Impact of anesthesia on cardiac function during echocardiography in mice

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Roth, David M., James S. Swaney, Nancy D. Dalton, Elizabeth A. Gilpin, and John Ross, Jr. Impact of anesthesia on cardiac function during echocardiography in mice. Am J Physiol Heart Circ Physiol 282: H2134–H2140, 2002; 10.1152/ajpheart.00845.2001.—Anesthetics provide sedation and immobility facilitating echocardiography in mice, but influence cardiac function. We studied the effects of intraperitoneal and inhaled anesthetic agents on echocardiographic measurements. Mice were anesthetized with intraperitoneal tribromoethanol (TBE), ketamine-midazolam (K/M), ketamine-xylazine (K/X), or inhaled isoflurane (Isf), and echocardiographic parameters were assessed at 5, 10, 15, and 20 min. In C57BL/6N mice, Isf produced high initial heart rates (HR) that decreased to levels comparable to TBE at 15–20 min (~450 beats/min) and the most stable percent fractional shortening (%FS) and end-diastolic dimension (EDD). With TBE, %FS initially was low, but increased comparable to Isf (~45%) at 15 min. K/M produced similar time trends but lower absolute values compared with TBE for all parameters. K/X produced cardiac depression evidenced by low HR and %FS, and increased EDD. Isf was the most reproducible in repeat studies at 12 days. In C57BL/6J mice, compared with C57BL/6N mice, K/M produced higher HR, and %FS and TBE produced smaller EDD. In conclusion, anesthetic agent, timing of echocardiographic measurements, and genetic background are all critical variables during echocardiography in mice.

left ventricular function; tribromoethanol; ketamine; midazolam; xylazine; isoflurane

TRANSTHORACIC ECHOCARDIOGRAPHY is a valuable and widely applied tool for the noninvasive serial assessment of cardiac function in mice (8). Given the increasing numbers of transgenic and surgical models of cardiovascular disease in the mouse, echocardiography has become a standard approach for defining cardiac phenotypes and screening transgenic mouse colonies (20). Anesthesia provides sedation and immobility of animals during echocardiography greatly facilitating reliable data acquisition, but it is well recognized to have significant effects on cardiovascular function (22). Various anesthetic agents have been used to achieve sedation for echocardiography in mice, including intraperitoneal injection of ketamine and xylazine (K/X) (20), tribromoethanol (TBE; Avertin) (18), chloral hydrate (20), barbiturates [pentobarbital (26), thiobutabarbitol (15)], or inhalation of volatile anesthetics such as isoflurane (Isf) (3, 19) or halothane (5). These agents have diverse pharmacological profiles and produce multiple and variable cardiovascular effects in animals (1, 7). Despite the importance of anesthetic effects on cardiac function, few studies (2, 10, 19, 26) have been published concerning anesthesia during echocardiography in mice. No studies have compared the temporal response of echocardiographic measurements with the use of intraperitoneal and inhaled anesthetics. In addition, although anesthetic effects of barbiturates (9, 11), benzodiazepines (9), and volatile anesthetics (17) vary among mouse strains, only one study (10), which evaluated only TBE, has compared anesthesia in different mouse strains during echocardiography.

Accordingly, the present study was designed to examine the time course of intraperitoneal and volatile anesthetic effects on cardiac function. The reproducibility of each anesthetic agent was assessed by the performance of repeat echocardiographic measurements after 12 days, and the cardiac effects of intraperitoneal and volatile anesthetics were compared in two strains of the commonly used C57BL/6 mouse.

MATERIALS AND METHODS

Experimental animals. Animals were handled according to National Institutes of Health and institutional animal care and use committee guidelines of the University of California-San Diego. Male C57BL/6NCrlBR (C57BL/6N) or C57BL/6J mice (8–10 wk) were obtained from Charles River Laboratories (Wilmington, MA) and Jackson Laboratory (Bar Harbor, ME), respectively.

Experimental protocols. Groups of eight C57BL/6N mice were anesthetized with intraperitoneal TBE, ketamine-midazolam (K/M), K/X, or inhaled Isf. In addition, groups of eight C57BL/6J mice were anesthetized with TBE, K/M, and Isf. Heart rate (HR), left ventricular (LV) fractional shortening (%FS), LV end-diastolic dimension (EDD), and aortic ejection fraction (11) were measured.

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time (C57BL/6N group only) were assessed in all mice with the use of an echocardiograph (Sonos 5500, Agilent) and L15/6-MHz transducer. With mice in the left lateral decubitus position, a parasternal short-axis view was obtained for LV M-mode imaging at the papillary muscle level and LV chamber dimensions were determined.

Echocardiographic measurements were performed by a single blinded observer (N. D. Dalton) at 5, 10, 15, and 20 min after the induction of anesthesia. The intraobserver variability of this observer has been reported previously (20) and is 0.9 ± 8.2% (means ± 2 SD) (n = 12) for LV chamber diameter measurements in C57BL/6 mice. Time to loss and restoration of the righting reflex also were determined for each anesthetic agent. The study was repeated in C57BL/6 mice after 12 days to assess reproducibility of echocardiographic parameters with each anesthetic. One C57BL/6N mouse in the TBE group died in its cage between the first and second anesthetic trial.

Body and heart weight comparison. Before each study, individual mice were weighed to ensure that mice of similar age and body weight were used and to calculate proper anesthetic dosage in each treatment group. All mice were euthanized 1 mo after the study was completed to assess differences in cardiac size and body weight between experimental groups. After the mice were euthanized, the hearts were removed for morphometric comparison of whole heart size.

Statistical analysis. For the C57BL/6N mice, a repeated-measures analysis of variance was applied to the various echocardiographic measures, testing for interactions between anesthetic (TBE, K/M, K/X, and Isf) and the time course (5, 10, 15, and 20 min, respectively) and between anesthetic with time course for the two trials (three-way interaction) (24). Significant interactions would indicate that different anesthetics showed a different time course or that the time course differed in reproducibility for anesthetics over the two trials. A similar analysis examined time to loss of righting reflex and duration of loss of the reflex for an interaction between anesthetic and trial.

To compare the two strains of mice, C57BL/6N and C57BL/6J, another repeated-measures analysis of variance study used three anesthetics (TBE, K/M, and Isf) and examined interactions among the following: 1) mouse strain and anesthetic, 2) mouse strain, anesthetic, and time course, and 3) among mouse strain, anesthetics, time course, and trial. However, to illustrate differences between mouse strains, only values at 15 min, generally the time of maximum cardiac effect and adequate reproducibility, are shown.

In RESULTS, a statement pointing out significant differences reflects a statistically significant interaction (P < 0.05) for the interaction effect being described. When interactions were significant, a limited number of modified tests with Bonferroni’s correction for multiple comparisons were carried out to establish differences between anesthetics (23). All analyses were carried out with the use of Statistical Analysis Software (SAS) (16). Data are represented as means ± SE.

RESULTS

Time trends for anesthetic agents. The effects of anesthetics on echocardiographic measurements in C57BL/6N mice over the 20-min study are shown in Fig. 1. Mice anesthetized with TBE showed a minimal

Fig. 1. Echocardiographic measurements of heart rate (HR) (A), end-diastolic dimension (EDD) (B), %fractional shortening (%FS) (C), and aortic ejection time (D) using tribromoethanol (TBE), ketamine-midazolam (K/M), ketamine-xyalazine (K/X), and isoflurane (Isf) at 5, 10, 15, and 20 min postinjection, respectively. At 15 min, HR (KX vs. Isf and K/M; P < 0.05), %FS (TBE vs. KX; P < 0.05), EDD (K/M vs. KX; P < 0.05), and aortic ejection time (KX vs. TBE, K/M, and Isf; P < 0.05) were measured. There was a highly significant interaction of time with anesthetic for both HR, %FS, and aortic ejection time (P < 0.0001), but not for EDD (P = 0.07).
increase in HR over time. However, TBE produced a large increase in %FS from a decreased level to a stable normal level (~45%) at 15 min and a moderate decrease in EDD over time. M-mode echocardiographic images provided in Fig. 2 illustrate the changes in cardiac function over time with TBE. Mice anesthetized with K/M showed gradual increases in HR and %FS to levels less than those with TBE at 15–20 min, but no changes were noted in EDD over time. The greatest cardiac depression was observed during anesthesia with K/X; HR was low initially and continued to slow, and the slow HRs coincided with reduced %FS and enlarged EDD values compared with the other anesthetic agents. Isf produced the highest HRs and the greatest stability in %FS over the 20-min study. HR and %FS were comparable for Isf and TBE at the 15-min time point. Aortic ejection time increased dramatically over the 20-min study in the K/X group but was relatively stable over time for the other anesthetics. These data indicate that the anesthetic agent and the time after induction of anesthesia directly affect echocardiographic measurements.

Reproducibility of anesthetic agents. The effects of TBE, K/M, and Isf on cardiac function in C57BL/6N mice during repeated echocardiographic examination 12 days apart are shown in Fig. 3. TBE and K/M showed similar values for HR, %FS, and EDD at some of the time points for each trial, but other values were dramatically different, with K/M showing more variability between the two studies at 15–20 min. Isf was the most reproducible compared with TBE and K/M such that there were no significant differences in HR, %FS, and EDD between the 2 separate trials. These data support the idea that the volatile anesthetic Isf provides the greatest reproducibility when anesthetizing mice during repeat studies.

Strain differences and anesthetic agents. The effects of anesthesia on cardiac function in C57BL/6N and C57BL/6J strains of mice are presented in Fig. 4. Fifteen minutes after induction of anesthesia with TBE, no difference in HR or %FS was noted between the strains; however, EDD was significantly smaller in C57BL/6J mice compared with C57BL/6N mice (Fig. 4A). Mice anesthetized with K/M showed significantly increased HR and %FS values and significantly decreased EDD in C57BL/6J compared with C57BL/6N mice (Fig. 4B). In contrast, no difference in HR, %FS or EDD was noted between groups during anesthesia with Isf (Fig. 4C). These data indicate that the anesthetic effect on cardiac function may vary with the strain of mouse studied.

Onset and duration of anesthesia. The onset and duration of anesthesia, assessed by the righting time, are shown in Table 1. Righting times between C57BL/6N and C57BL/6J mouse strains showed no significant difference in the time to loss of righting reflex for any of the anesthetics used. However, the time to regain righting reflex was significantly shorter for C57BL/6N mice when anesthetized with TBE and significantly longer with K/M, demonstrating variability in anesthetic effect between the two strains. The analysis of anesthetics within strains TBE, K/M, and K/X produced nearly identical times for onset of anesthesia for C57BL/6N mice; however, anesthesia duration was markedly increased for K/X. In C57BL/6J mice, there was no difference in time to onset of anesthesia using TBE and K/M; however, anesthesia with TBE produced the longest duration to recovery. Anesthesia with Isf exhibited the most rapid loss and regaining of righting reflex in either strain of mouse except for K/M in the C57BL/6J group.

Body weight and heart weight. Body weights at the time of echocardiographic measurement and final heart weights for C57BL/6N and C57BL/6J are shown in Table 1. There were no significant differences in initial
Our study demonstrates that the type of anesthesia and the timing of echocardiographic measurements after anesthesia can have significant effects on echocardiographic parameters in mice. Reproducibility of echocardiographic measurements twelve days later was not substantially different between anesthetics. Differences in anesthetic effects on righting reflex, HR, and echocardiographic measurements were found between mouse strains.

Comparison of intraperitoneal versus inhaled volatile anesthesia. Intraperitoneal injection of the dissociative anesthetic ketamine combined with the $\alpha_2$-agonist xylazine (K/X) (19, 20, 26) and TBE (6, 12, 18), formerly available under the trade name Avertin, have been used by many investigators during echocardiographic studies of the mouse. K/X can produce bradycardia and hypotension in mice (25) and produced the greatest cardiac depression in our study. Intraperitoneal TBE produces less hemodynamic compromise in the mouse; however, toxicity and mortality have been reported (25, 27) that may be eliminated by proper storage of the drug (4°C and dark conditions) (14). The benzodiazepine midazolam produces minimal cardiac depression when used alone (7). Midazolam combined with ketamine in our study produced less cardiac depression than K/X but greater cardiac depression than TBE. All intraperitoneal agents showed early depression of cardiac function and exhibited an increase in %FS over the 20-min study. All intraperitoneal agents except K/X were associated with increasing HR.

Isf is being used in mice during echocardiography (3, 19). Inhalation of volatile anesthetics obviates needle injection and provides a steady-state level of anesthetic concentration that theoretically may lessen the time dependence of echocardiographic measurements. Isf generally produces minimal cardiac depression and has a higher molecular stability compared with other volatile anesthetics such as halothane and methoxyflurane, which limits metabolism and reduces the potential for toxicity (1). Isf in our study showed less variation over time in %FS compared with TBE and K/M. Chaves et al. (2) recently found less variability in echocardiographic measurements in mice with inhaled halothane compared with intraperitoneal K/X.

Timing of measurements. A novel finding of our study was the significant variability of echocardiographic measurements over time after induction of anesthesia. This finding was most striking for the
intraperitoneal anesthetics. Most studies do not report the time after anesthesia that echocardiographic images are acquired. However, it is clear from our study that time is a critical variable. Time of measurements should be controlled for and reported in investigations to allow for comparison of cardiac function between experimental groups in a single study and between studies by different investigators.

Animal strain. The anesthetic effects of barbiturates, benzodiazepines, and volatile anesthetics vary among mouse strains (9, 11, 17). Kiatchoosakun et al. (10) compared TBE anesthesia during echocardiography in three mouse strains and found differences in LV EDDs between C57BL/6J and A/J mice but concluded that the degree of cardiac depression resulting from TBE anesthesia was statistically similar in C57BL/6J, A/J, and FVB/N strains. Interestingly, despite comparing two closely related strains of C57BL/6 mice, we found TBE produced similar HR and %FS values; however, EDD varied significantly between strains. K/M produced significant differences for all cardiac parameters and a doubling in the time to regain righting reflex when the two strains were compared. Variation in sleep time between mouse strains following midazolam.

Table 1. Data for time to loss and regaining of righting reflex, initial mouse body weight before study, and final whole heart weight for mice anesthetized with TBE, K/M, K/X, and Isf

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<th>C57BL/6N Mice</th>
<th>C57BL/6J Mice</th>
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<td>TBE</td>
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<td>TBE</td>
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<td>Righting time, min</td>
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<tr>
<td>Loss</td>
<td>1.5 ± 0.3(8)</td>
<td>1.8 ± 0.1(8)</td>
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<td>Regain</td>
<td>51.0 ± 7.9(8)</td>
<td>45.1 ± 7.3(8)</td>
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<td>Initial body weight, g</td>
<td>25.7 ± 0.2(8)</td>
<td>25.4 ± 0.4(8)</td>
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<tr>
<td>Final whole heart weight, mg</td>
<td>123 ± 3.7(6)</td>
<td>128 ± 3.7(8)</td>
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Values are group means ± SE; values in parentheses represent the number of animals in each group. TBE, tribromoethanol; K/M, ketamine-midazolam; K/X, ketamine-xylazine; Isf, isoflurane. *P < 0.05 vs. C57BL/6N TBE group; †P < 0.01 vs. C57BL/6N K/M group.
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lam may vary >300% (9). These findings may reflect a lighter plane of anesthesia with K/M between C57BL/6J and C57BL/6N mice and subsequently less cardiac depression. Isf produced no differences in any of the cardiac parameters between the two strains. These differences show that one anesthetic agent may not be suitable for all mouse strains and emphasize the need for strain-matched control groups in echocardiographic studies.

Ideal approach to echocardiography in mice. The ideal anesthesia for mouse echocardiography should be easy to administer, reproducible, rapid in onset and recovery, cause minimal HR and cardiac depression, and have low toxicity. Identification of an ideal agent for all studies is difficult, but Isf and TBE came closest to these goals. K/M showed less reproducibility and slower HR and %FS, although values approached normal in one mouse strain (C57BL/6J).

Some suggest echocardiography in conscious mice is preferable to using anesthetics. Yang et al. (26) found significant decreases in HR and cardiac contractility with intraperitoneal pentobarbital sodium or KX compared with conscious mice. Awake measurements required training sessions to prevent bradycardia during transducer placement and awake heart rates averaged 658 beats/min, a value significantly higher than some HR quoted for unrestrained mice during telemetry (568 beats/min) (21), suggesting the possibility of elevated sympathetic tone during awake measurements. Similar HR and %FS values were reported by Esposito et al. (4) during conscious echocardiography in mice. Kiatchoosakun et al. (10) reported HR of 612, 680, and 732 beats/min in conscious A/J, C57BL/6J, and FVB/N mouse strains, respectively, and were unable to adequately perform Doppler measurements in conscious mice despite training and manual restraint. Takuma et al. (19) found %FS was not different between Isf anesthesia and conscious mice and noted in awake mice variations in probe position may have affected the results. The necessity of training regimens, problems with transducer placement, variations in sympathetic and parasympathetic tone during restraint, and relatively high HR make measurements in conscious mice less than ideal for all applications of echocardiography. Thus an ideal method for echocardiography in mice appears not to be available because studies in conscious mice have higher HR and the best anesthetics produce somewhat lower heart rates than those in unrestrained mice, and HR in mice correlate well with cardiac contractility (13).

Choice of anesthetic. We evaluated anesthetics only in mice with normal cardiac function. The “ideal” anesthetic for mice with cardiomyopathy may differ depending on the study goals, the type and extent of cardiomyopathy, and the strain of mouse. For example, anesthetics with less cardiac depression such as Isf and TBE (or perhaps conscious measurements) may be used to establish whether or not cardiac function and morphology are normal in transgenic or knockout mice. Isf or TBE would be the anesthetic of choice if cardiac function were known to be depressed. If repeated or prolonged studies were needed under stable conditions in a single study, Isf would clearly be preferable. On the other hand, K/X may be advantageous to reduce HR and cardiac contractility in evaluating echocardiographic and Doppler measurements in models of hypertrophic cardiomyopathy, in which there is hyperfunction and impaired cardiac relaxation.

Limitations of current study. We examined only one dose of each anesthetic, although doses were selected to be in agreement with published reports. Given the time constraint of repeat measurements at 5 min, we were able to make only one Doppler measurement (aortic ejection time). Future studies will be necessary to evaluate the full impact of anesthesia on Doppler measurements in mice.

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