Effects of Intravenous Anesthetics on Function and Metabolism in the Reperfused Working Rat Heart

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ABSTRACT—We investigated the comparative effects of ketamine, flunitrazepam, diazepam and midazolam on function and metabolism in reperfused rat hearts. Seventy-two hearts were rapidly excised and perfused with buffer as a Neely's working model. Whole heart ischemia was induced for 15 min followed by reperfusion for 20 min. Four intravenous anesthetics in 2 different concentrations (10 and 50 times of therapeutic concentrations) were administered during reperfusion. The data were compared to a control group in which intravenous anesthetics were not used. At the end of reperfusion, myocardial metabolites were measured by liquid chromatography. Cardiac outputs in the both groups given lower and higher doses of ketamine and flunitrazepam and in the groups given the higher dose of diazepam and midazolam were significantly lower than that in the control group [at the end of reperfusion: control: 60.4; ketamine: 48.8 (lower) and 14.6 (higher); flunitrazepam: 50.2 (lower) and 50.6 (higher); diazepam: 62.6 (lower) and 42.5 (higher); midazolam: 59.5 (lower) and 51.2 (higher), ml/min]. The levels of ATP in all higher concentration anesthetic groups were significantly lower than those in the control group (control: 23.7, ketamine: 17.8, flunitrazepam: 17.8, diazepam: 17.7, midazolam: 17.7, μmol/g). These results suggest that ketamine and flunitrazepam moderately depress cardiac function more than diazepam and midazolam when they are given during reperfusion.

Keywords: Intravenous anesthetic, Cardiac function, Myocardial metabolism, Reperfusion, Isolated heart

Flunitrazepam, diazepam and midazolam are extensively employed for anesthesia in cardiac surgery. Recently, ketamine has been reevaluated as a proper anesthetic for use in cardiac surgery, including emergency coronary artery bypass surgery (1). In emergency coronary artery bypass surgery for acute myocardial infarction, coronary perfusion of the ischemic myocardium is interrupted before the cardio-pulmonary bypass, and anesthetic agents would reach the ischemic area just after reperfusion. The same condition could occur in patients with acute myocardial infarction undergoing anesthesia during percutaneous transluminal coronary angioplasty. However, the direct effects of these anesthetics on myocardial function and metabolism have not been well examined in the setting of post-ischemic reperfusion.

In previous studies, hemodynamic responses to induction of anesthesia with these anesthetics have been examined in patients with myocardial ischemic disease (2–5). Rossetti et al. reported that diazepam delayed the onset of exercise-induced myocardial ischemia in patients with coronary artery disease and that the anti-ischemic action of diazepam appeared to be mediated by a reduction in myocardial oxygen consumption (6). In contrast with this ischemic condition, the administration of intravenous anesthetics only during reperfusion may impair the post-ischemic functional recovery. In the reperfused heart, luxury perfusion does not always work protectively. In our previous study where we investigated the myocardial effects of thiopental against aerobic and reperfused hearts (7), cardiac function was decreased by 24% under the aerobic condition, whereas cardiac contraction was not observed when it was administered during reperfusion. Therefore, it is important to investigate the direct effects of these anesthetics on reperfused heart to determine whether these agents are safe for anesthesia during reperfusion period. We hypothesized in this study that the intravenous anesthetics would impair the post-ischemic recovery of the myocardium when they are given only during reperfusion. The present study was carried out to compare the effects of intravenous anesthetic agents on
myocardial function and metabolism, and to determine which agent was appropriate for anesthesia during the reperfusion period. Previously, we examined the effects of 10 and 100 times the therapeutic dose of ketamine, flunitrazepam, diazepam and midazolam on the aerobic rat heart-lung preparation (8). Therefore, in this experiment, we employed the same species and a similar concentration range of each anesthetic to compare the present results with the previous aerobic data.

MATERIALS AND METHODS

These experiments were approved by the Animal Ethical Committee of the Yamanashi Medical University. Seventy-two 3-month-old male Wistar rats weighing 280–320 g (SLC, Shizuoka) were used. In a plastic cage, the animals were anesthetized with isoflurane with non-intubated spontaneous respiration. The hearts were rapidly excised and put into ice-cold saline. The aorta, which was transected 4–5 mm above the aortic valve, was mounted on a steel cannula and a retrograde perfusion of the coronary arteries was started (Fig. 1). Non-recirculating modified Krebs-Henseleit bicarbonate buffer (KHB) was used as the perfusate. The perfusate was maintained at 37.0±0.3°C and contained: 118 mM NaCl, 4.7 mM KCl, 2.0 mM CaCl2, 1.2 mM MgSO4, 1.2 mM KH2PO4, 25 mM NaHCO3, 0.5 mM di-NaEDTA and 11 mM glucose. The solution was equilibrated with a gas mixture of 95% O2 and 5% CO2. During the retrograde perfusion, the left atrium was connected to an angled steel cannula via a pulmonary vein. The remaining pulmonary veins were ligated to avoid leakage. After this preliminary perfusion, retrograde perfusion was stopped by clamping the tube from the preperfusion reservoir, and antegrade perfusion was started by perfusing the left atrium and by releasing the aortic outflow for 10 min. The KHB was used also during perfusion in the working heart systems.

Left ventricular pressure was measured with a transducer (P10EZ; Gould, Oxnard, CA, USA) connected to a thin catheter (18G, Argyle Intramedicut Catheter; Sherwood, Tokyo) inserted into the left ventricle through the mitral valve from the angled steel cannula in the left atri-
um. Rates of tension development (dP/dt) were electronically measured as the first derivative of left ventricular pressure. Aortic outflow was recorded with an electromagnetic blood flow meter (MFV-3200; Nihon Kohden, Tokyo). Coronary flow was measured by timed collection of the pulmonary artery outflow and surface runoff of the heart resulting from the coronary sinus and Thebesian vessel drainage. Cardiac output was estimated as the sum of the aortic and coronary outflows. The coronary effluent was not recycled as the perfusate.

Aortic oxygen tension was measured by sampling perfusate from the atrial bubble trap on the left atrial line with a gas-tight syringe. For measurement of oxygen tension of coronary effluent, a catheter was placed in the pulmonary artery, from which samples were obtained with a gas-tight syringe. The oxygen tension was measured in an intermittently self-calibrating blood gas analyzer system (Model 1306; Instrumentation Laboratory, Lexington, MA, USA). Myocardial oxygen consumption, MVO₂ (µmol/min/g), was calculated as O₂ solubility multiplied by coronary flow per gram heart tissue multiplied by the difference between the inflow and outflow O₂ tensions. Oxygen delivery (DO₂) was calculated from the inflow O₂ tension multiplied by O₂ solubility multiplied by coronary flow per gram heart tissue.

After the 10 min of stabilization, whole heart ischemia was induced by clamping the one-way aortic valve bypass for 15 min. Since the largest fraction of coronary flow occurs during diastole, this one-way valve severely restricted coronary perfusion, but did not influence aortic

### Table 1. Hemodynamic data

<table>
<thead>
<tr>
<th></th>
<th>Aerobic 0 min</th>
<th>Ischemic 15 min</th>
<th>Reperfusion 20 min</th>
<th>Reperfusion 25 min</th>
<th>Reperfusion 30 min</th>
<th>Reperfusion 35 min</th>
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<tbody>
<tr>
<td><strong>Cardiac output (ml/min)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>70.0 ± 4.0</td>
<td>0.5 ± 0.2</td>
<td>4.2 ± 9.8</td>
<td>39.7 ± 16.9</td>
<td>56.1 ± 8.0</td>
<td>60.4 ± 6.2</td>
</tr>
<tr>
<td>HK</td>
<td>69.4 ± 6.9</td>
<td>0.5 ± 0.3</td>
<td>7.4 ± 9.0</td>
<td>31.2 ± 14.4</td>
<td>42.1 ± 11.6*</td>
<td>48.8 ± 8.6*</td>
</tr>
<tr>
<td>LF</td>
<td>67.2 ± 4.1</td>
<td>0.6 ± 0.5</td>
<td>5.0 ± 5.0</td>
<td>8.2 ± 5.9*</td>
<td>11.8 ± 6.9*</td>
<td>14.6 ± 10.0*</td>
</tr>
<tr>
<td>LF</td>
<td>64.9 ± 5.6</td>
<td>0.5 ± 0.5</td>
<td>8.4 ± 12.4</td>
<td>34.7 ± 18.7</td>
<td>47.7 ± 8.3*</td>
<td>50.2 ± 8.1*</td>
</tr>
<tr>
<td>HD</td>
<td>65.6 ± 5.2</td>
<td>0.6 ± 0.6</td>
<td>7.9 ± 10.5</td>
<td>33.7 ± 16.2</td>
<td>45.5 ± 12.0*</td>
<td>50.6 ± 6.6*</td>
</tr>
<tr>
<td>LD</td>
<td>69.5 ± 7.5</td>
<td>0.4 ± 0.1</td>
<td>9.5 ± 17.0</td>
<td>43.9 ± 19.8</td>
<td>60.7 ± 8.0</td>
<td>62.6 ± 6.6</td>
</tr>
<tr>
<td>HD</td>
<td>66.3 ± 2.8</td>
<td>0.5 ± 0.5</td>
<td>3.8 ± 3.2</td>
<td>22.3 ± 12.0*</td>
<td>38.4 ± 13.2*</td>
<td>42.5 ± 5.2*</td>
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<tr>
<td>LM</td>
<td>71.2 ± 7.5</td>
<td>0.5 ± 0.2</td>
<td>23.7 ± 24.0</td>
<td>52.3 ± 12.5</td>
<td>60.9 ± 7.0</td>
<td>59.5 ± 5.5</td>
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<tr>
<td>HM</td>
<td>69.8 ± 6.2</td>
<td>0.4 ± 0.1</td>
<td>14.9 ± 18.6</td>
<td>34.4 ± 22.1</td>
<td>51.9 ± 11.8</td>
<td>51.2 ± 8.3*</td>
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<td><strong>Left ventricular dP/dt max (mmHg/sec)</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>4850 ± 302</td>
<td>138 ± 52</td>
<td>900 ± 872</td>
<td>3200 ± 899</td>
<td>3850 ± 338</td>
<td>4225 ± 333</td>
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<tr>
<td>HK</td>
<td>4700 ± 312</td>
<td>155 ± 84</td>
<td>1325 ± 573</td>
<td>2838 ± 661</td>
<td>3400 ± 612*</td>
<td>3800 ± 518</td>
</tr>
<tr>
<td>LF</td>
<td>5025 ± 462</td>
<td>188 ± 99</td>
<td>1200 ± 745</td>
<td>1188 ± 753*</td>
<td>1488 ± 858*</td>
<td>1688 ± 1001*</td>
</tr>
<tr>
<td>LF</td>
<td>4643 ± 328</td>
<td>150 ± 78</td>
<td>1188 ± 921</td>
<td>2925 ± 776</td>
<td>3638 ± 302</td>
<td>3903 ± 288</td>
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<tr>
<td>HD</td>
<td>5025 ± 354</td>
<td>163 ± 106</td>
<td>1600 ± 964</td>
<td>3030 ± 974</td>
<td>3675 ± 810</td>
<td>4125 ± 463</td>
</tr>
<tr>
<td>LD</td>
<td>4813 ± 442</td>
<td>168 ± 73</td>
<td>1580 ± 1147</td>
<td>3600 ± 701</td>
<td>4303 ± 440*</td>
<td>4463 ± 487</td>
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<td>190 ± 78</td>
<td>1405 ± 492</td>
<td>3013 ± 894</td>
<td>3940 ± 468</td>
<td>4025 ± 459</td>
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<tr>
<td>LM</td>
<td>4663 ± 338</td>
<td>190 ± 81</td>
<td>2373 ± 1206*</td>
<td>4008 ± 613*</td>
<td>4400 ± 485*</td>
<td>4438 ± 521</td>
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<tr>
<td>HM</td>
<td>5213 ± 323</td>
<td>188 ± 64</td>
<td>1650 ± 1649</td>
<td>3488 ± 1498</td>
<td>4388 ± 755</td>
<td>4288 ± 645</td>
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<tr>
<td><strong>Coronary flow (ml/min)</strong></td>
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<tr>
<td>Control</td>
<td>17.4 ± 1.2</td>
<td>0.5 ± 0.2</td>
<td>13.5 ± 1.1</td>
<td>14.9 ± 0.7</td>
<td>15.2 ± 0.7</td>
<td>15.3 ± 0.8</td>
</tr>
<tr>
<td>HK</td>
<td>18.5 ± 2.9</td>
<td>0.5 ± 0.3</td>
<td>14.8 ± 2.9</td>
<td>16.0 ± 3.4</td>
<td>16.1 ± 3.0</td>
<td>16.3 ± 3.5</td>
</tr>
<tr>
<td>LF</td>
<td>17.1 ± 1.6</td>
<td>0.6 ± 0.5</td>
<td>13.9 ± 2.9</td>
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<tr>
<td>LF</td>
<td>17.4 ± 1.1</td>
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<td>14.0 ± 1.2</td>
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<tr>
<td>HD</td>
<td>17.1 ± 1.4</td>
<td>0.6 ± 0.6</td>
<td>14.1 ± 1.1</td>
<td>14.7 ± 1.9</td>
<td>15.2 ± 1.7</td>
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<td>15.6 ± 2.0</td>
<td>15.5 ± 1.2</td>
</tr>
<tr>
<td>LM</td>
<td>18.4 ± 1.5</td>
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<td>17.8 ± 2.8</td>
<td>17.4 ± 1.0*</td>
<td>17.2 ± 1.6</td>
</tr>
<tr>
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<td>0.4 ± 0.1</td>
<td>14.4 ± 1.3</td>
<td>15.5 ± 1.9</td>
<td>17.2 ± 1.1*</td>
<td>17.1 ± 2.4</td>
</tr>
</tbody>
</table>

LK and HK = lower and higher dose of ketamine, LF and HF = lower and higher dose of flunitrazepam, LD and HD = lower and higher dose of diazepam, LM and HM = lower and higher dose of midazolam. *P < 0.05, as compared with control values. Values are mean ± S.D. (n=8).
output or ventricular afterload (9). Only during the ischemic period, the heart was paced at 333 beats/min. Reperfusion of the heart after this ischemic period of 15 min was performed by declamping the one-way aortic valve bypass tube and maintained for 20 min.

Hearts prepared for this study were randomly assigned to one of nine groups as follows (each group: n=8): control (C: no anesthetics), 24 or 120 mg/l of ketamine (Sankyo Co., Ltd., Tokyo) (LK and HK); 0.6 or 3 mg/l of flunitrazepam (Nihon Roche Co., Ltd., Tokyo) (LF and HF); 6 or 30 mg/l of diazepam (Takeda Yakuhin Kogyo Co., Ltd., Osaka) (LD and HD); and 6 or 30 mg/l of midazolam (Yamanouchi Seiyaku Co., Ltd., Tokyo) (LM and HM). These concentrations are considered to be 10 and 50 times the therapeutic doses that are required for hypnosis and amnesia during surgery (3, 10-15). All drugs were obtained commercially. Flunitrazepam and diazepam had been already dissolved in benzyl alcohol and ethyl alcohol, and each drug was diluted in KHB (final concentration: benzyl alcohol 9.0-90 µg/ml, ethyl

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Fig. 2. Changes in the oxygen delivery (DO2) to myocardial oxygen consumption (MVO2) ratio of the control and groups given two levels of the ketamine, flunitrazepam, diazepam and midazolam as a function of time. The X-axis represents the perfusion time in minutes. At zero time, ischemia was induced by the one-way aortic valve procedure. Perfusion as an ischemic heart was continued for 15 min followed by reperfusion for 20 min. During reperfusion, the heart was exposed to the perfusate without (control group) or with each anesthetic. Filled columns: control, high density hatched columns: ketamine, dotted columns: flunitrazepam, low density hatched columns: diazepam, open columns: midazolam. Each point represents the mean ± S.D. for 8 hearts. *P < 0.05, as compared with the control group. $P < 0.05, as compared with the value at 0 min of each group.
alcohol 48–600 µg/ml). Before the experiment, each solution was equilibrated with a gas mixture of 95% O₂ and 5% CO₂ for 30 min. During reperfusion, the perfusate was changed to KHB without (control group) or with ketamine, flunitrazepam, diazepam or midazolam.

At the end of reperfusion, the heart was quickly frozen in a liquid nitrogen and was freeze-dried for 6 days. We weighed pieces of freeze-dried samples used for measurements of metabolites. An aliquot was extracted with perchloric acid and centrifuged at 3000 r.p.m. Concentrations of high energy phosphates (ATP, ADP and AMP) were measured by a high performance liquid chromatography according to the modified method of those described by Wynants and Van Belle (16). The values were expressed as µmol per gram dry heart weight. Energy charge as a fundamental metabolic control parameter was calculated as described by Atkinson and Walton (17).

The data are expressed as means ± S.D. Testing for significant differences between the control and the anesthetic groups was accomplished by one-way ANOVA, followed by Dunnett's test. Intragroup comparisons of DO₂ to MVO₂ ratio were performed by two-way ANOVA for repeated measures, followed by paired *t*-tests with the Bonferroni correction. A probability of *P*<0.05 was regarded as statistically significant.

**RESULTS**

Before reperfusion, there were no significant differences in the coronary flow, cardiac output and left ventricular dP/dt maximum (LVₐₐₘₓ) between the control and

![Fig. 3. ATP, ADP, AMP and energy charge of the control and groups given two levels of ketamine, flunitrazepam, diazepam and midazolam. Energy charge was calculated as follows: \( \text{Energy Charge} = \frac{\text{ATP} + \frac{1}{2} \text{ADP}}{\text{ATP} + \text{ADP} + \text{AMP}} \). Filled columns: control, high density hatched columns: ketamine, dotted columns: flunitrazepam, low density hatched columns: diazepam, open columns: midazolam. Each point represents the mean ± S.D. for 8 hearts. *P*<0.05, as compared with the control group.](image)
the anesthetic groups (Table 1).

During reperfusion, cardiac output in the LK and LF groups were significantly lower than that in the C group (at the end of reperfusion: LK -19%, LF -17%, LD +4% and LM -2%, compared with control group value) (Table I). Cardiac output in all higher concentration anesthetic groups was significantly lower than that in the C group (at the end of reperfusion: HK -76%, HF -16%, HD -30% and HM -15%, compared with the control group value). Changes in LV\textsubscript{max} of ketamine groups were almost similar to those in cardiac output (Table I). Coronary flow in the LD, LM, HM groups were significantly higher than that in the C group (Table I).

Figure 2 shows the relationship between DO\textsubscript{2} and MVO\textsubscript{2}. We observed the elevation of oxygen supply-demand ratio in the early phase of reperfusion compared with the preischemic value in all groups. There were almost no significant differences in DO\textsubscript{2} to MVO\textsubscript{2} ratio among all groups. Only at the end of reperfusion, DO\textsubscript{2} to MVO\textsubscript{2} ratio in the HK group was significantly greater than that in the C group.

There were no significant differences in the myocardial high energy phosphates between the C and the lower concentration anesthetic hearts. On the other hand, the levels of ATP in all higher concentration anesthetic groups were significantly lower than that in the C group. The levels of energy charge in the higher concentration anesthetic groups were also lower than that in the C group (Fig. 3).

DISCUSSION

At 10 and 50 times of the therapeutic concentrations required for hypnosis and amnesia during surgery, the results in this study are summarized as follows: First, ketamine was the strongest in delaying the recovery of cardiac function during reperfusion. Secondly, higher concentration of ketamine increased the oxygen supply-demand ratio at the end of the reperfusion period (20 min after reperfusion). In contrast, flunitrazepam, diazepam and midazolam had no effect on this ratio. Thirdly, 10 times the therapeutic concentrations of these four anesthetics did not affect the myocardial metabolism in terms of the high energy phosphates. With regard to the higher concentration, they showed deleterious effects on post-ischemic metabolic recovery.

The cardiovascular action of these agents remains controversial (18–23). Ketamine produces sympathomimetic pressor effects mediated via an intact autonomic nervous system (11). Riou et al. (21) showed a dose-related positive inotropic response to ketamine even when adrenergic transmission was pharmacologically removed. In contrast, laboratory investigation on isolated guinea pig heart demonstrated that ketamine was more potent than midazolam in directly depressing contractility at equivalent doses for induction (18). Kashimoto et al. (24) indicated that ketamine markedly depressed cardiac function in the presence of moderate hemorrhage. Rusy et al. (25) showed that ketamine inhibition of transsarcolemmal Ca\textsuperscript{2+} influx played a major role in the negative inotropic action and that ketamine had relatively little effect on the availability of Ca\textsuperscript{2+} stored in and released from the sarcoplasmic reticulum. On the other hand, it is also suggested that ketamine produces positive inotropic effects by increasing Ca\textsuperscript{2+} influx (23). Recently, Endou et al. (26) have reported that these diverse effects could be explained by the consistent suppression of the transsarcolemmal ionic currents of ketamine and by the species and tissue differences in membrane ionic current systems. Thus the species, tissue differences, in vivo or in vitro, or Ca\textsuperscript{2+} concentration at which the studies were conducted may contribute to the conflicting interpretations of the effect of ketamine on contractile function. As for benzodiazepines, Reves et al. (27) showed a dose-related decrease in LV\textsubscript{max} after administration of diazepam or midazolam. They suggested that midazolam was 1.5-fold more potent than diazepam. In contrast to these reports, our previous study indicated that 10 and 100 times the therapeutic doses of ketamine, flunitrazepam, diazepam and midazolam on the heart-lung preparation did not have any depressant property on cardiac function (8). In the present study, we employed the same species as our previous experiment to compare reperfused heart with aerobic heart.

There are human studies showing effects of these agents on coronary artery disease (5, 28, 29). In unpremedicated patients with diseased coronary artery, the mean arterial pressure decreased 5% to 10% with diazepam (28) and 5% to 20% with flunitrazepam (29) and midazolam (5).

In some situations with stable angina, functional suppressive effects with anesthetics reduce myocardial oxygen demand and may work beneficially. On the other hand, the presence of intravenous anesthetics only during reperfusion may affect the heart deleteriously (7). Therefore, it was needed to investigate the direct effects of these anesthetics on reperfused heart to determine whether these agents were safe or not for anesthesia during the reperfusion period.

We quantitated the effects of intravenous anesthetics on myocardial performance during reperfusion in the working rat heart model, which eliminates interfering neurohumoral effects of in vivo studies and is independent of systemic vascular changes. We employed 10 and 50 times the therapeutic doses of each anesthetic to compare the results of our previous experiment, in which no cardiac depression was observed under aerobic isolated lung-heart preparation (8). In the present study using 50
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times the therapeutic concentrations, diazepam and midazolam depressed functional recovery of cardiac output and $dP/dt_{max}$ during reperfusion. The precise mechanism for these depressions by benzodiazepines remains unclear. It may involve attenuation of transsarcolemmal Ca$^{2+}$ flux (18). These anesthetics at 10 times their therapeutic concentrations had no deleterious effects on functional recovery during the post-ischemic reperfusion. These results suggest that clinical doses of diazepam and midazolam may be safe when they are administered during reperfusion. On the other hand, ketamine showed pronounced depression of cardiac output and negative inotropic effects expressed by decreased $dP/dt_{max}$. Rusy et al. (25) examined the effects of ketamine in isolated rabbit papillary muscles under conditions wherein the contractile activity depends predominantly on either the transsarcolemmal influx of Ca$^{2+}$ or the release of Ca$^{2+}$ stored in the sarcoplasmic reticulum. Their results suggest that ketamine exerts its negative inotropic effect mainly by reducing the transsarcolemmal influx of Ca$^{2+}$. In contrast to our previous study demonstrating no cardiac depression with the same dose ranges of the drugs (8), it is suggested that the presence of a higher dose of ketamine during the reperfusion phase may be deleterious as compared with the aerobic condition and that ketamine is relatively much more potent than flunitrazepam, diazepam and midazolam in directly depressing contractility.

If coronary autoregulation is intact, a decrease in coronary flow would be expected to occur concomitantly with reduction of oxygen consumption. However, the higher concentration of ketamine decreased oxygen demand and little affected coronary flow, and it increased the ratio of oxygen supply to demand. On the other hand, flunitrazepam, diazepam and midazolam did not alter this ratio at 20 min after reperfusion so that autoregulation was not affected by these three drugs. Stowe et al. (18) demonstrated in aerobic isolated guinea pig hearts that ketamine and midazolam attenuated autoregulation at high concentrations as demonstrated by an increased ratio of oxygen delivery to consumption. Although our results are consistent with those of Stowe et al. on the effects of ketamine, there is an inconsistency between their results and ours on midazolam. We observed the elevation of the oxygen supply-demand ratio in the early phase of reperfusion compared with the preischemic value even in the control group, suggesting that ischemia itself would attenuate coronary autoregulation. In contrast to their aerobic study, our study was performed under the reperfused condition; therefore, the present data could not support the findings from Stowe et al. (18) with regard to midazolam. Midazolam, flunitrazepam and diazepam may not cause further blunting of autoregulation when used during reperfusion, since the O$_2$ supply-demand ratio did not differ from that of the control.

In the present study, reperfusion was performed for 20 min, which might be a somewhat short time to compare functional recovery. However, we had to remove the hearts and measure myocardial metabolites to determine whether exposure to anesthetics affects recovery of myocardial energy metabolism during reperfusion. We expected that the myocardial high energy phosphate would fully recover after a long reperfusion, and it might be hard to compare the recovery rate among the groups. Previously, we reported that the ATP, ADP and AMP contents of isolated rat heart under the aerobic state were 25.0, 5.6 and 1.3 μmol/g dry wt., respectively (8). Therefore, it seems that the myocardial energy metabolism in the present control hearts had not recovered yet. In such a reperfusion, ATP contents and energy charge were decreased with all higher doses of intravenous anesthetics. However, intravenous anesthetics, even at 100 times the therapeutic doses, did not affect myocardial high energy phosphates unless the heart is ischemic (8). These results indicate that the presence of intravenous anesthetics would suppress the postischemic recovery of myocardial energy levels.

In humans, the anesthetic plasma concentration of ketamine, calculated as the mean of the ketamine concentrations during steady-state anesthesia, is 2.4 mg/l (11). The therapeutic concentration for a deep hypnotic effect is reported to be 0.6 mg/l for midazolam (12). Diazepam is approximately as potent as midazolam (3, 13). The plasma diazepam level after induction is 0.7 mg/l (10). In terms of sedative and hypnotic potencies, flunitrazepam is said to be about five to ten times as potent as midazolam and diazepam (3, 14, 15). Therefore, we considered that the concentrations employed in the present study were approximately 10 and 50 times the therapeutic concentrations required for hypnosis and amnesia during surgery.

The direct implication of our data to the clinical setting may be questioned, because there is the question of extrapolation from reperfusion of global ischemia to that of regional ischemia. Moreover, we employed a high concentration for this study, but such high doses are not usually used in clinical reperfusion after the cardio-pulmonary bypass procedure. Furthermore, many factors affect these plasma concentrations (e.g., speed of injection, volume of distribution, plasma protein concentration, pH), and our concentrations may not be accurately equi-potent among the four anesthetics. A limitation of our use of the rat heart preparation is that it is not quite an appropriate model for the clinical situation. Especially, for ketamine, rat heart is not entirely a suitable species for studying its negative inotropic effect (8, 26). However, even in such an animal model, ketamine impaired recov-
ery during reperfusion. There is also potential hemodynamic depression when benzodiazepines are administered in combination with other anesthetic agents (30, 31), the mechanism of which remains unclear. Besides our results, hemodynamic interactions of these agents with other drugs used in anesthesia remain to be examined.

In conclusion, at 50 times the therapeutic concentration, diazepam and midazolam depressed functional and metabolic recovery during reperfusion. However, they had no deleterious effects when 10 times the therapeutic doses were administered during the post-ischemic reperfusion. Ketamine had a stronger depressive effect on cardiac function than diazepam and midazolam did during reperfusion, and the depressive effect of flunitrazepam was moderate. As compared to our previous study wherein ketamine did not have any depressant property on cardiac function in the aerobic isolated rat heart (8), the administration of ketamine during reperfusion phase was harmful for post-ischemic recovery.

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