Propofol Attenuates Hydrogen Peroxide-induced Mechanical and Metabolic Derangements in the Isolated Rat Heart

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Background: Oxygen-derived free radicals are involved in tissue damage during myocardial ischemia and reperfusion. Recent in vitro studies have demonstrated that a beneficial effect of propofol lies on its free radical scavenging properties. The current study, therefore, examined whether propofol is effective against the mechanical and metabolic damage induced by exogenously administered hydrogen peroxide in the isolated rat heart.

Methods: Rat hearts were perfused aerobically with Krebs-Henseleit bicarbonate buffer at a constant flow rate according to Langendorff’s technique, while being paced electrically. Hearts were studied in control Krebs-Henseleit bicarbonate buffer, with Intralipid vehicle, with 25 μM or 50 μM propofol for 40 min, and with 50 μM propofol for 30 min followed by Intralipid for 10 min. A similar set of hearts was treated with hydrogen peroxide for 4 min, either in the absence of or beginning 10 min after Intralipid or propofol infusion. Left ventricular pressure was recorded as an index of mechanical function. The tissue concentrations of adenosine triphosphate, adenosine diphosphate, adenosine monophosphate, and creatine phosphate were measured as indices of energy metabolism. The tissue concentration of malondialdehyde was measured to evaluate lipid peroxidation.

Results: Hydrogen peroxide (600 μM) significantly increased the left ventricular end-diastolic pressure, decreased the left ventricular developed pressure (i.e., produced mechanical dysfunction), and decreased tissue concentrations of adenosine triphosphate and creatine phosphate (i.e., metabolic damage). Hydrogen peroxide also increased the tissue concentration of malondialdehyde. These mechanical and metabolic alterations induced by hydrogen peroxide were significantly attenuated by propofol (25 μM or 50 μM), while the increase in malondialdehyde was completely suppressed by propofol.

Conclusions: The current study demonstrates that in the isolated heart, propofol attenuates both mechanical and metabolic changes induced by exogenously applied hydrogen peroxide. The beneficial action of propofol is probably correlated with the reduction of the hydrogen peroxide-induced lipid peroxidation. (Key words: Anesthetics, intravenous: propofol. Heart: energy metabolism; mechanical function. Measurement technique: high-performance liquid chromatography; malondialdehyde. Metabolism: lipid peroxidation. Toxicity: hydrogen peroxide.)

REACTIVE oxygen species, such as superoxide anion, hydroxyl radical, and hydrogen peroxide (H₂O₂), have been shown to participate in tissue damage in various diseases. For example, it is well known that oxygen free radicals are generated intracellularly and extracellularly in the myocardium and endothelium during ischemia and reperfusion. These reactive oxygen species cause lipid peroxidation of the cell membrane and intracellular Ca²⁺ overload, which are responsible for mechanical and metabolic damage. In fact, H₂O₂ induces intracellular Ca²⁺ accumulation in the cardiomyocyte. We have demonstrated that treatment with H₂O₂ produces mechanical dysfunction and metabolic changes, including lipid peroxidation in the isolated perfused heart.

Propofol has a chemical structure similar to phenol-based free radical scavengers such as butylated hydroxytoluene and the endogenous antioxidant vitamin E. The phenolic structure is responsible for an antioxidant activity because the hydroxyl group can release hydrogen and thereafter be converted into a less active radical by the resonance of the aromatic ring. Recent in vitro studies with isolated organelles have demonstrated that propofol inhibits the lipid peroxidation induced by oxidative stress in the rat liver mitochondria, microsomes, and brain synaptosomes. Therefore, propofol exhibits the antioxidant action in some organelles.
Nevertheless, it is unclear whether propofol attenuates the changes induced by reactive oxygen metabolites in the heart. In the current study, we produced myocardial damage by exogenous administration of H$_2$O$_2$ as a source of reactive oxygen species in the Langendorff rat heart. Hydrogen peroxide and ischemia-reperfusion are similar in that both produce myocardial damage and induce accumulation of lipid peroxides in the myocardium. We examined the effect of propofol on the H$_2$O$_2$-induced myocardial damage in terms of both mechanical and metabolic dysfunctions. Furthermore, we measured the myocardial concentration of malondialdehyde (MDA), a product of lipid peroxidation, to delineate the protective effect of propofol against H$_2$O$_2$-induced myocardial damage.

Materials and Methods

Heart Preparation

The protocol of animal experiments in the current study was approved by the Ashikawa Medical College Committee on Animal Research. Male Sprague-Dawley rats (weighing 300–350 g, aged 9–10 weeks) were used. The animals were anesthetized with 50 mg·kg$^{-1}$ intraperitoneal sodium pentobarbital. After thoracotomy, the heart and aortic arch were rapidly excised and immersed in a cold Krebs-Henseleit bicarbonate (KHB) buffer (4°C). The composition of the KHB buffer was (in mm): NaCl 118, KCl 4.7, KH$_2$PO$_4$ 1.2, MgSO$_4$ 1.2, CaCl$_2$ 2.5, NaHCO$_3$ 25, and glucose 11. The heart was perfused via retrograde cannulation of the aorta with the KHB buffer at a constant pressure of 80 cmH$_2$O. The buffer was equilibrated with a gas mixture of 95% O$_2$–5% CO$_2$, which produced a P$_O_2$ range of 500–550 mmHg, a P$_CO_2$ range of 36–42 mmHg, and a pH value of 7.38–7.46. Temperature was maintained at 37°C throughout the experiment with a thermostatically controlled recirculating water bath. About 10 min after constant-pressure perfusion, the flow was switched to constant-flow perfusion (10 ml·min$^{-1}$) using a microtube pump (Eyela MP-3, Tokyo-Rikakikai Instruments, Tokyo, Japan), and this flow was maintained at a constant rate throughout the experiment. Constant-flow perfusion was regulated to maintain a constant supply of oxygen, H$_2$O$_2$, propofol, and Intralipid (Kabi Pharmacia, Uppsala, Sweden) to the heart throughout the experiment. The heart rate was kept constant by pacing the heart at 300 beats·min$^{-1}$ throughout the study via stimuli (3F46, San-Ei Instrument, Tokyo, Japan) applied to the left ventricle (4 V, two time threshold; 2-ms duration).

For measurement of left ventricular pressure, a saline-filled polyethylene cannula connected to a pressure transducer was inserted into the left ventricular cavity via the left atrium. The end of the polyethylene cannula in the left ventricle was open to the left ventricular cavity. The left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), and left ventricular developed pressure (LVDP) were employed as indices of myocardial mechanical function. The values of LVSP, LVEDP, and LVDP were determined from the left ventricular pressure curves recorded continuously during the course of the study. The coronary perfusion pressure (CPP) was measured by a pressure transducer connected to the aortic cannula.

Experimental Protocol

The effects of propofol and/or vehicle solution on the mechanical function and energy metabolism in the H$_2$O$_2$-untreated hearts (normal heart experiments) and the H$_2$O$_2$-treated hearts (H$_2$O$_2$-treated heart experiments) were investigated. The normal and H$_2$O$_2$-treated heart groups were then divided into five groups: the KHB group, Intralipid group, 25-μm propofol group, 50-μm propofol group, and 50-μm propofol/Intralipid group. In these groups, all hearts were initially allowed to stabilize for 20 min under the condition of constant-flow perfusion before the observation period. The experimental protocol during the observation period is illustrated in figure 1.

H$_2$O$_2$-treated Heart Experiments. Each of the KHB, Intralipid, 25-μm propofol, and 50-μm propofol groups was divided into two subgroups. In the first subgroup, KHB buffer, Intralipid, or propofol was infused for 40 min to evaluate the mechanical function and energy metabolism. In the second subgroup, KHB buffer, Intralipid, or propofol was infused for 25 min to evaluate lipid peroxidation. In the 50-μm propofol/Intralipid group, the hearts were infused with 50 μm propofol for 30 min followed by Intralipid for 10 min to evaluate the mechanical function and energy metabolism. KHB buffer, Intralipid, or propofol was infused into the aortic cannula at a constant flow rate of 0.05 ml·min$^{-1}$. Hydrogen peroxide was infused for 4 min into the aortic cannula at a constant flow rate of 0.1 ml·min$^{-1}$ from 10 min after the start of infusion of KHB buffer, Intralipid, or propofol.

Normal Heart Experiments. Each of the KHB, Intralipid, 25-μm propofol, and 50-μm propofol groups
1. H$_2$O$_2$-treated heart experiments

- **KHB group**
  - KHB buffer
  - H$_2$O$_2$

- **Intralipid group**
  - Intralipid

- **Propofol 25µM group**
  - Propofol 25 µM

- **Propofol 50µM group**
  - Propofol 50 µM

- **Propofol 50µM / Intralipid group**
  - Propofol 50 µM
  - Intralipid

2. Normal heart experiments

- **KHB group**
  - KHB buffer
  - H$_2$O$_2$

- **Intralipid group**
  - Intralipid

- **Propofol 25µM group**
  - Propofol 25 µM

- **Propofol 50µM group**
  - Propofol 50 µM

- **Propofol 50µM / Intralipid group**
  - Propofol 50 µM
  - Intralipid

Fig. 1. Schematic representation of experimental protocol. Starting time of infusion with Krebs-Henseleit bicarbonate (KHB) buffer, Intralipid, or propofol was taken as 0 min. The effects of propofol on the mechanical function and energy metabolism were investigated in the H$_2$O$_2$-treated and normal heart experiments. In the H$_2$O$_2$-treated heart experiments, each of the Krebs-Henseleit bicarbonate, Intralipid, 25-µM propofol, and 50-µM propofol groups was divided into two subgroups; in the first subgroup the infusion was continued for 40 min, and in the second subgroup it was continued for 25 min. In the normal heart experiments, each of the Krebs-Henseleit bicarbonate, Intralipid, 25-µM propofol, and 50-µM propofol groups was divided into three subgroups; in the first, second, and third subgroups the infusion was continued for 40, 25, and 10 min, respectively. In the 50-µM propofol group of both H$_2$O$_2$-treated and normal heart experiments, propofol was infused for 30 min and then Intralipid was infused for 25 min. H$_2$O$_2$ (600 µM as a final concentration in perfusate) or distilled deionized water (DDW) was infused from 10 min after to 14 min after the start of infusion with Krebs-Henseleit bicarbonate buffer, Intralipid, or propofol. H$_2$O$_2$ was infused for 4 min. All the hearts were frozen (F) for measurements of energy metabolites or malondialdehyde at the end of each infusion with Krebs-Henseleit bicarbonate buffer, Intralipid, or propofol.

was divided into three subgroups. In the first subgroup, KHB buffer, Intralipid, or propofol was infused for 40 min to evaluate the mechanical function and energy metabolism. In the second subgroup, KHB buffer, Intralipid, or propofol was infused for 25 min to evaluate lipid peroxidation. In the third subgroup, KHB buffer, Intralipid, or propofol was infused for 10 min to evaluate energy metabolism. In the 50-µM propofol group, the hearts were infused with 50 µM propofol for 20 min followed by Intralipid for 20 min to evaluate mechanical function and energy metabolism. The experimental conditions and protocol were almost the same as those in the H$_2$O$_2$-treated heart experiments, except that distilled deionized water was infused instead of H$_2$O$_2$.

In each experiment, LVSP, LVEDP, LVDP, and CPP were continuously recorded during a 40-min observation period. To measure the tissue concentrations of adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), creatine phosphate (CrP), the hearts were frozen immediately before H$_2$O$_2$ infusion (the third subgroup in the normal heart experiment) or at the end of the observation period for 40 min (the first subgroup in the H$_2$O$_2$-treated and normal heart experiments) with freezing clamps previously chilled in liquid nitrogen. Other hearts were frozen 11 min after the cessation of H$_2$O$_2$ infusion (the second subgroup in the H$_2$O$_2$-treated and normal heart experiments) for measurements of the tissue concentrations of MDA.

**Measurements of Metabolites**

The frozen myocardial samples were stored in liquid nitrogen (at −173°C) until the biochemical analysis was performed. Each sample was pulverized in liquid nitrogen with a mortar and pestle. To measure the tissue water content and dry weight of the tissue, a part of the pulverized tissue powder was weighed and allowed to stand in a warm oven overnight. The remainder of the powder was used for determination of the tissue concentrations of ATP, ADP, AMP, CrP, and MDA.

ATP, ADP, AMP, and CrP were extracted from the samples with 6% perchloric acid. The tissue extract was centrifuged at 10,000g for 10 min at 4°C. The supernatant was neutralized with 70% potassium hydroxide and centrifuged at 10,000g for 10 min at 4°C again. The resultant solution was used for determinations of ATP, ADP, AMP, and CrP. These metabolites were measured according to the standard enzymatic

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procedures. Values were expressed as micromoles per gram of dry weight.

Malondialdehyde, a product of lipid peroxidation, was measured using high-performance liquid chromatography instead of using thiobarbituric acid because the latter method may measure not only MDA but also other substances. High-performance liquid chromatographic procedure was performed according to the method described by Koller and Bergmann. Malondialdehyde was extracted from the pulverized tissue sample with 10 mm phosphate buffer (pH 8.0), and the tissue extract was centrifuged at 10,000 for 15 min at 4°C. The supernatant was filtered through a Millipore filter (UFC3125C, Nihon Millipore Kogyo, Yonezawa, Japan), at 2,000 g for 10 min. The filtered solution (20 μl) was injected into the high-performance liquid chromatography system (LC-9A, Shimadzu, Kyoto, Japan), equipped with a guard column (G-ODS 0.4 × 1 cm, Shimadzu) and an analytic column (CLC-ODS 0.46 × 25 cm, Shimadzu). Malondialdehyde was separated with 15% acetonitrile (high-performance liquid chromatography grade; Nacalai tesque, Kyoto, Japan) containing 50 mm myristyltrimethylammonium bromide (Aldrich Chemical, Milwaukee, WI) and 1.0 mm Na2HPO4 adjusted to pH 6.8, at a flow rate of 1.0 ml · min⁻¹. The effluent was monitored at 267 nm using a spectrophotometric detector (SPD-2A, Shimadzu). The quantitative analysis was performed by comparison with standard curves. Malondialdehyde standards were prepared by acid hydrolysis of malondialdehyde bis(dimethyl acetel) (Aldrich Chemical, Milwaukee, WI). An additional experiment was performed to determine whether propofol itself interferes with MDA measurement. The mixture of various concentrations of MDA standards and 50 μm propofol in the 10-mm phosphate buffer (pH 8.0) was incubated for 30 min. The concentration of MDA in the buffer in the presence or absence of propofol was measured by high-performance liquid chromatography according to the method described earlier.

The following drugs were used: propofol (2,6-diisopropylphenol, Tokyo Kasei Kogyo, Tokyo, Japan), 10% Intralipid (Kabi Pharmacia, Uppsala, Sweden), sodium pentobarbital (Nacalai tesque, Kyoto, Japan), and H2O2 (Santoku Chemical, Tokyo, Japan). The 10% Intralipid is a solvent of commercially available propofol, and contains 10% soy bean oil, 1.2% egg lecithin, and 2.25% glycerol. Propofol was dissolved in Intralipid and further diluted with distilled deionized water. The concentration of Intralipid in the drug solution was 2%. H2O2 was diluted with distilled deionized water. Propofol or Intralipid was infused into the inflow tube connected to the side arm of the aortic cannula at a flow rate of 0.05 ml · min⁻¹ using an infusion pump. Hydrogen peroxide solution was also infused to the inflow tube at a flow rate of 0.1 ml · min⁻¹ using another infusion pump. The final concentration of propofol in the perfusate (KHB buffer perfused at a flow rate of 10 ml · min⁻¹ plus propofol solution infused at a flow rate of 0.5 ml · min⁻¹) was 25 or 50 μm. The concentration of propofol changed from 25 to 24.8 μm or from 50 to 49.5 μm when H2O2 was added to the perfusate, because total flow rate of perfusate increased from 10.05 to 10.15 ml · min⁻¹ during H2O2 infusion, but these small changes in the concentration of propofol did not affect left ventricular pressure or CPP. The final concentration of H2O2 in the perfusate including infusion solution was 600 μm. The reagents and enzymes used for biochemical analysis were purchased from Sigma Chemical Co. (St. Louis, MO).

**Statistical Analysis**

All values were expressed as mean ± SEM (standard error of the mean). Statistical analysis of the results was performed with one-way analysis of variance. This was followed by Fisher's protected least-significant difference for multiple comparisons. A P value of less than 0.05 was considered statistically significant.

**Results**

**Effects of Propofol on Mechanical Function and Energy Metabolism in Normal Heart**

Experiments were performed to examine how propofol affects myocardial mechanical function and energy metabolism in normal hearts (hearts not treated with H2O2) during 40 min of perfusion with KHB, Intralipid, 25 μm propofol, or 50 μm propofol (tables 1 and 2). In the KHB and Intralipid groups (n = 8 for each), myocardial mechanical function and energy metabolism did not change significantly throughout the experimental period. Propofol significantly decreased LVSP, LVPD, and CPP in a dose-dependent fashion, whereas it did not affect LVEDP and energy metabolism. In the 50-μm propofol/Intralipid group, LVSP, LVPD, and CPP decreased significantly during the period of infusion with 50 μm propofol but returned to the initial level (0 min value) within 10 min after the cessation of propofol infusion.
## Table 1. Effect of Propofol and/or Intralipid on the Mechanical Function and Coronary Resistance in Normal Hearts

<table>
<thead>
<tr>
<th>Min</th>
<th>LVSP (mmHg)</th>
<th>LVEDP (mmHg)</th>
<th>LVDP (mmHg)</th>
<th>CPP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KHB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>71.5 ± 1.1</td>
<td>0.6 ± 0.2</td>
<td>70.9 ± 1.2</td>
<td>61.0 ± 1.1</td>
</tr>
<tr>
<td>10</td>
<td>72.1 ± 1.0</td>
<td>0.8 ± 0.2</td>
<td>71.4 ± 1.1</td>
<td>62.5 ± 0.8</td>
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<td>40</td>
<td>72.6 ± 1.2</td>
<td>0.8 ± 0.3</td>
<td>71.9 ± 1.3</td>
<td>63.0 ± 1.1</td>
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<tr>
<td>Intralipid</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>72.8 ± 0.9</td>
<td>0.5 ± 0.2</td>
<td>72.3 ± 1.0</td>
<td>62.4 ± 0.7</td>
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<tr>
<td>10</td>
<td>74.8 ± 1.5</td>
<td>0.9 ± 0.1</td>
<td>73.9 ± 1.4</td>
<td>63.4 ± 1.1</td>
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<tr>
<td>40</td>
<td>76.3 ± 1.6</td>
<td>0.8 ± 0.3</td>
<td>75.5 ± 1.5</td>
<td>65.1 ± 1.2</td>
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<td>Propofol 25 µM</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>72.0 ± 0.6</td>
<td>0.5 ± 0.2</td>
<td>71.5 ± 0.6</td>
<td>60.9 ± 0.8</td>
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<tr>
<td>10</td>
<td>68.0 ± 0.6*</td>
<td>0.8 ± 0.2</td>
<td>67.3 ± 0.6*</td>
<td>58.1 ± 0.6†</td>
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<tr>
<td>40</td>
<td>66.1 ± 0.7†</td>
<td>0.9 ± 0.3</td>
<td>65.3 ± 0.8†</td>
<td>56.4 ± 0.5†</td>
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<td>Propofol 50 µM</td>
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<td></td>
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<td>72.1 ± 0.8</td>
<td>0.8 ± 0.2</td>
<td>71.4 ± 0.8</td>
<td>61.4 ± 1.1</td>
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<tr>
<td>10</td>
<td>62.0 ± 1.4†</td>
<td>0.5 ± 0.2</td>
<td>61.5 ± 1.3†</td>
<td>53.1 ± 0.5†</td>
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<td>61.8 ± 1.5†</td>
<td>0.9 ± 0.2</td>
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<td>52.9 ± 0.5†</td>
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<td>Propofol 50 µM/Intralipid</td>
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<td></td>
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<td></td>
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<td>71.9 ± 0.5</td>
<td>61.5 ± 0.3</td>
</tr>
<tr>
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<td>60.4 ± 0.5†</td>
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<td>59.6 ± 0.8</td>
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</table>

Values are mean ± SEM of eight hearts for each experiment. Values were obtained at 0, 10, 30, and 40 min after the start of KHB buffer, Intralipid, or propofol infusion. LVSP = left ventricular systolic pressure; LVEDP = left ventricular end-diastolic pressure; LVDP = left ventricular developed pressure; CPP = coronary perfusion pressure; KHB = Krebs-Henseleit bicarbonate.

* P < 0.05 versus KHB group.
† P < 0.01 versus KHB group.

## Table 2. Effect of Propofol and/or Intralipid on the Energy Metabolism in Normal Hearts

<table>
<thead>
<tr>
<th>Min</th>
<th>CrP (µmol/g dry wt)</th>
<th>ATP (µmol/g dry wt)</th>
<th>ADP (µmol/g dry wt)</th>
<th>AMP (µmol/g dry wt)</th>
</tr>
</thead>
<tbody>
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<td>KHB</td>
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<td></td>
<td></td>
<td></td>
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<td>10</td>
<td>22.9 ± 0.7</td>
<td>19.4 ± 0.6</td>
<td>4.5 ± 0.2</td>
<td>1.1 ± 0.1</td>
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<td>40</td>
<td>17.7 ± 0.7</td>
<td>18.9 ± 0.5</td>
<td>5.2 ± 0.2</td>
<td>1.2 ± 0.1</td>
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<tr>
<td>Intralipid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>21.1 ± 0.5</td>
<td>18.8 ± 0.6</td>
<td>4.6 ± 0.0</td>
<td>1.0 ± 0.0</td>
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<tr>
<td>40</td>
<td>17.9 ± 1.1</td>
<td>19.2 ± 0.7</td>
<td>5.4 ± 0.2</td>
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<tr>
<td>Propofol 25 µM</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>10</td>
<td>21.9 ± 1.9</td>
<td>19.7 ± 0.1</td>
<td>4.6 ± 0.1</td>
<td>1.0 ± 0.0</td>
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<td>40</td>
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<tr>
<td>40</td>
<td>19.4 ± 0.6</td>
<td>19.0 ± 0.8</td>
<td>4.8 ± 0.2</td>
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<td>Propofol 50 µM/Intralipid</td>
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<td></td>
<td></td>
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<tr>
<td>40</td>
<td>17.4 ± 0.6</td>
<td>18.5 ± 0.3</td>
<td>4.8 ± 0.1</td>
<td>1.2 ± 0.0</td>
</tr>
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</table>

Values are mean ± SEM of eight hearts for each experiment. Values were obtained at 10 and 40 min after the start of KHB buffer, Intralipid, or propofol infusion. Values of "10 min" in the propofol 50 µM/Intralipid group were not determined because they correspond with values of "10 min" in the propofol 50 µM group. No significant differences were detected when compared with the value in the KHB group (P > 0.05).

CrP = creatine phosphate; ATP = adenosine triphosphate; ADP = adenosine diphosphate; AMP = adenosine monophosphate; KHB = Krebs-Henseleit bicarbonate.

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Fig. 2. Effect of propofol in Intralipid or Intralipid alone on mechanical function in hydrogen peroxide-treated heart. Changes of left ventricular pressure in Krebs-Henseleit bicarbonate (A), Intralipid (B), 25-μM propofol (C), 50-μM propofol (D), or 50-μM propofol/Intralipid (E) groups are recorded. Open symbols indicate left ventricular systolic pressure, and solid symbols indicate left ventricular end-diastolic pressure. Each value represents mean ± SEM (n = 8 in each group). *P < 0.05 and **P < 0.01 when compared with value in the Krebs-Henseleit bicarbonate group.

Effects of Propofol on H$_2$O$_2$-induced Changes of Mechanical Function and Coronary Resistance
Changes in left ventricular pressure induced by H$_2$O$_2$ are shown in figure 2. Before starting the H$_2$O$_2$ infusion, there were no significant differences in mechanical function between the normal and H$_2$O$_2$-treated heart groups. In both KHB (n = 8) and Intralipid (n = 8) groups, H$_2$O$_2$ induced a temporary but severe decrease in LVSP (figs. 2A and 2B). Left ventricular end-diastolic pressure also increased markedly after the H$_2$O$_2$ infusion, and the increase that was accompanied by elevation of the LVSP to the pre-H$_2$O$_2$ level, lasted throughout the observation period. Consequently, LVDP decreased significantly after the H$_2$O$_2$ infusion and did not recover to the pre-H$_2$O$_2$ level by the end of the experiment (fig. 3). These results indicate that H$_2$O$_2$ produced a long-lasting mechanical dysfunction, although LVSP returned to the control level within 15 min of the cessation of H$_2$O$_2$ infusion.

Before the start of H$_2$O$_2$ infusion, propofol induced a dose-dependent decrease in LVSP. In the presence of propofol, H$_2$O$_2$ was infused for 4 min. Hydrogen peroxide produced a temporary and severe decrease in LVSP in both the 25- and 50-μM propofol groups (n = 8 for each group; figs. 2C and 2D). Increases in LVEDP and LVSP after the end of H$_2$O$_2$ infusion, however, were significantly attenuated by propofol when compared with the KHB and the Intralipid groups. After 40 min of infusion, there was no significant difference in LVSP between the KHB and the 25-μM propofol groups (P > 0.05), whereas LVSP in the 50-μM propofol group was significantly lower than that in the KHB group (P < 0.05). Consequently, LVDP in the 25-μM propofol group was significantly higher than that in the 50-μM propofol and KHB groups (fig. 3).

There is a possibility that the depressed recovery of LVSP and LVDP in the 50-μM propofol group is due to the cardiodepression induced by propofol itself. For

Fig. 3. Effect of propofol in Intralipid or Intralipid alone on left ventricular developed pressure in hydrogen peroxide-treated heart. Hearts are those in figure 2. Each value represents mean ± SEM (n = 8 in each group). *P < 0.05 and **P < 0.01 when compared with value in the Krebs-Henseleit bicarbonate group. †P < 0.05 and ‡P < 0.01 when compared with value in the 50-μM propofol group.
this purpose, in the H₂O₂-treated heart experiments, we perfused the heart with 50 μM propofol for 30 min and then with Intralipid for 10 min (50-μM propofol/ Intralipid group, n = 8), and compared the recovery of mechanical function between when the 50 μM propofol infusion was performed during the whole course of the experiment (40 min) and when it was performed for 30 min and stopped. Changes in LVSP, LVEDP, and LVEDP during propofol infusion in the 50-μM propofol/ Intralipid group were similar to those in the 50-μM propofol group. LVSP and LVDP increased greatly after the cessation of propofol infusion; these values at the end of experiment in the 50-μM propofol/Intralipid group were significantly higher than those in the 50-μM propofol group and similar to those in the 25-μM propofol group (figs. 2 and 3). These results indicate that the cessation of propofol infusion does not reduce the recovery of mechanical function, or rather, it improves mechanical function (LVSP and LVDP) that has been suppressed by propofol.

Figure 4 shows changes in CPP throughout the experimental period. Hydrogen peroxide produced a transient decrease followed by a long-lasting increase in CPP in each of the five groups. Before the H₂O₂ infusion, there was a statistically significant decrease in CPP in both the 25- and 50-μM propofol groups when compared with the KHB and the Intralipid groups. Increase in CPP after H₂O₂ infusion was significantly attenuated in either the 25-μM propofol, the 50-μM propofol, or the 50-μM propofol/Intralipid group.

Effect of Propofol on H₂O₂-induced Changes of Myocardial Energy Metabolism

Figures 5 and 6 show the tissue concentrations of ATP, ADP, AMP, and CrP in the five groups 10 and 40 min after KHB buffer, Intralipid, or propofol infusions. There were no significant differences in the tissue concentrations of energy metabolites at 10 min (immediately before the H₂O₂ infusion) among the KHB, Intralipid, or propofol groups. The tissue concentrations of ATP, ADP, and CrP at the end of the experiment in the propofol 25- and 50-μM groups were higher than those in the KHB and Intralipid groups (40 min in figs. 5 and 6). Decreases in ATP and ADP induced by H₂O₂ were significantly attenuated by propofol. In contrast, CrP did not change after H₂O₂ infusion in the 25-μM propofol group, whereas it increased in the 50-μM propofol group. The tissue concentrations of ATP and CrP at the end of experiment in the 50-μM propofol/Intralipid group were higher than those in the KHB and the Intralipid groups, but lower than those in the 50-μM propofol group (40 min in figs. 5 and 6). The ratio of wet/dry tissue weight calculated for quantitative analysis of energy metabolites was not different among all groups in the both normal and H₂O₂-treated heart experiments.

Effect of Propofol on Lipid Peroxidation Induced by H₂O₂

To examine whether the beneficial effects of propofol on the H₂O₂-induced mechanical dysfunction and metabolic changes correlate with reduction of lipid peroxidation, the tissue concentration of MDA was measured 25 min after the start of infusion (i.e., 11 min after the end of H₂O₂ infusion; fig. 7). The reason that a 25-min interval was chosen is that H₂O₂ produced the greatest increase in LVEDP in the KHB and the Intralipid groups around that time (fig. 2). In the KHB and Intralipid groups, H₂O₂ produced a significant increase in the MDA concentration by approximately 5 and 7 times their control values without H₂O₂, respectively. In contrast, the tissue concentration of MDA in both 25- and 50-μM propofol groups did not increase even after H₂O₂ infusion. Propofol (25 and 50 μM) also decreased the tissue MDA concentration in the normal heart (fig. 7). Nevertheless, propofol did not interfere with measurement of MDA (data not shown). These results suggest that propofol prevents the H₂O₂-induced lipid peroxidation.
Discussion

Many studies have addressed the actions of substances that protect the myocardium from reactive oxygen-induced damage. For example, nifedipine and propranolol inhibit the lipid peroxidation of the sarcolemmal membrane induced by oxidative stress.\(^{15}\) Catalase, lidocaine,\(^{7}\) and agonists for adenosine A2 receptor\(^{16}\) attenuate the mechanical dysfunction and metabolic changes induced by H\(_2\)O\(_2\) in the isolated perfused heart. In the current study, we examined the effect of propofol on mechanical dysfunction, metabolic changes, and lipid peroxidation induced by H\(_2\)O\(_2\) in the isolated perfused rat heart.

The reasons we used H\(_2\)O\(_2\) as a source of reactive oxygen metabolite can be summarized as follows: (1)
H$_2$O$_2$ and its metabolite, the hydroxyl radical, are known to be important in the pathogenesis of myocardial damage induced by ischemia-reperfusion; and (2) H$_2$O$_2$ penetrates the cell membrane, reaching intracellular sites and may therefore induce severe damage to the myocardial cell. The current study demonstrated that H$_2$O$_2$ produced mechanical dysfunction (as evidenced by an increase in LVEDP and a decrease in LVDP), an increase in coronary resistance (as evidenced by an increase in CPP), and metabolic changes (as evidenced by a decrease in the tissue ATP and CrP concentrations). These alterations induced by H$_2$O$_2$ were attenuated by 25 $\mu$m and 50 $\mu$m propofol, suggesting that propofol has a beneficial effect on the H$_2$O$_2$-induced mechanical dysfunction and metabolic derangements.

We found that the recovery of LVDP after H$_2$O$_2$ infusion was better in the 25-$\mu$m propofol group than that in the 50-$\mu$m propofol group. However, high-energy phosphate after the H$_2$O$_2$ infusion was better preserved in the 50-$\mu$m propofol group than that in the 25-$\mu$m propofol group. This is probably because propofol itself induced dose-dependent decrease in mechanical function, which may preserve energy in the H$_2$O$_2$-treated hearts. In fact, the cessation of infusion with propofol in the 50-$\mu$m propofol/Intralipid group greatly increased LVSP and LVEDP, and the tissue concentrations of ATP and CrP in the 50-$\mu$m propofol/Intralipid group were lower than those in the 50-$\mu$m propofol group.

According to biochemical studies, the hydroxyl radical generated from H$_2$O$_2$ by the Fenton reaction easily removes a hydrogen atom from a methylene carbon of an unsaturated fatty acid of membrane phospholipids. Peroxidation of lipids then would inactivate membrane-associated enzymatic proteins, such as Na$^+$-K$^+$-adenosinetriphosphatase and Ca$^{2+}$-adenosinetriphosphatase, and increase membrane permeability. These biochemical and physicochemical alterations of the cell membrane may be responsible for mechanical and metabolic derangements.

To test the hypothesis that the beneficial effects of propofol on the H$_2$O$_2$-induced mechanical dysfunction and metabolic changes correlate with reduction of lipid peroxidation induced by H$_2$O$_2$, we measured the tissue MDA concentration 11 min after the H$_2$O$_2$ infusion, when H$_2$O$_2$ produced the greatest increase in LVEDP in the KHB and Intralipid groups. In the KHB and the Intralipid groups, H$_2$O$_2$ increased the tissue concentration of MDA significantly. The increase in the tissue MDA concentration in the Intralipid group was greater than that in the KHB group. There may be some interaction between H$_2$O$_2$ and Intralipid, because Intralipid contains egg lecithin (phosphatidylcholine). Nevertheless, both 25 and 50 $\mu$m propofol completely suppressed the H$_2$O$_2$-induced increase in tissue MDA. Our findings suggest that the beneficial effects of propofol on H$_2$O$_2$-induced myocardial damage correlate with inhibition of lipid peroxidation. According to reports on propofol's antioxidant effect, it is assumed that propofol behaves in the same way as phenol-based antioxidants, which scavenge free radical species (e.g., hydroxyl radical and lipid peroxyl radical) by a process of hydrogen abstraction and thereby themselves become a less reactive phenoxyl radical.

It should be noted, however, that propofol did not attenuate completely the H$_2$O$_2$-induced mechanical dysfunction (an increase in LVEDP and a decrease in LVDP) and metabolic derangements (a decrease in the tissue ATP and CrP concentrations), in spite of the complete inhibition of lipid peroxidation. Recently, Kong et al. reported that prevention of lipid peroxidation does not prevent myocardial contractile dysfunction induced by reactive oxygen in isolated rabbit hearts. Therefore, factors other than lipid peroxidation...
may play a important role in myocardial damage induced by reactive oxygen. In fact, oxidation of sulfhydril groups in protein or nonprotein has been demonstrated to contribute to myocardial damage induced by reactive oxygen.25 These biochemical alterations, which are not attributed to lipid peroxidation of the cell membrane, may account for the incomplete action of propofol on H₂O₂-induced mechanical and metabolic derangements.

Propofol inhibited preferentially the H₂O₂-induced increase in LVEDP rather than the H₂O₂-induced decrease in LVDP. The beneficial action of propofol on the increase in LVEDP may be due to inhibition of intracellular Ca²⁺ accumulation, because myocardial contracture induced by H₂O₂ is, at least in part, mediated by an increase in intracellular Ca²⁺.26 Cook and Housmans19 suggested that propofol inhibits the slow inward I-type Ca²⁺ current across the sarcolemma. Although the beneficial role of nisoldipine and flunarazine against H₂O₂-induced mechanical dysfunction has been demonstrated, there is no direct evidence to show that the beneficial effect of propofol to attenuate the H₂O₂-induced mechanical dysfunction and metabolic derangements is caused by inhibition of the Ca²⁺ current. Further studies are needed to determine the detailed mechanism of the protective action of propofol on the H₂O₂-induced changes.

Peak plasma concentration of propofol after an intravenous induction dose increases to as much as 44 µM,27 whereas stable plasma concentrations of approximately 10–20 µm are observed during a maintenance period.28 Furthermore, more than 90% of propofol is bound to plasma protein.29 The concentration range tested in this study therefore is considerably greater than that likely to be encountered during clinical practice. However, even trace plasma concentrations of propofol may play a crucial role in protecting the cell membrane against oxidative stress as well as vitamin E. It may also be that propofol that is bound to proteins or that which is present in membranes may still be able to perform its antioxidant role in these settings, so that free drug concentration is less important than in receptor or partitioning studies.

In conclusion, the results of the current study demonstrate that propofol attenuates both mechanical dysfunction and metabolic derangements induced by exogenous H₂O₂. Furthermore, an increase in the tissue concentration of MDA induced by H₂O₂ was completely suppressed by either 25 or 50 µm propofol after the H₂O₂ infusion. The results also suggest that the beneficial effects of propofol correlate with inhibition of H₂O₂-induced lipid peroxidation.

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