Preferable Anesthetic Conditions for Echocardiographic Determination of Murine Cardiac Function

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Abstract. Ketamine and xylazine are routinely used for measurement of hemodynamics of mice and rats by echocardiography. The anesthetic agents produce low heart rate (HR) in the animals, which may result in misleading data in the hemodynamic profiles of the small animals. The purpose of the present study was to select an appropriate anesthetic condition in the evaluation of mouse and rat cardiac function by echocardiography. Echocardiographic measurement was performed in male C57BL6 mice anesthetized with an intraperitoneal injection of 30 or 40 mg/kg pentobarbital (P30 or P40) or a combination of 60 mg/kg ketamine and 6 mg/kg xylazine (KX) and in male Wistar rats with an intraperitoneal injection of 40 or 50 mg/kg pentobarbital (P40 or P50) or a combination of 100 mg/kg ketamine and 10 mg/kg xylazine (KX). Basal HR of P30-anesthetized mice and P40-anesthetized were comparable to those in the conscious state, whereas KX-anesthetized mice and rats were 38% and 74% of those of the conscious animals, respectively. Fractional shortening (FS) and cardiac output index (COI) of the P30-anesthetized mice or the P40-anesthetized rats were greater than those of KX-anesthetized animals. Intraperitoneal injection of dobutamine at 0.3 and 1 mg/kg increased HR, FS, and COI of the P30-anesthetized mice and the P40-anesthetized rats, respectively, whereas the percent responses of these parameters in KX animals were greater than those in pentobarbital-anesthetized ones due to the lower basal values for the cardiac functional parameters. Anesthesia with P30 for the mouse and P40 for the rat rather than ketamine/xylazine may be relevant to the evaluation of cardiac function using echocardiography.

Keywords: anesthesia, blood flow, cardiac function, echocardiography, heart rate

Introduction

Recently, small animals, particularly mice with genetic alterations, have been created using sophisticated transgenic techniques. The animals are then used for the extrapolation of human diseases and the resulting information is utilized for the development of new drugs. Rats have also been used for the similar purposes in various fields of study. To evaluate physiological, pathophysiological, and pharmacological profiles of cardiac and hemodynamic function in animals, cardiac functional parameters such as fractional shortening, ejection fraction, cardiac output index, and stroke volume index are generally determined (1–3). The transthoracic echocardiography is one of the most efficient methods, with which cardiac morphology and performance in humans and animals without invasive damage are determined (2–6). This measurement is preferable with respect to a reduction in the number of experimental animals. Accordingly, echocardiography has become a standard for defining cardiac phenotype and screening transgenic animals, especially mice and rats (4, 6–10).

In the measurement of cardiac function in experimental animals, various anesthetic agents such as pentobarbital (1, 11), urethane (12–14), and ketamine (1, 5, 6, 10, 13, 15) have been employed to induce sedation and immobility. Apparently, the anesthetic...
agents directly and indirectly affect cardiac performance and hemodynamics (5, 11, 15). Therefore, anesthetic agents are an important factor when evaluating changes in these parameters under physiological and pathological conditions. Several anesthetic agents such as pentobarbital, tribromoethanol, isoflurane, and a combination of ketamine and xylazine are usually employed for the determination of cardiac function of mice or rats by echocardiography. Although gaseous anesthetic agents like isoflurane easily control the depth of anesthesia during operation, an appropriate apparatus is required for their use. Recently, tribromoethanol may not be recommended as an anesthetic agent for mice because of its harmful effects on morbidity and mortality for mice (16). A combination of ketamine and xylazine is frequently used as an anesthetic to determine echocardiographic parameters in mice (13, 15). However, the treatment exerts negative chronotropic and inotropic effects in the animal (10, 11, 13 – 15). In the present study, we examined the effects of anesthetic conditions with ketamine plus xylazine and pentobarbital on echocardiographic parameters in mice and rats to investigate preferable anesthetic conditions in small animals.

Materials and Methods

Animals

Male C57BL6 mice (SLC, Hamamatsu) weighing 20 – 25 g and male Wistar rats (SLC) weighing 220 – 250 g were used in the present study. The animals were conditioned according to The Guide for the Care and Use of Laboratory Animals as promulgated by the National Research Council. The protocol of this study was approved by the Committee of Animal Use and Welfare of Tokyo University of Pharmacy and Life Science.

Agents and anesthesia

The following agents were purchased: pentobarbital (Dainippon Pharm. Co., Osaka) and ketamine, xylazine, dobutamine, and atenolol (Sigma Chem, St. Louis, MO, USA). In the present study, mice were anesthetized with an intraperitoneal injection of 30 mg/kg pentobarbital (P30), 40 mg/kg pentobarbital (P40), or a combination of 60 mg/kg ketamine and 6 mg/kg xylazine (KX). Rats were similarly anesthetized with an intraperitoneal injection of 40 mg/kg pentobarbital (P40), 50 mg/kg pentobarbital (P50), or a combination of 100 mg/kg ketamine and 10 mg/kg xylazine (KX). The doses for ketamine and xylazine were selected according to the reports of others (17, 18). The dose for pentobarbital was set in consideration of the results showing heart rate (HR) similar to that of in vivo conscious animals in a preliminary study.

Echocardiography

After anesthesia, the left hemithorax of the mouse or rat was shaved. The animals were pre-warmed with a panel heater to maintain their rectal temperature at 37°C during the determination of cardiac parameters by echocardiography. Transthoracic echocardiography was performed by using ProSound 5500® (Aloka, Tokyo) with a 13-MHz linear transducer for mice and 10-MHz for rats in a phased array format, which offers 0.35-mm lateral resolution and 0.25-mm axial resolution, real time digital acquisition, storage, and review capabilities. The heart was first imaged in the two-dimensional mode in the parasternal long-axis view. From this view, an M-mode cursor was positioned perpendicular to the interventricular septum and posterior wall of the left ventricle at the level of the papillary muscles. Chamber dimensions were determined by the M-mode tracings. In contrast, a two-dimensional short-axis view of the mid-left ventricle at the chordal level was assessed by the B-mode tracings. Wall thickness was determined from this dimensional short-axis view.

Velocities of aortic and pulmonary arterial flows were measured with a pulsed wave Doppler in the parasternal long axis view (19 – 21). The transducer was positioned parallel to the aortic or pulmonary arterial root to receive the interrogation beam, which can obtain the maximal velocities of aortic or pulmonary arterial flow, and then the flow was monitored.

Cardiac parameters

The left ventricular internal diameters at end diastole (LVEndD) and systole (LVEndS) were measured by the M-mode tracings, and left ventricular posterior wall thickness diastole (LVPWd) and inter ventricular septal thickness diastole (IVSd), by the B-mode tracings. During diastole, LVIDd and wall thickness (LVPWd and IVSd) were estimated from the results on the maximum chamber cavity. During systole, LVIDs was estimated from the results on the maximal anterior motion of the posterior wall.

The left ventricular mass (LV mass) was calculated according to the following equation (15):

$$LV\ mass = 1.055 \times [\frac{IVSd + LVIDd + LVPWd}{3} - (LVIDd)^3]$$

where 1.055 is the specific gravity of the myocardium. The derived LV mass was normalized for body weight (LV/Body).

The left ventricular fractional shortening (FS), a measure of left ventricular systolic function, was
calculated from the M-mode echocardiogram of the left ventricular dimensions using the following equation (15):

\[ FS (%) = \left[ \frac{LVIDd - LVIDs}{LVIDd} \right] \times 100 \]

Ejection fraction (EF) was calculated from the left ventricular volume of the 2D long-axis view using the following equation (18):

\[ EF (%) = \left[ \frac{EDV - ESV}{EDV} \right] \times 100 \]

In this equation, EDV, a left ventricular end-diastolic volume, and ESV, a left ventricular systolic volume, represent the left ventricular diastolic and systolic volumes, respectively. EDV and ESV were calculated using the following equations (18, 22):

\[ EDV = (LVIDd)^3 \]
\[ ESV = (LVIDids)^3 \]

After the determination of aortic or pulmonary arterial flow, velocity-time integral (VTI) and aortic (AoD) or pulmonary arterial (PAD) diameters were measured. Cardiac output (CO) and stroke volume (SV) were calculated from the following equations (15):

\[ CO = \frac{Aod}{2 \pi \times VTI \times HR} \]
\[ SV = CO / HR \]

Then, CO and SV were normalized to the body weight of the animal to express CO and SV indices (COI and SVI), respectively.

**Effects of dobutamine on cardiac parameters of anesthetized mice or rats**

To elucidate the reactivity of anesthetized animals to a cardiotonic agent, the effects of a β₁-adrenoceptor stimulant, dobutamine, on HR, FS, and COI of mice anesthetized with P30, P40, and KX (n = 8 each) were examined. Rats were similarly anesthetized with an intraperitoneal injection of P40, P50, and KX (n = 8 each). Fifteen minutes after anesthesia with ketamine /xylazine or pentobarbital, 1 mg/kg dobutamine for mice or 0.3 mg/kg for rats was intraperitoneally injected. Then, 0.1 or 1 mg/kg atenolol was intraperitoneally injected to mice or rats, respectively. Ten minutes later 1 or 0.3 mg/kg dobutamine was administered intraperitoneally to mice or rats, respectively. Changes in cardiac parameters were monitored throughout the experiments with the echocardiographic system.

**Statistics**

The results are expressed as the means ± S.E.M. The statistical significance of differences in cardiac parameters was estimated using a one- or two-way analysis of variance (ANOVA) followed by Fisher’s PLSD correction for multiple comparisons. Differences with a probability of less than 5% were considered to be significant (P<0.05).

**Results**

**Anesthetic agents on mouse cardiac parameters**

Table 1 shows cardiac parameters determined by the echocardiographic system for the effects in P30-, P40-, and KX-anesthetized mice. The basal HR of conscious mice was 659 ± 22 beats/min and the basal blood pressure was 134 ± 2/91 ± 2 mmHg (n = 10). HRs of the mice anesthetized with P30, P40, and KX were 676 ± 29 (P = 0.921 vs Basal HR), 365 ± 15 (P < 0.001 vs Basal HR), and 251 ± 18 (P < 0.001 vs Basal HR) beats/min (n = 12 each), respectively. Treatment with P40 or KX resulted in a significant reduction in HR. The left panels in Fig. 1 show representative M-mode echocardiograms of mice anesthetized with P30, P40, and KX. Consistent with the reduction in HR, FS and EF were much more reduced in P40- and KX-anesthetized mice than P30-anesthetized mice. CO and COI values of the P30-anesthetized mice were greater than those of the other two groups, whereas the SV and SVI values of those mice were lower. LVIDd, LVIDs, ESV, and EDV values were smaller in the P30-anesthetized mice than KX-anesthetized mice. These values were slightly smaller in the P40-anesthetized mice than in KX-anesthetized mice.
Effects of anesthetic agents on rat cardiac parameters  
Table 2 shows cardiac parameters determined by the echocardiographic system in P40-, P50-, and KX-anesthetized mice. The HR of conscious rats was 402 ± 10 beats/min and the blood pressure was 148 ± 3/104 ± 2 mmHg (n = 10). KX anesthesia reduced HR to 296 ± 13 beats/min (P < 0.001 vs Basal HR, n = 10). The HR of the P40-anesthetized rats was 397 ± 4 beats/min (P = 0.799 vs Basal HR, n = 10), whereas that of the P50-anesthetized animals was 374 ± 3 beats/min (P = 0.011 vs Basal HR, n = 10). The right panels in Fig. 1 show representative M-mode echocardiograms of rats anesthetized with various anesthetic agents such as P40, P50, and KX. EF, FS, and COI values were lower in P50- and KX-anesthetized rats than P40-anesthetized rats. In contrast, the SVI value was similar among P40-, P50-, and KX-anesthetized rats.

Effects of xylazine on HR and duration of anesthesia  
Figure 2 shows effects of xylazine on HR (upper panel) and duration of anesthesia (lower panel) in 60 mg/kg ketamine-treated mice (left panel) and 100 mg/kg ketamine-treated rats (right panel). Xylazine at doses ranging from 0.2 to 20 mg/kg decreased HR of mice in a dose-dependent manner, which was accompanied by a prolongation of the anesthesia (righting reflex). Similarly, a decrease in HR and prolongation of anesthesia were observed in ketamine-treated rats with increased doses of xylazine.

Determination of cardiac output  
To determine cardiac output of mice or rats, blood flows in both aorta and pulmonary artery were measured using the color Doppler method. As shown in the left panel of Fig. 3, the COI value calculated from the pulmonary arterial flow (700 ± 36 µl/min per g, n = 12) was similar to that in the aorta (682 ± 78 µl/min per g, n = 12). It took approximately 5 min to determine the aortic blood flow of a mouse with echocardiography. In contrast, it took less than 1 min to determine the pulmonary arterial flow of a mouse.

The CO estimated from the pulmonary artery was similar to that derived from the aortic flow in rats. Similarly, the COI value estimated from the pulmonary arterial flow (410 ± 14 µl/min per g, n = 10) was similar to that determined by aortic flow (388 ± 36 µl/min per g, n = 10) (right panel in Fig. 3).

Effects of β1-adrenoceptor stimulant on cardiac parameters  
Figure 4a shows effects of 1 mg/kg dobutamine on HR, FS, and COI of the P30-, P40-, or KX-anesthetized mice. Dobutamine in the KX-anesthetized mice increased HR, FS, and COI to approximately 155%, 150%, and 160% of the corresponding pre-treatment value, respectively. Treatment of the P40-anesthetized mice with the β1-stimulant also increased the three parameters to approximately 180%, 175%, and 140% of the corresponding pre-treatment value, respectively. Although

Table 1. Cardiac parameters determined by the echocardiographic system in 30 mg/kg pentobarbital (P30)-, 40 mg/kg pentobarbital (P40)-, and 60 mg/kg ketamine plus 6 mg/kg xylazine (KX)-anesthetized mice

<table>
<thead>
<tr>
<th></th>
<th>P30</th>
<th>P40</th>
<th>KX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bw (g)</td>
<td>22.4 ± 0.5</td>
<td>22.1 ± 0.6</td>
<td>22.9 ± 0.6</td>
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<tr>
<td>LVIdd (mm)</td>
<td>3.03 ± 0.09</td>
<td>3.72 ± 0.07*</td>
<td>4.03 ± 0.07*</td>
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<tr>
<td>LVIds (mm)</td>
<td>1.70 ± 0.09</td>
<td>2.61 ± 0.06*</td>
<td>2.84 ± 0.06*</td>
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<td>FS (%)</td>
<td>44.0 ± 1.6</td>
<td>29.8 ± 1.0*</td>
<td>29.5 ± 0.8*</td>
</tr>
<tr>
<td>ESV (µl)</td>
<td>5.56 ± 1.17</td>
<td>18.10 ± 1.21*</td>
<td>23.49 ± 1.41*</td>
</tr>
<tr>
<td>EDV (µl)</td>
<td>28.81 ± 2.71</td>
<td>52.36 ± 3.14*</td>
<td>66.32 ± 3.33*</td>
</tr>
<tr>
<td>EF (%)</td>
<td>80.7 ± 3.8</td>
<td>65.4 ± 3.1*</td>
<td>64.6 ± 2.6*</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>676 ± 29</td>
<td>365 ± 15*</td>
<td>251 ± 18*</td>
</tr>
<tr>
<td>CO (ml/min)</td>
<td>16 ± 1</td>
<td>13 ± 1*</td>
<td>10 ± 1*</td>
</tr>
<tr>
<td>CO index (µl/min per g)</td>
<td>700 ± 36</td>
<td>586 ± 34*</td>
<td>457 ± 32*</td>
</tr>
<tr>
<td>SV (µl)</td>
<td>23 ± 1</td>
<td>33 ± 1*</td>
<td>37 ± 1*</td>
</tr>
<tr>
<td>SV index (µl/g)</td>
<td>1.03 ± 0.04</td>
<td>1.47 ± 0.05*</td>
<td>1.62 ± 0.06*</td>
</tr>
<tr>
<td>LV mass (mg)</td>
<td>87 ± 4</td>
<td>85 ± 4</td>
<td>91 ± 3</td>
</tr>
<tr>
<td>LV mass/Bw (mg/g)</td>
<td>3.88 ± 0.16</td>
<td>3.86 ± 0.14</td>
<td>3.96 ± 0.08</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of 12 animals. *Significantly different from the P30 group (P < 0.05).

Abbreviations: Bw, body weight; LVIdd, left ventricular internal diameter diastole; LVIds, left ventricular internal diameter systole; FS, fractional shortening; ESV, end-systolic volume; EDV, end-diastolic volume; EF, ejection fraction; HR, heart rate; CO, cardiac output; SV, stroke volume; LV mass, left ventricular mass.
treatment of the P30-anesthetized mice with the β1-stimulant increased the three parameters, the degree of the increase rate was smaller than that for the P40- or KX-anesthetized animals (approximately 105%, 120%, or 120%, respectively). Dobutamine-mediated changes in these cardiac parameters were abolished almost completely by treatment with 0.1 mg/kg of a β1-adrenoceptor antagonist, atenolol, the dose of which per se did not affect the cardiac parameters of the anesthetized mice.

Similarly, effects of dobutamine on cardiac parameters of anesthetized rats were examined (Fig. 4b). When the KX-anesthetized rats were treated with 0.3 mg/kg dobutamine, HR, FS, and COI were increased to approximately 160%, 165%, and 170% of the corresponding pre-treatment value, respectively. Dobut-
amine administered to the P50-anesthetized rats increased these three parameters to approximately 150%, 140%, and 160% of the pre-treatment values, respectively. Treatment of the P40-anesthetized rats with the $\beta_1$-stimulant also resulted in increases (approximately 130%, 135%, and 145% of the corresponding pre-treatment values). These positive chronotropic and inotropic effects of dobutamine were abolished by treatment with 1 mg/kg atenolol, regardless of treatment with any anesthetic agent.

### Discussion

In the present study, we studied the measurement of rodent cardiac function by echocardiography. In the determination of mouse cardiac function using an echocardiographic system, a combination of ketamine and xylazine is generally used for anesthesia (6, 11, 15). This anesthesia is convenient for the determination of cardiac parameters because of an appreciable reduction in HR of the experimental animals, which can determine several echocardiographic measures more conveniently. HR of small animals is reported to be very fast as compared with that of large animals or humans (15). In fact, we observed that HR of conscious mice was approximately 660 beats/min, which is so fast that the aortic flow of the animals is only assessable with much effort using an echocardiographic system. Although ketamine per se does not affect cardiac performance, its effective period is very short (less than 5 min). However, as seen in Fig. 2, xylazine greatly potentiated the anesthesia when combined with ketamine. Furthermore, the depth of xylazine anesthesia was dose-dependently associated with the decreased HR of ketamine-anesthetized animals. The results suggest that a decrease in HR, which substantially influences cardiac and...
hemodynamic parameters, is inevitable whenever a combination of ketamine and xylazine is used for anesthesia. Accordingly, it is doubtful whether the parameters for cardiac function determined under ketamine/xylazine anesthesia actually represent the physiological status of the animals. We found in the present study that values for FS, EF, and COI were markedly depressed in KX-anesthetized mice as compared with P30-anesthetized mice. Although anesthesia with P40 in mice also decreased cardiac pump function, the degree of the P40-evoked decreases was less than in the KX-anesthetized mice. Furthermore, we observed that anesthesia with pentobarbital, unlike KX-anesthesia, did not induce a decrease in HR and that the
Fig. 4. Effect of dobutamine on the heart rate (HR, upper panels), fractional shortening (FS, middle panels), and cardiac output index (COI, lower panels) of mice and rats. 

a: Mice were anesthetized with 30 mg/kg pentobarbital (P30, left panels), 40 mg/kg pentobarbital (P40, middle panels), and 60 mg/kg ketamine plus 6 mg/kg xylazine (KX, right panels) (n = 8 each). Intra-peritoneal administration of 1 mg/kg dobutamine (Dob) induced a significant increase in HR, FS, and COI in the mice. Pretreatment with an intraperitoneal injection of 0.1 mg/kg atenolol (Ate) abolished these increases almost completely (D + A).

b: Rats were anesthetized with 40 mg/kg pentobarbital (P40, left panels), 50 mg/kg pentobarbital (P50, middle panels), and 100 mg/kg ketamine plus 10 mg/kg xylazine (KX, right panels) (n = 8 each). HR, FS, and COI before drug treatment were determined at 15 min after anesthesia (Pre). Intrapertoneal administration of 0.3 mg/kg dobutamine induced significant increases in HR, FS, and COI in the rats. Pretreatment with an intraperitoneal injection of 1 mg/kg atenolol (Ate) abolished these increases almost completely (D + A). *Significantly different from mice or rats before drug treatment (Pre) (P<0.05). 

#Significantly different from dobutamine-treated mice or rats (Dob) (P<0.05).
HR of the P30-anesthetized mice was comparable to that of conscious animals. Thus, anesthesia with P30, rather than P40 or KX, may be suitable for evaluating mouse cardiac function using an echocardiographic system with respect to the maintenance of physiological levels of HR.

In another set of experiments, we examined the effects of anesthesia on cardiac parameters in rats. Results similar to those from the experiments in mice were obtained. That is, KX treatment resulted in a marked reduction in HR and decreases in cardiac pump function in rats, whereas P50 treatment caused relatively small reduction in HR and P40 treatment retained HR similar to that in the conscious state. As shown in Tables 1 and 2, HR directly affected the COI value of the animals, indicating that the anesthetic agent employed is a critical factor when assessing cardiac parameters by echocardiography. The results suggest that pentobarbital may be a preferable anesthetic agent to determine cardiac functional parameters of mice and rats in that these animals show physiological HR.

Generally, COI is calculated by dividing CO derived from blood flow of the ascending aorta with body weight (21, 24). Since the ascending aorta is located behind the pulmonary artery, it is difficult to directly position the transducer parallel to the aorta in mice and rats, which is required to measure the aortic flow. Accordingly, it usually takes approximately 5 min to determine the aortic flow. It is theoretically conceived that the blood flow of the ascending aorta is the same as that of the pulmonary artery. Thus, we might replace the aortic flow with the pulmonary arterial flow in the determination of COI. Since the pulmonary artery is located near the surface of the thoracic cavity, this location might also be a benefit to the measurement of blood flow with an echocardiographic system. We compared the pulmonary arterial flow with the ascending aortic blood flow to determine the COI of the anesthetized animal. As shown in Fig. 3, we observed that the standard errors for COIs in mice and rats derived from pulmonary arterial flow were small as compared with those determined from aortic flow, suggesting that values detectable by the former method are more consistent. Furthermore, we found that the time spent determining the pulmonary arterial flow was shorter. Accordingly, it is suggested that pulmonary arterial flow, instead of aortic flow, is suitable for the determination of COI in anesthetized small animals.

We examined the responsiveness of anesthetized animals to cardiotonic agents. For this purpose, the effects of anesthesia on β1-adrenoceptor agonist-mediated changes in mouse or rat cardiac function were determined by echocardiography. The HR, FS, and COI of the KX-anesthetized mice increased up to 50–60% as compared with the pre-treatment values when 1 mg/kg dobutamine was administered. In contrast, the dobutamine-mediated increase in HR, FS, and COI of the P30-anesthetized mice was less than 20% above the pre-treatment values. However, the absolute values for these parameters in response to dobutamine were higher in the pentobarbital-anesthetized than KX-anesthetized animals. The reduction in the rate of dobutamine-mediated increase in HR of the pentobarbital-anesthetized mice seems to be due to lower values for the basal HR of the KX-anesthetized mice in the pre-treatment period. In the case of rats, similar results and suggestions were obtained. Therefore, dobutamine-induced cardiotonic effects may be artificially overestimated in animals anesthetized with KX. The findings also indicate a significant role of physiological levels of HR in the determination of cardiac function of small rodents by echocardiography.

In conclusion, we found preferable anesthetic conditions, intraperitoneal administration of P30 anesthesia in mice and P40 anesthesia in rats, to measure cardiac performance by echocardiography. According to our method, cardiac function with conscious levels of HR could be conveniently determined.

References


