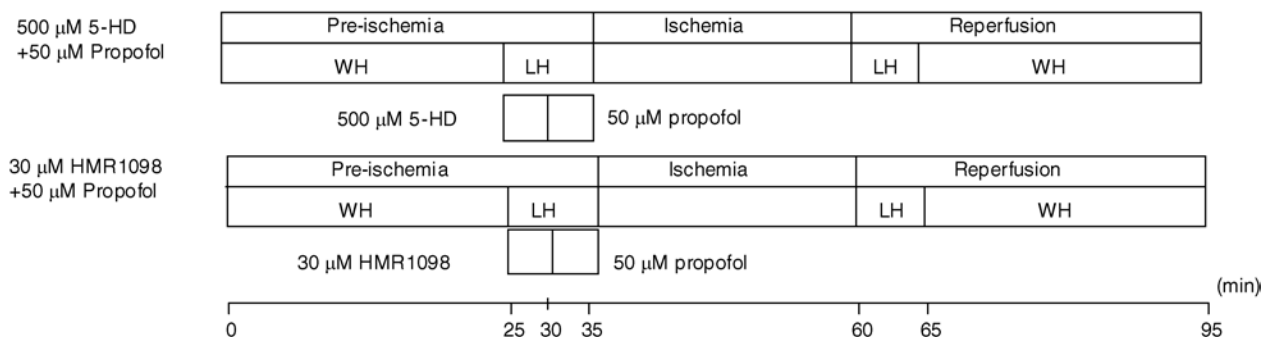


FIGURE 1 Scheme of experimental protocols

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.<sup>4</sup> 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 ( $\pm$  dP/dt<sub>max</sub>) 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<sup>-1</sup> 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<sup>-1</sup> using a silver bipolar electrode (4V, two-time threshold; 2 msec dura-

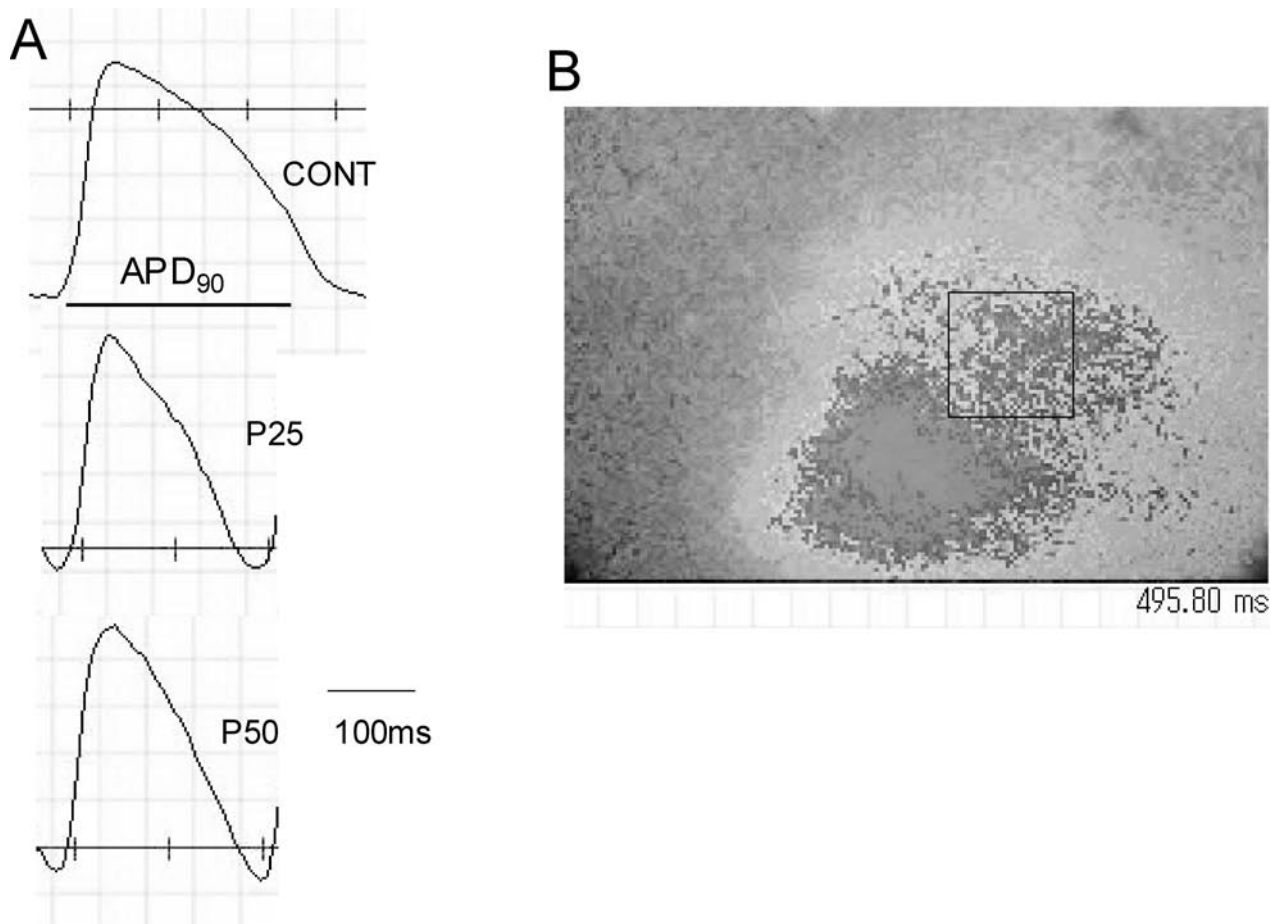


FIGURE 2 Optical recording of ventricular action potential durations in isolated guinea pig heart

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  $\mu\text{M}$  propofol group; P50 = 50  $\mu\text{M}$  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  $\mu\text{M}$  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- $\{\beta\}$ -[2(di-*n*-butylamino)-6-naphthylvinylpyridinium (Di-4-ANEPPS, 1  $\mu\text{M}$ ; 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%).<sup>18</sup> 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,<sup>18</sup> and were paced at a rate of 300 beats·min<sup>-1</sup> from the right ventricle. Before 25-min of global ischemic arrest, the hearts were incubated with propofol (25 and 50  $\mu\text{M}$ ) 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  $\times$  128 points, 3.7 msec time resolution, Brain Vision, Japan) at emission and excitation wavelengths of > 700 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 $K_{\text{ATP}}$ CHANNEL OPENING?

We investigated the effect of propofol on  $K_{\text{ATP}}$  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  $\mu\text{M}$  5-hydroxydecanoate (5-HD, a mitochondrial  $\text{K}_{\text{ATP}}$  blocker; Sigma-Aldrich, Tokyo, Japan) or with 30  $\mu\text{M}$  HMR1098 (a sarcolemmal  $\text{K}_{\text{ATP}}$  blocker; Aventis Pharma Deutschland GmbH, Frankfurt, Germany) for five minutes, and then with 50  $\mu\text{M}$  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-3 $\beta$ 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  $\mu\text{M}$ ) 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  $\times$  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  $\mu\text{g}$  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-3 $\beta$  or  $^{\text{p}}\text{Ser}$ -phospho-GSK-3 $\beta$  (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.<sup>19</sup>

#### Statistical analysis

To determine differences in functional recovery and

APD<sub>90</sub> 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-3 $\beta$  / total GSK-3 $\beta$  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  $\pm$  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  $-\text{dP}/\text{dt}$  did not significantly differ among the control, 25, and 50  $\mu\text{M}$  propofol groups. (Table I) After 35 min of reperfusion, LVDP was restored to  $55 \pm 11\%$  of the baseline value in the control group. In hearts pretreated with 25 and 50  $\mu\text{M}$  propofol, LVDP was restored to  $73 \pm 11\%$  and  $75 \pm 13\%$  of the baseline value, respectively. Propofol (25 and 50  $\mu\text{M}$ ) 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,  $-\text{dP}/\text{dt}$  in the control group was  $62 \pm 12\%$  of the baseline value. Prior exposure to 25 and 50  $\mu\text{M}$  propofol restored  $-\text{dP}/\text{dt}$  to  $86 \pm 14\%$  and  $86 \pm 12\%$  of the baseline value, respectively. At both concentrations, propofol similarly attenuated the increase of  $-\text{dP}/\text{dt}$  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  $257 \pm 145\%$  of the baseline value in the control group. In hearts pretreated with 25 and 50  $\mu\text{M}$  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 <sup>#</sup>	58 ± 12 <sup>#</sup>	56 ± 10 <sup>#</sup>
	P50	81 ± 7	78 ± 7	63 ± 12 <sup>#</sup>	55 ± 9 <sup>#</sup>	62 ± 14 <sup>#</sup>	60 ± 9 <sup>#</sup>
LVEDP (mmHg)	CONT	4 ± 3	1 ± 4	18 ± 11	7 ± 5	6 ± 2	5 ± 1
	P25	3 ± 1	1 ± 1	1 ± 1 <sup>#</sup>	3 ± 1 <sup>#</sup>	2 ± 1 <sup>#</sup>	3 ± 1 <sup>#</sup>
	P50	1 ± 1	1 ± 2	3 ± 1 <sup>#</sup>	5 ± 3	4 ± 2 <sup>#</sup>	3 ± 2
HR (beats·min <sup>-1</sup> )	CONT	220 ± 30	290 ± 24	240 ± 53	230 ± 45	250 ± 24	250 ± 24
	P25	230 ± 45	200 ± 31 <sup>#</sup>	260 ± 31	210 ± 50	200 ± 31 <sup>#</sup>	210 ± 33 <sup>#</sup>
	P50	230 ± 24	210 ± 33 <sup>#</sup>	220 ± 31	220 ± 49	200 ± 48 <sup>#</sup>	210 ± 32 <sup>#</sup>
-dP/dt (mmHg·sec <sup>-1</sup> )	CONT	852 ± 160	1285 ± 212	779 ± 181	399 ± 95	533 ± 122	510 ± 91
	P25	862 ± 88	1188 ± 153	737 ± 338	737 ± 153 <sup>#</sup>	750 ± 181 <sup>#</sup>	735 ± 129 <sup>#</sup>
	P50	972 ± 135	1115 ± 140	828 ± 182	676 ± 85 <sup>#</sup>	774 ± 91 <sup>#</sup>	818 ± 102 <sup>#</sup>
+dP/dt (mmHg·sec <sup>-1</sup> )	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 <sup>-1</sup> )	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 <sup>#</sup>	12.6 ± 6.0 <sup>#</sup>

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; <sup>#</sup>*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 <sup>#</sup>	72 ± 5 <sup>#</sup>	72 ± 6 <sup>#</sup>	70 ± 6 <sup>#</sup>
	P-P50	79 ± 7	75 ± 5	81 ± 3 <sup>#</sup>	70 ± 8 <sup>#</sup>	70 ± 10 <sup>#</sup>	72 ± 7 <sup>#</sup>
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 <sup>#</sup>	4 ± 2 <sup>#</sup>	4 ± 3
	P-P50	2 ± 3	3 ± 3	4 ± 3	2 ± 4 <sup>#</sup>	2 ± 3 <sup>#</sup>	3 ± 3 <sup>#</sup>
HR (beats·min <sup>-1</sup> )	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 <sup>-1</sup> )	P-CONT	823 ± 119	1275 ± 190	1109 ± 204	574 ± 237	537 ± 197	532 ± 173
	P-P25	882 ± 140	1218 ± 114	1171 ± 177	969 ± 61 <sup>#</sup>	993 ± 83 <sup>#</sup>	944 ± 129 <sup>#</sup>
	P-P50	944 ± 149	1100 ± 136	1140 ± 127	976 ± 137 <sup>#</sup>	1027 ± 225 <sup>#</sup>	924 ± 163 <sup>#</sup>
+dP/dt (mmHg·sec <sup>-1</sup> )	P-CONT	1460 ± 295	1673 ± 211	1440 ± 154	937 ± 180	870 ± 202	853 ± 178
	P-P25	1416 ± 334	1418 ± 178	1320 ± 172	1210 ± 192 <sup>#</sup>	1342 ± 138 <sup>#</sup>	1356 ± 197 <sup>#</sup>
	P-P50	1465 ± 301	1588 ± 158	1445 ± 130	1273 ± 166 <sup>#</sup>	1293 ± 168 <sup>#</sup>	1385 ± 142 <sup>#</sup>
COF (mL·min <sup>-1</sup> )	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 <sup>#</sup>	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 <sup>#</sup>	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; <sup>#</sup>*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  $116 \pm 44\%$  and  $127 \pm 58\%$  of the baseline value, respectively.

In another set of experiments, hearts ( $n = 6$  per group) were paced at  $300 \text{ beats} \cdot \text{min}^{-1}$  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  $65 \pm 16\%$  of the baseline value in the control group and to  $94 \pm 8\%$  and  $91 \pm 8\%$  of baseline value in hearts preincubated with 25 and 50  $\mu\text{M}$  propofol, respectively. Propofol (25 and 50  $\mu\text{M}$ ) 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  $122 \pm 62\%$  of the baseline value in the control group. In hearts pretreated with 25 and 50  $\mu\text{M}$  propofol, COF was restored to  $85.5 \pm 18\%$  and  $92.3 \pm 16\%$  of the baseline value, respectively.

*Propofol shortens APD during early reperfusion (Figure 2)*  
Before ischemia, the  $\text{APD}_{90}$  among control, 25, and 50  $\mu\text{M}$  propofol groups did not significantly differ ( $P = 0.31$ ). The  $\text{APD}_{90}$  of the control group was similar to the baseline value at five minutes of reperfusion. At five minutes of reperfusion, propofol (25 and 50  $\mu\text{M}$ ) significantly shortened  $\text{APD}_{90}$  ( $P = 0.033$  and  $P = 0.014$ , respectively). At 35 min of reperfusion,  $\text{APD}_{90}$  was similar to the baseline value in both propofol groups and did not significantly differ among all groups ( $P = 0.18$ ) (Table III).

*Propofol does not exert cardioprotective effects by opening  $K_{\text{ATP}}$  channels*

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

Prior incubation with 25 and 50  $\mu\text{M}$  propofol significantly reduced infarct size as compared with con-

TABLE III Summary of action potential duration

	Treatment	Re-5	Re-35
APD90 (msec)			
CONT	$152 \pm 7$	$145 \pm 13$	$145 \pm 7$
P25	$145 \pm 11$	$125 \pm 21^{\#}$	$153 \pm 13$
P50	$144 \pm 12$	$121 \pm 13^{\#}$	$143 \pm 7$

Values are mean  $\pm$  SD ( $n = 6$  per group). Re-5 = five minutes after reperfusion; Re-35 = 35 min after reperfusion. CONT = control group; P-25 = 25  $\mu\text{M}$  propofol; P-50 = 50  $\mu\text{M}$  propofol;  $\text{APD}_{90}$  = action potential duration at 90% repolarization.  $\#P < 0.05$  vs CONT.

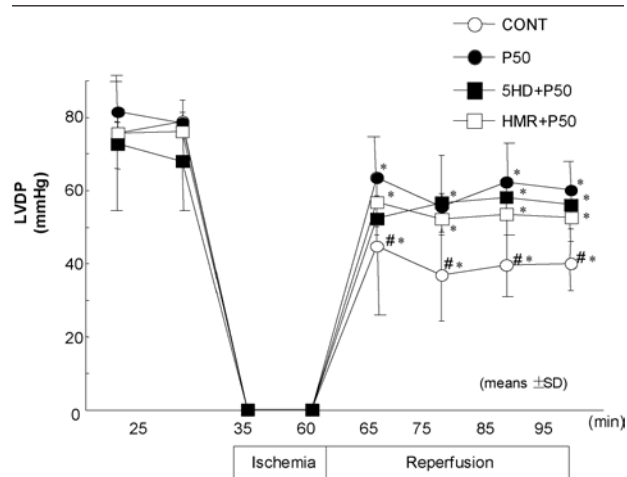


FIGURE 3 Effects of  $K_{\text{ATP}}$  channels on functional recovery induced by propofol

Hearts were stabilized by perfusion with Krebs-Ringer solution for 25-min, incubated with 500  $\mu\text{M}$  5-HD or 30  $\mu\text{M}$  HMR1098 for five minutes, 50  $\mu\text{M}$  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  $\mu\text{M}$  propofol group; 5HD = 5-hydroxydecanoate; HMR = HMR1098. Values are means  $\pm$  SD ( $n = 6$  per group). \*,  $P < 0.05$  vs corresponding baseline value;  $\#P < 0.05$  vs P50 group.

rol hearts ( $P < 0.0001$ ). Such protection induced by 50  $\mu\text{M}$  propofol was not blocked by either 500  $\mu\text{M}$  5-HD or 30  $\mu\text{M}$  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-3 $\beta$  Ser9 residue*

At five minutes of reperfusion, propofol (25 and 50  $\mu\text{M}$ ) significantly increased levels of phospho-GSK-3 $\beta$  compared to the control group ( $P = 0.038$  and  $P = 0.019$ , respectively) (Figure 4). The ratios of phospho-GSK-3 $\beta$ / total GSK-3 $\beta$  were  $0.27 \pm 0.17$  in the control group and  $0.72 \pm 0.32$  and  $0.79 \pm 0.47$  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  $\mu$ M propofol group; P-50 = 50  $\mu$ M propofol; P-CONT = control with pacing; P-P25 = 25  $\mu$ M propofol with pacing; P-P50 = 50  $\mu$ M propofol with pacing; 5-HD = 5-hydroxydecanoic acid; HMR = HMR1098. # $P < 0.05$  vs CONT.

groups pre-incubated with 25 and 50  $\mu$ M propofol, respectively.

### Discussion

We found that 25 and 50  $\mu$ M 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-3 $\beta$  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-3 $\beta$  inhibition by activation of the  $K_{ATP}$  channel.

In the current study, propofol (25 and 50  $\mu$ M) 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.<sup>3,4</sup> Ko *et al.*<sup>3</sup> demonstrated that propofol (30 and 100  $\mu$ M) improved the recovery of LVDP and  $dP/dt$  and significantly lowered LVEDP in isolated rat hearts. Kokita *et al.*<sup>4</sup> reported that propofol (25 and 50  $\mu$ M) 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 obvi-

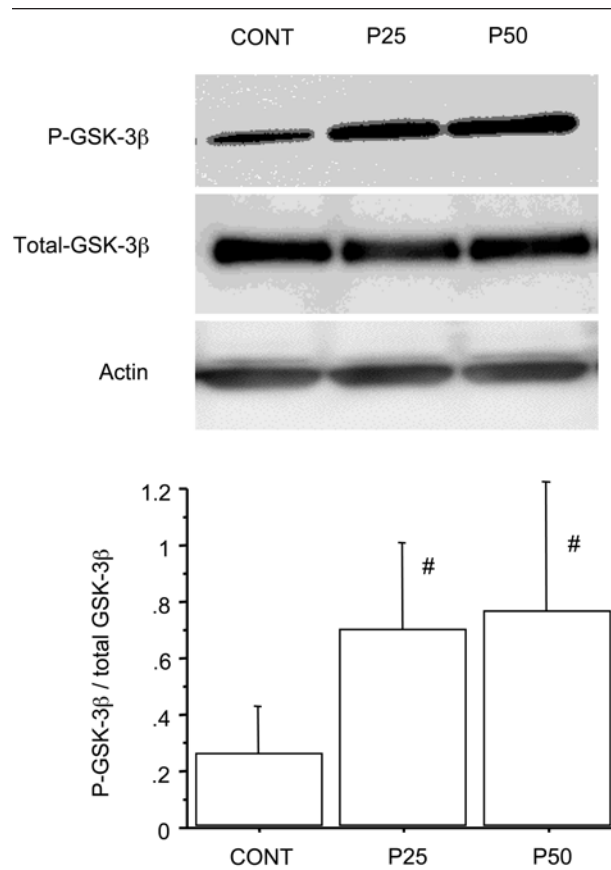


FIGURE 4 Effects of propofol on phosphorylation of Ser<sup>9</sup> residue on GSK-3 $\beta$  in ischemia-reperfused hearts. Upper and lower panels show representative immunoblots and summarized phospho-GSK-3 $\beta$  (p-GSK-3 $\beta$ ) levels, respectively. Levels of GSK-3 $\beta$  phosphorylation in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

ously important in various cardioprotective mechanisms, for example, preconditioning,<sup>10,11</sup> 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.<sup>20-22</sup> Vincent *et al.*<sup>23</sup> 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.*<sup>24</sup> 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.*<sup>25</sup> reported that



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.*<sup>26</sup> 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  $\mu$ M 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.<sup>27-29</sup> 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  $\mu$ M propofol significantly shortened APD<sub>90</sub> at five minutes of reperfusion. Prior incubation with HMR1098 did not affect the ability of 50  $\mu$ M 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.<sup>5,30</sup> 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.<sup>7-9</sup> Wickley *et al.*<sup>9</sup> recently found that propofol stimulates PKC- $\alpha$ , PKC- $\delta$ , PKC- $\epsilon$ , and PKC- $\zeta$  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<sup>2+</sup> overload are several key factors involved in conferring myocardial protection through medication. Fang *et al.*<sup>31</sup> reported that PKC activation induces GSK-3 $\beta$  phosphorylation, and that PKC- $\delta$  interacts with GSK-3 $\beta$  in several cell lines.<sup>31,32</sup> In contrast, PKC $\epsilon$  might indirectly regulate GSK through phosphatidylinositol-3-kinase (PI3K), because the induction of a dominant-negative PKC $\epsilon$  blocks the activity of PI3K induced by

phorbol ester and insulin.<sup>33</sup> Therefore, the selective PKC isoform responsible for modulating GSK-3 $\beta$  remains an area of interest for future studies.

A recent study has shown that GSK-3 $\beta$  mediates the convergence of myocyte protection signalling through inhibiting the opening of mPTP.<sup>34</sup> Reperfusion injury salvage kinases afford protection against ischemia and phosphorylate GSK-3 $\beta$  during reperfusion.<sup>13,35</sup> Pharmacological GSK-3 $\beta$  inhibition reduces infarct size and improves postischemic function.<sup>13</sup> Opioids induce cardioprotection by inactivating GSK-3 $\beta$  at Ser<sup>9</sup>,<sup>36</sup> and GSK inhibition enhances the protective effect of isoflurane-induced post-conditioning via an mPTP-dependent mechanism *in vivo*.<sup>37</sup> The present study found that propofol (25 and 50  $\mu$ M) reduced infarct size and increased levels of p-GSK-3 $\beta$ . Thus, GSK-3 $\beta$  inhibition by phosphorylation at the N-terminal serine residue, Ser<sup>9</sup>, might act as a central mediator of the cardioprotective effects of propofol. Sztark *et al.*<sup>38</sup> reported that propofol might directly inhibit mPTP at concentrations above those applied clinically. Javadov *et al.*<sup>39</sup> 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-3 $\beta$ , due to the mitochondrial localization of GSK-3 $\beta$ , remain unknown. However, our data showed that propofol enhances GSK-3 $\beta$  phosphorylation upon reperfusion, suggesting that propofol inactivates GSK-3 $\beta$  during this process. These data also suggest that GSK-3 $\beta$ , 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-3 $\beta$  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-3 $\beta$  plays a critical role in myocardial protection against ischemia-reperfusion injury. Selective inhibition of GSK-3 $\beta$  mimicked the beneficial actions of ischemic preconditioning<sup>13</sup> and opioid-induced myocardial protection during reperfusion.<sup>36</sup> Further study is required to clarify the relationships between the cardioprotective effects of propofol and GSK-3 $\beta$  phosphorylation. However, we believe that we are the first to assess the effects of propofol on GSK-3 $\beta$  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%,<sup>40</sup> 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  $\mu\text{M}$  and stable levels at approximately 10–25  $\mu\text{M}$  during maintenance infusion.<sup>41</sup> Additional factors such as speed of injection, volume of distribution, and *pH* 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  $\text{K}_{\text{ATP}}$  channel blockers did not interfere with these benefits. Furthermore, we demonstrated that a propofol-induced cardioprotective effect is involved in the inhibition of GSK-3 $\beta$ .

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