A Brief Regional Ischemic-reperfusion Enhances Propofol-induced Depression in Left Ventricular Function of in situ Rat Hearts

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Background: Propofol is short-acting intravenous general anesthetics that reduces cardiovascular hemodynamics. The effects of propofol on intrinsic myocardial contractility, however, remain debatable. The aim of the current study was to test the hypothesis that inhibitory effects of propofol on left ventricular (LV) contractility and mechanical work capability of in situ ejecting rat hearts are attenuated after a brief regional ischemia and reperfusion.

Methods: The authors obtained steady-state LV pressure-volume loops and intermittently obtained LV end-systolic pressure-volume relation and evaluated effects of propofol on LV function by end-systolic pressure-volume area (PVA_{mLVV}) at midrange LV volume (mLVV).

Results: Propofol (5.2 ± 0.3–11.1 ± 3.7 μg·ml⁻¹) significantly decreased ESP_{mLVV} to 78 ± 12%–64 ± 13% of prepropofol and PVA_{mLVV} to 76 ± 13%–63 ± 16% of prepropofol in normal hearts, whereas propofol at a lower concentration (4.1 ± 1.0 μg/ml) did not. Although brief ischemic-reperfusion per se did not affect LV function, propofol after that, even at a lower concentration (4.1 ± 1.0 μg/ml), significantly decreased ESP_{mLVV} to 70 ± 27% of prepropofol and PVA_{mLVV} to 68 ± 33% of prepropofol. Pretreatment with a protein kinase C (PKC) inhibitor, bisindoylmaleimide reduced the propofol (4.1 ± 1.0 μg/ml)-induced greater decreases in ESP_{mLVV} and PVA_{mLVV} after brief ischemic-reperfusion to 94 ± 33% and 92 ± 39% of prepropofol. In the propofol-infused hearts after brief ischemic-reperfusion, protein kinase C-ε translocation to the nucleus-myofibril fraction was found.

Conclusion: In contrast to the study hypothesis, brief ischemic-reperfusion enhanced the inhibitory effects of propofol on LV systolic function; this enhancement is attributable to activation of protein kinase C.

Propofol is frequently used as a short-acting intravenous general anesthetic. However, the effects of propofol on intrinsic myocardial contractility remain debatable.1 No cardiac protective effects2 and concentration-dependent cardiac protective effects3,4 of propofol against various myocardial reperfusion injuries have been reported in isolated rat hearts. In clinical settings, propofol anesthesia should be available without any risks for patients with various past histories such as brief or long ischemia and reperfusion. It is especially important to clarify how a brief regional ischemia and reperfusion modulate propofol-induced actions on cardiac function. If cardiac function appeared normal after a brief regional ischemia and reperfusion, such past history might be overlooked.

Propofol causes a negative inotropic effect in rat ventricular myocytes under baseline conditions as well as during activation of β-adrenoreceptors. In the latter case, the site of action of propofol involves the activation of protein kinase C (PKC)-α.5 Recent studies have demonstrated that propofol activated PKC in rat cardiomyocytes results in phosphorylation of several myofibrillar proteins such as myosin light chain 2 and troponin I and an increase in myofilament Ca²⁺ sensitivity.6 Propofol increased myofilament Ca²⁺ sensitivity, at least in part by increasing pH via PKC-dependent activation of Na⁺/H⁺ exchange.7 Therefore, PKC activation can trigger several cellular mechanisms that can increase myofilament Ca²⁺ sensitivity, resulting in changes in cardiac inotropy and lusitropy. On the other hand, it is known that brief ischemia initiates events that lead to PKC activation and protection against a longer ischemia.

The aim of the current study was to test the hypothesis that a brief regional ischemia and reperfusion associated with no apparent changes of cardiac function offsets the negative inotropic action of propofol via PKC-dependent pathway on left ventricular (LV) function of in situ ejecting rat hearts. For evaluating LV contractility and work capability, we used the end-systolic pressure-volume relation (ESPVR)-systolic pressure-volume area (PVA) framework, i.e., LV ESP (ESP_{mLVV}) and PVA (PVA_{mLVV}) at midrange LVV (mLVV).8,9

Materials and Methods

LV Volumetric Conductance Catheter System

We used the previously developed type of a miniaturized 3-French conductance catheter and a conductance catheter signal processing apparatus (S1 Medico-tech Co., Ltd., Osaka, Japan) for rats.10 The summed conductance G (t) of the segmental conductance (G_1, G_2, and G_3) is theoretically proportional to the LV blood volume.

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Instantaneous intraventricular conductance volume $V(t) = [V_1(t) + V_2(t) + V_3(t)]$ is obtained from the measured conductance $G(t)$. Absolute blood volume is obtainable by subtracting a constant offset volume $V_c$.

**Surgical Preparation**

The investigation conformed with the Guiding Principles for the Care and Use of Animals approved by the Council of the Physiologic Society of Japan (Tokyo, Japan).

Retired breeder male Wistar rats ($n = 32$) weighing 400–500 g were anesthetized with pentobarbital sodium (50 mg/kg intraperitoneally). Additional doses of pentobarbital sodium (10–30 mg/kg intraperitoneally) were administered as needed, resulting in constant anesthetic throughout the experiment. The trachea was intubated, and each rat was ventilated with room air ($FIO_2 = 0.21$) to maintain $P_{O_2}$, $P_{CO_2}$, and $pH$ approximately 7.4 by adding supplemental O$_2$ or by changing the respiratory rate as needed. The chest was opened via a midline sternotomy and the pericardium was dissected to expose the heart. A conductance catheter (custom-made by Inter Medical Co., Ltd., Tokyo, Japan) was inserted into the LV through an apical stab. The positioning of this catheter is critical to obtain reliable LVV data, i.e., synchronous segmental conductance volume changes.$^8$–$^{11}$

A 2.5-French catheter-tip micromanometer (Millar Instruments, Houston, Texas) was also inserted through the apex into the LV. A polyethylene tube (3-French) was inserted into the external jugular vein for intravenous injection of the drug. A string occluder was placed loosely around the ascending aorta. The respirator was stopped during data acquisition to avoid respiratory fluctuation of cardiac signals. After 3-min occlusion of the left anterior descending coronary artery to perform a brief regional ischemia, the heart was reperfused for 60 min (bIR).

**Experimental Protocols**

At first, specific resistivity of sampled blood was measured as previously reported.$^8$–$^{11}$ When hemodynamics were stable, a series of LV P-V loops was obtained during increasing afterload by a gradual ascending aortic occlusion within a few seconds to evaluate LV ESPVR.$^8$–$^{11}$ After collecting the baseline P-V data, we continuously infused propofol via the polyethylene tube inserted in the external jugular vein with syringe pump. From the initiation of experiment, it takes 1 h to start propofol infusion. The animals were divided into four groups (I, II, IV, and V, each $n = 6, 10, 10$, and 6, respectively), as shown in fig. 1A. In group II, propofol was infused at 40 mg·kg$^{-1}$·h$^{-1}$ for 30 min. In group IV, 60 min after bIR propofol was infused at 40 mg·kg$^{-1}$·h$^{-1}$ for 30 min. In group V, pretreatment with a broad range protein kinase C inhibitor (PKC-I, bisindolylmaleimide (Sigma Chemical, St. Louis, MO) 1 mg/kg intravenously$^{12}$ was performed 10 min before bIR, and 60 min after bIR, propofol was infused at 40 mg·kg$^{-1}$·h$^{-1}$ for 30 min. Propofol 1% intralipid emulsion is commercially available (AstraZeneca K.K., Osaka, Japan). Previous study revealed that there was no effect of the lipid emulsion in which propofol is dissolved.$^{15}$

The data collection was performed before and 10, 20, and 30 min after starting of infusion of propofol in the II, IV, and V groups and 10, 20, 30, 45, and 60 min after bIR in the IV and V groups to investigate the effect of bIR per se (IV) and the effect of PKC-I and bIR per se (V) on LV mechanical and hemodynamic parameters. Group I ($n = 6$).
6) was time-control without any intervention and thus the data collection was performed for 90 min, indicating that ESP, PVA, effective arterial elastance (Ea), end-diastolic volume (ESV), end-dia-stolic volume (EDV), stroke volume (SV), and heart rate were not significantly different.

At the end of the experiment, the rat blood was sampled and stored at −80°C (n = 11) for measurement of blood concentration of propofol. Propofol blood concentration was measured by high-pressure liquid chromatography. Each blood concentration of propofol at 10, 20, and 30 min after infusion was 4.1 ± 1.0 µg/ml, 5.2 ± 0.3 µg/ml, and 11.1 ± 3.7 µg/ml. Therefore, the elapsed time indicates the approximate blood concentration of propofol, although the relation between the time and concentration of propofol was not proportional.

In the final part of each experiment, Vc was measured by injecting hypertonic saline (10% NaCl solution; 0.020–0.025 ml) into the pulmonary artery to change transiently the resistivity of the blood in the LV. The LV and the three individual segmental conductance volume signals were digitized in a 12-bit accuracy at a sampling frequency of 500 Hz and stored in a computer system (PC-9801 FA; NEC, Tokyo, Japan) for later analyses.

At the end of each experiment, each rat was injected with a lethal dose of pentobarbital sodium. The LV including the interventricular septum was excised and weighed after both the atria and right ventricular free wall were trimmed off. The LV of the rats weighed 0.718 ± 0.054 g, ranging between 0.818 and 0.615 g. These LV weights were used to normalize LVV by 1g of LV myocardium in individual hearts.

**Data Analysis**

In rat LV in situ, a curvilinear ESPVR is obtained by drawing an upper enveloping curve on a series of P-V loops in a manner similar to our previous method.8,9 The LV ESP-V data on the left-upper shoulder of all the P-V loops were plotted and fitted by the method of the least-squares using the exponential equation.8,9 We obtained the best-fit ESPVR curve in each of the 30 hearts.

LV PVA is a measure of the total mechanical energy generated by an LV contraction.14 PVA of an isovolumic contraction represents the maximal capability of exter-nal mechanical work of the LV at a given preload.15 In the current study, PVA was defined as the area in the P-V relation diagram surrounded by the already determined best-fit ESPVR curve, the volume axis (instead of negligibly small end-diastolic P-V relation curve), and the vertical isovolumic P-V line at any preloaded LVV (figs. 1B and 1C). Exclusion of the end-diastolic P-V relation curve measure might affect the interpretation of data for ESP and PVA in in situ hearts, when a lusitropic effect on diastolic function is predicted. However, as shown in figure 2, end-diastolic P-V relation curves are very close to the volume axis and thus the influence by the exclusion might be very small. PVA as a function of LVV was obtained by integrating the exponential function from the extrapolated Vo along the volume axis.8,9 LVV ranged between Vo and approximately 0.26 ml/g.

ESPmlLV on the curvilinear ESPVR is preferable to evaluate the shift of the ESPVR.8,9,11 PVAmlLV on the curvilinear PVA-V relations is preferable to evaluate the upward and downward shifts of the PVA-V relations and hence the increase and decrease in LV work capabili-ty8,9,11 (fig. 1B). In the current study, we chose a value of 0.08 ml/g as mlLV as in a previous study9 because the mean value of [Vo + (maximum ESV − minimum ESV) on the ESPVR × 1/2] was approximately 0.079 ± 0.015 ml/g. The mean V0 value was 0.017 ± 0.011 ml/g. There were no significant differences among the four groups (I, II, IV, and V).

ESP at ESV (ESPESV) was specifically defined to differentiate from ESPmlLV. Ea is defined as the ESP/SV ratio of the LV under stable hemodynamics,16 and thus Ea de-pends on SV. SV was obtained by (LV EDV − LV ESV) (fig. 1B). Ejection fraction (EF) was obtained by SV/(LV EDV − Vo).

In situ Hybridization Histochemistry

The heart from the group I (time-control, n = 3) and group III (bIR, n = 8) (fig. 1A) was rapidly removed and immediately frozen using powdered dry ice within 1 min. Frozen sections, 10 µm thick, were cut in a cryo-stat, thaw-mounted on silane-coated slides, and stored at

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**Fig. 2. Representative left ventricular (LV) pressure (P)-volume (V) loops and end-systolic pressure-volume relations (dotted curves) during an aortic occlusion in ejecting in situ hearts without (A and B) and with brief ischemia and reperfusion (bIR) (C and D). (A) and (C) Prepropofol infusion (Pre). (B) and (D) 30-min propofol infusion at 40 mg·kg⁻¹·h⁻¹, Volume axes indicate normalized absolute LV volume for 1 g LV mass. Vo value was not subtracted from LV volume value.**
−80°C until use. They were fixed in 4% paraformaldehyde/0.1M phosphate buffer pH 7.4 for 15 min at room temperature, rinsed in 2 × standard saline citrate, and then dried with a graded ethanol series. After drying, the slides were stored at −80°C until hybridized.

The probe sequences were as follows: heat shock protein (Hsp) 70 (30-mer), complementary to nucleotides 621–892 of rat Hsp70 mRNA; 17 Hsp27 (40-mer), complementary to nucleotides 621–660 of rat Hsp27 mRNA.18 Computer-assisted homology search revealed no identical sequences in any genes in the GenBank database. The probes were labeled with 35S-DATP using terminal deoxynucleotidyl-transferase (Takara, Otsu, Japan). The specific activity of each probe was 5–10 × 108 cpm/μg. Excess (>100) amounts of nonlabeled probes completely eliminated the hybridization signals for their respective mRNAs to show that these signals were specific. Sections were hybridized overnight at 37°C in 100 μl of buffer containing 4 × standard saline citrate, 50% formamide, 0.12 M phosphate buffer, 0.1 M dithiothreitol with 5% dextran sulfate, and 0.1 M sodium dodecylsulfate, 250 μg/ml yeast tRNA, 10% dextran sulfate, and 0.1 M dithiothreitol with 5 × 108 cpm of labeled probe per slide. After hybridization, they were washed four times for 20 min at 55°C in 1 × standard saline citrate, immersed briefly in distilled water, and dehydrated with a graded ethanol series and then dried. Film autoradiography was performed using Bioimaging-analyzer BAS2500 (Fuji Film, Tokyo, Japan). All the slides for the same probe were processed simultaneously.

Immunoblotting and Quantification of Protein Kinase C

To localize PKC isoforms of the LV free wall (n = 6 each) in group I (time-control), group II (propofol-infusion), and group IV (bIr and propofol-infusion) (fig. 1A), Western blotting of the subcellular fractions (S and P1 and P2) was performed as previously described12,19 by the use of anti-PKC-α, β, γ, δ, and ε antibodies (Transduction Laboratory, Inc., Lexington, KY), anti-PKCζ antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), and the secondary antibodies (Promega Co., Madison, WI and Amersham Pharmacia Biotec Ltd., Buckinghamshire, UK).

Statistical Analysis

All data were expressed as mean ± SD. Statistical comparisons were performed using one-way analysis of variance with repeated measures and post hoc Dunnett test and one-way analysis of variance with post hoc Fisher protected least significant difference test. In all statistical tests, P values < 0.05 were considered significant.

Results

Figure 1B shows each control series of LV P-V loops of an in situ rat heart while changing afterload by a gradual aortic occlusion. Ea is obtainable from steady-state P-V loops. We drew enveloping curves on the series of P-V loops as curvilinear baseline ESPVR in normal rats. Then, we obtained ESP_{mlLV} and PVA_{mlLV} (fig. 1C). We obtained similar results in the other 31 rat hearts.

Comparison of ESPVR between Group II and Group IV

Although each ESPVR described in a low LVP range appears to be linear, the ESPVR is virtually curvilinear in a wide LVP range in control and during propofol infusion (fig. 2). After 30 min of onset of propofol infusion, an enveloping curve appeared to shift downward from an enveloping curve in prepropofol infusion (figs. 2A and 2B), whereas an enveloping curve appeared not to shift from the control during saline infusion (data not shown). An ESPVR appeared not to change at all after bIr for 60 min (fig. 2C) but it more markedly shifted downward after 30 min of propofol infusion (fig. 2D) than in a heart without bIr (fig. 2B).

Effects of Propofol on LV Mechanics and Hemodynamics in Groups II, IV, and V

Mean ESP_{0.08} and mean PVA_{0.08} in group II were not significantly reduced at 10-min (P10) propofol infusion
Table 1. (Continued)

<table>
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<th>P10</th>
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<th>P30</th>
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<th>P10</th>
<th>P20</th>
<th>P30</th>
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<td>100 ± 39</td>
<td>69 ± 26†</td>
<td>51 ± 19†</td>
<td>141 ± 28</td>
<td>132 ± 48</td>
<td>100 ± 42</td>
<td>92 ± 33†</td>
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<td>3.45 ± 1.84*</td>
<td>2.37 ± 1.28†</td>
<td>1.66 ± 0.94†</td>
<td>4.79 ± 0.85</td>
<td>3.96 ± 1.59</td>
<td>3.36 ± 1.97</td>
<td>2.53 ± 1.03*</td>
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<td>0.081 ± 0.047†</td>
<td>0.091 ± 0.046†</td>
<td>0.097 ± 0.045†</td>
<td>0.035 ± 0.007</td>
<td>0.057 ± 0.039</td>
<td>0.066 ± 0.045</td>
<td>0.075 ± 0.060</td>
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<td>0.251 ± 0.092</td>
<td>0.233 ± 0.097</td>
<td>0.226 ± 0.107</td>
<td>0.161 ± 0.018</td>
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<tr>
<td>0.150 ± 0.030†</td>
<td>0.122 ± 0.003</td>
<td>0.109 ± 0.041</td>
<td>0.126 ± 0.013</td>
<td>0.111 ± 0.026</td>
<td>0.102 ± 0.017</td>
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<td>77.7 ± 11.2†</td>
<td>73.5 ± 10.6†</td>
<td>68.7 ± 11.3†</td>
<td>91.0 ± 4.52</td>
<td>86.5 ± 9.45</td>
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<td>444 ± 128</td>
<td>409 ± 93*</td>
<td>373 ± 91*</td>
<td>476 ± 81†</td>
<td>537 ± 117†</td>
<td>525 ± 97†</td>
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<td>408 ± 40</td>
<td>372 ± 65</td>
<td>336 ± 61</td>
<td>324 ± 56*</td>
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</table>

bIR: brief ischemic-reperfusion. Ea: effective arterial elastance. EDV: end-diastolic volume. EF: ejection fraction. ESP$_{0.08}$: end-systolic pressure at mLV (r = 0.08 ml/g). ESV: end-systolic volume. HR: heart rate. P10, P20, and P30: 10-, 20-, and 30-min propofol infusion. PKC-I: protein kinase C inhibitor. Pre-bIR: pre-brief ischemic-reperfusion. Pre-P: pre-propofol. PVA$_{0.08}$: systolic pressure-volume area at mLV (r = 0.08 ml/g). Time-course from Pre-bIR to bIR60 in Group IV corresponds to that in Group III. * $P < 0.05$ versus Pre-P. † $P < 0.05$ versus II. The comparison between the group IV and V is omitted.

but were significantly reduced at 20-min (P20) and 30-min propofol infusion (P30). Although mean ESP$_{0.08}$, PVA$_{0.08}$, Ea, and EF did not change for 60 min after bIR (bIR10 ~ bIR60) (although EF value was originally large in the rat LV), mean PVA$_{0.08}$ in group IV was significantly reduced at P10, P20, and P30 versus prepropofol (Pre-P), and mean ESP$_{0.08}$ and mean PVA$_{0.08}$ in group IV were significantly reduced at P20 and P30 versus corresponding values in group II. Pretreatment with PKC-I and bIR did not affect mean PVA$_{0.08}$ (4.4 ± 1.2 ~ 4.8 ± 0.9 mmHg·ml·bear$^{-1}$·g$^{-1}$) and mean EF (91 ± 5%) for 60 min. The following propofol significantly reduced mean PVA$_{0.08}$ only at P30 from Pre-P in group V but did not significantly reduce it compared to P30 of group II. Mean EF was significantly decreased at P20 and P30 from Pre-P in group II and was significantly decreased at P10, P20, and P30. Significant percentage differences but no expression of Hsp70 and Hsp27 mRNA was observed in LV free walls of three hearts without bIR (group IV, fig. 1A), and PVA$_{0.08}$ (to 78 ± 12% of prepropofol and PVA$_{0.08}$) and ESP$_{0.08}$ (76 ± 13% ~ 16% of prepropofol in normal hearts, whereas propofol at a lower concentration (4.1 ± 1.0 µg/ml) did not. Mean percentage values of PVA$_{0.08}$ and ESP$_{0.08}$ at P10, P20, and P30 to each prepropofol control were significantly reduced at P10, P20, and P30 in group IV compared with those in group II but not in group V (table 1).

There were no significant differences in mean Ea (reflecting total peripheral resistance) at P10, P20, and P30 in group II, but mean Ea at P20 and P30 in group IV, although not in group V, was significantly reduced from Pre-P. Mean end-diastolic volume did not show any differences but mean ESV increased significantly at P10, P20, and P30 in group IV compared with those in group II. Mean SV significantly decreased from each Pre-P at P10, P20, and P30 in the three groups except for P10 and P20 and group V but did not differ among the three groups. Although heart rate significantly decreased at P30 or P20 in either group, it was maintained within the physiologic range (table 1).

**Comparison of Mean Percentage Values of PVA$_{0.08}$ and ESP$_{0.08}$ among the Three Groups (II, IV, and V)**

Mean percentage values of PVA$_{0.08}$ and ESP$_{0.08}$ at P20 and P30 to each propofol control in group II were significantly smaller than prepropofol control, indicating that propofol (5.2 ± 0.3 µg/ml ~ 11.1 ± 3.7 µg/ml) significantly decreased ESP$_{0.08}$ to 78 ± 12% ~ 64 ± 13% of prepropofol and PVA$_{0.08}$ to 76 ± 13% ~ 16% of prepropofol in normal hearts, whereas propofol at a lower concentration (4.1 ± 1.0 µg/ml) did not. Mean percentage values of PVA$_{0.08}$ and ESP$_{0.08}$ at P10, P20, and P30 to each prepofol control were significantly smaller in group IV (bIR + propofol) than those in group II (propofol), indicating that propofol after bIR even at a lower concentration (4.1 ± 1.0 µg/ml) significantly decreases ESP$_{0.08}$ (to 70 ± 27% of prepropofol) and PVA$_{0.08}$ (to 68 ± 33% of prepropofol) (fig. 3). Although these mean percentage values in group V were significantly larger than those in group IV only at P30, they did not significantly differ from those in group II at P10, P20, and P30 (fig. 3). This indicates that pretreatment with PKC-I decreased the greater negative inotropic effects of propofol induced by bIR to the original propofol effect.

In situ Hybridization Histochemistry

The marked expression of Hsp70 (n = 3 of 8) and Hsp27 (Hsp27) mRNA (n = 4 of 8) was identified in eight LV free walls of eight hearts with bIR (group III, fig. 1A), but no expression of Hsp70 and Hsp27 mRNA was observed in LV free walls of three hearts without bIR (group I, fig. 1A, fig. 4). This indicated that even brief (3-min) regional ischemia and reperfusion (bIR) was effective to express Hsp70 and Hsp27 mRNA and the expression was maintained for 90 min, although the expression was not found in the remaining hearts with bIR.

**Effects of Propofol Alone or bIR + Propofol on PKC-α, β, γ, δ, ε, ξ Translocation**

Figure 5 showed each representative set of Western blotting of PKC-α, PKC-δ, and PKC-ε in P1 (nucleus-myofibril), P2 (membrane), and S (cytosol) fractions of the group I (time-control), II (propofol), and IV (bIR + propofol). The amount of PKC-ε expression in P1 fraction moderately increased only in group IV but not in
group II compared with group I (lowermost panel in fig. 5), whereas the amount of expressions of PKC-α and PKC-δ did not differ among groups I, II, and IV (uppermost and middle panels in fig. 5).

Summarized data are shown in figure 6. The mean amount of expression of PKC-ε in P1 fraction was significantly larger in group IV compared with those in group I ($P < 0.05$) and in group II ($P < 0.05$), although the mean amount of expression of PKC-ε in S fraction was not significantly smaller in group IV. PKC-ε seems to be translocated to the nucleus and myofibril. There was no difference in the mean amount of expressions of PKC-ε in P1 and S fractions between groups I and II. The amounts of expressions of PKC-γ and PKC-ζ in all fractions were not significantly different among groups I, II, and IV (data not shown). The expression of PKC-β was not detected.

**Discussion**

The most striking finding in the current study was that propofol significantly more intensely depressed LV systolic function at a lower concentration ($4.1 \pm 1.0 \mu g/ml$) after 3-min regional ischemia and 60-min reperfusion.
(bIR) than in the hearts without bIR, although 3-min regional ischemia per se did not affect LV systolic function and hemodynamics for 60-min reperfusion. This finding did not support our hypothesis that a brief regional ischemia and reperfusion associated with no apparent changes of cardiac function offsets the negative inotropic action of propofol via PKC-dependent pathway on LV function of in situ ejecting rat hearts, although the presumed PKC-dependent pathway contributed to the current results.

The shape of ESPVR in the rat LV was an upward convex curve. We cannot use Es (maximal elastance) to evaluate LV contractility; instead we used ESP$_{0.08}$ and PVA$_{0.08}$ in rat hearts. Moreover, PVA closely and linearly correlates with cardiac oxygen consumption per beat under a variety of loading conditions in a stable contractility in the cross-circulated rat hearts. Therefore, the PVA$_{0.08}$ can reasonably be used as a measure of total mechanical energy as well as work capability as a function of preload of a ventricular contraction even under a nonlinear ESPVR in a rat LV.

Although it has been reported that even at clinically relevant concentrations (1~2 μg/ml) propofol was not cardiodepressant in isolated rat hearts, a negative inotropic effect of propofol can be observed at concentrations higher than 5.2 ± 0.3 μg/ml in the current study. The negative inotropic action of propofol is mediated mainly by inhibition of transsarcolemmal Ca$^{2+}$ influx accompanied by shortening of action potential duration in isolated rat and guinea pig papillary muscles, inhibition of Ca$^{2+}$ uptake capacity of sarcoplasmic reticulum in homogenates of rat ventricles, or decreasing cytosolic free Ca$^{2+}$ in isolated rat myocardial cells.

On the other hand, the cardioprotective effects of propofol remain debatable. A clinically relevant concentration (1~2 μg/ml) of propofol had no protective effect against myocardial reperfusion injury by 30-min regional ischemia and 2 h reperfusion in isolated rat hearts.

Propofol at 4.4~17.7 μg/ml attenuated mechanical, biochemical, and histologic injurious changes caused by 15~25-min global ischemic and 30~30-min reperfusion in isolated rat hearts.

In the current study, it was confirmed that 3-min regional ischemia per se induced no detectable cardiac mechanical and hemodynamic changes during the following 60-min reperfusion (bIR) by measuring an intermittent ESPVR and continuous P-V loops (group IV, table 1). Propofol showed the greater inhibitory effect on LV contractions and mechanical work capability after bIR. A PKC-I decreased this greater effect of propofol after the bIR, indicating that PKC-dependent pathway is involved in this mechanism.

It might be possible that the bIR activates PKC and p38 mitogen-activated protein kinase (p38MAPK), thereby inducing Hsp genes. We revealed that 3-min brief ischemia can induce the genes for Hsp27 and Hsp70 without beneficial effects on LV function, although in the short-term current study we could not detect expressions of any heat shock proteins.

A recent article by Kanaya et al. reported that 10-min propofol (30 μl) treatment to cardiac cells caused a dramatic translocation of PKC-α from cytosolic to membrane fraction (corresponding to P1 and P2 in the current study), but the amount of expression of PKC-α did not differ among groups I, II, and III at all in the current study. This discrepancy may be attributable to the difference in actions of propofol in in vitro cardiac cells and in vivo whole hearts.

Furthermore, it has been reported ischemic-preconditioning is mediated by translocation of PKC-δ and PKC-ε and that anesthetic-preconditioning is mediated by PKC-δ but not by PKC-δ, both of which are members of the Ca$^{2+}$-independent, diacylglycerol-activated novel...
PKC subfamily.\(^{31,32}\) Although we assumed that the translocation of PKC-\(\delta\) or PKC-\(\varepsilon\) would occur in either bIR or propofol infusion, neither propofol nor bIR caused translocation of any PKC isoforms.

However, the combination of bIR and propofol-infusion caused the translocation of only PKC-\(\varepsilon\) to the nucleus and myofibrils (P1) fraction. It has been reported that ischemia-reperfusion induced the PKC-\(\varepsilon\) translocation to the myofilaments,\(^{33}\) thereby enhancing the phosphorylation in troponin-I, troponin-T, and troponin-C.\(^{34,35}\) In these studies, phorbol esters have been shown to inhibit contractility through PKC-induced phosphorylation of troponin-I, troponin-T, and troponin-C, resulting in the reduced actomyosin MgATPase activity.\(^{34,35}\) Therefore, it is plausible that bIR and propofol synergistically induced PKC-\(\varepsilon\)-mediated phosphorylation of troponin-I, troponin-T, and troponin-C, which causes contractile depression mediated via reduced actomyosin MgATPase.

Limitations of the Current Study
We studied in situ ejecting rat hearts, which are much more physiologic than the excised cross-circulated isovolumically contracting hearts, but the myocardium suffered from some injuries by LV catheterization. The effect of propofol on myocardium shows species differences and thus we cannot make any conclusions on patients from the results of experimental studies in rats. Furthermore, the current method for evaluating LV function could not completely rule out the possibility of indirect effects of propofol on sites other than myocardium.

High-dose pentobarbital anesthesia had negative inotropic effects.\(^{36}\) Although the possibility that pentobarbital anesthesia and injuries had already depressed LV contractility cannot be excluded, pentobarbital anesthesia probably did not modify the negative inotropic effect of propofol, as the threshold mean concentration (5.2 ± 0.3 \(\mu g/ml\)) that induced the negative inotropic effect is higher than the clinically relevant concentration (1~2 \(\mu g/ml\)) without pentobarbital anesthesia.

Conclusion
In contrast to our hypothesis, the current study indicates that the brief regional ischemia is not effective to cause any preconditioning effects on the propofol-induced negative inotropic action. The downstream mechanism of PKC activation would be different from that of preconditioning. On the other hand, there is a report showing that brief ischemia changes the effect of the drug (the anthracyclins, epirubicin) in isolated rat heart; brief ischemia attenuates an acute cardiotoxic effect.\(^{37}\) This report may support the current results showing that the brief regional ischemia enhanced the propofol-induced negative inotropic action. Further studies are needed for the clinical perspective from the current results.

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