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Effects of Tribromoethanol Anesthesia on Echocardiographic Assessment of Left Ventricular Function in Mice

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Background and Purpose: **Pentobarbital and ketamine-xylazine anesthesia in mice result in markedly decreased left ventricular fractional shortening and cardiac output. However, to the authors' knowledge, the effect of short-acting, alcohol-based anesthesia on these parameters is unknown.**

Methods: **Fifteen mice (FVB/N, C57Bl/6J, A/J, n = 5 each) underwent high-resolution (15 MHz) 2-dimensional-directed M-mode echocardiography before and after undergoing 2.5% tribromoethanol anesthesia (0.01 ml/g of body weight).**

Results: **Tribromoethanol anesthesia resulted in significant heart rate slowing (29%) and left ventricular enlargement (20%), and a more modest (12%) reduction in left ventricular fractional shortening. Cardiac output was unchanged. The differences in left ventricular function between conscious and tribromoethanol studies were similar for each of the three strains of mice.**

Conclusions: **Tribromoethanol anesthesia induced only modest effects on M-mode estimates of basal cardiac function and did not influence cardiac output. The effects to tribromoethanol anesthesia were similar among three commonly used mice strains.**

Echocardiography is a well-established non-invasive procedure used to assess cardiac morphology and function in anesthetized mice (1-3). However, anesthesia may affect cardiac function by direct effects on the cardiovascular system and indirect effects on circulatory control (4). Recently, it was documented that, compared with responses in conscious mice, pentobarbital and ketamine-xylazine anesthesia in mice results in markedly decreased left ventricular fractional shortening and reduced cardiac output (5).

Tribromoethanol (often inappropriately referred to as Avertin, the commercial drug, which is no longer available in the United States) is a short-acting, alcohol-based anesthetic used frequently for echocardiographic studies in mice because of its rapid onset of effect, wide range of tolerance, and safety (2, 6). However, the effect of tribromoethanol on left ventricular function and the existence of strain-dependent effects in adult mice are unknown. Therefore, we performed M-mode echocardiographic studies in three common mouse strains in the conscious state and after tribromoethanol anesthesia.

Materials and Methods

All experiments were conducted in accordance with institutional guidelines, and the Institutional Animal Care and Use Committee at Case Western Reserve University approved the experimental protocol. Animals were housed in an AAALAC-approved facility, using static microisolator cages at 22°C, 40 to 60% humidity, and time-controlled (12:12 h) lighting. All animals were specific pathogen free and were test negative for murine viruses and parasites. Cage bedding was changed weekly and was handled in laminar flow hoods, using Clidox disinfectant.

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Mice: Fifteen mice (*Mus musculus*) of three strains (FVB/N [Charles River Laboratories, Wilmington, Mass.] and C57Bl/6J and A/J [The Jackson Laboratory, Bar Harbor, Maine]) were studied. Mice (n = 5 for each strain) of either sex, 10 weeks old, and weighing 24.6 ± 2.5g, were "trained" for 5 to 10 min on three occasions over a period of three to five days. Training consisted of acclimating the mouse to manual restraint and application of the echocardiographic probe. Images obtained during the training period were not recorded.

For conscious studies, the mouse was restrained by the nape of the neck and held firmly in one hand. The hair over the thorax was shaved, and pre-warmed ultrasound transmission gel (Parker Laboratories, Inc., Fairfield, N.J.) was applied to skin over the precordium. Two-dimensionally (2D) directed — mode echocardiography was then performed (Fig. 1). All mice were restrained for fewer than 5 min for conscious measurements.

Anesthesia: Tribromoethanol was formulated by mixing 10 g of tribromomethyl alcohol with 10 ml of tertiary amyl alcohol (Aldrich Chemical Company, Milwaukee, Wis.). This stock solution was diluted to a 2.5% solution by addition of sterile water, and was stored in the dark at 2 to 4°C. Tribromoethanol anesthesia (2.5%, 0.01ml/g of body weight) (7) was subsequently administered intraperitoneally within 15 min of conscious measurements, using a sterile 29-gauge needle. The extremities were secured to the examining surface with paper tape. A warming pad (Deltaphase Isothermal Pad, Braintree Scientific, Inc., Braintree, Mass.) was used to maintain normothermia, then the echocardiographic study was repeated (Fig. 1). All animals completely recovered and were observed for 10 to 14 days. Anesthesia-related morbidity or mortality was not found during this study.

Echocardiographic studies were performed, using a 15-MHz (15L8) phased array transducer (Acuson Sequoia, Acuson Corporation, Mountain View, Calif.). Mice were imaged in the shallow left lateral decubitus position and short- and long-axis view

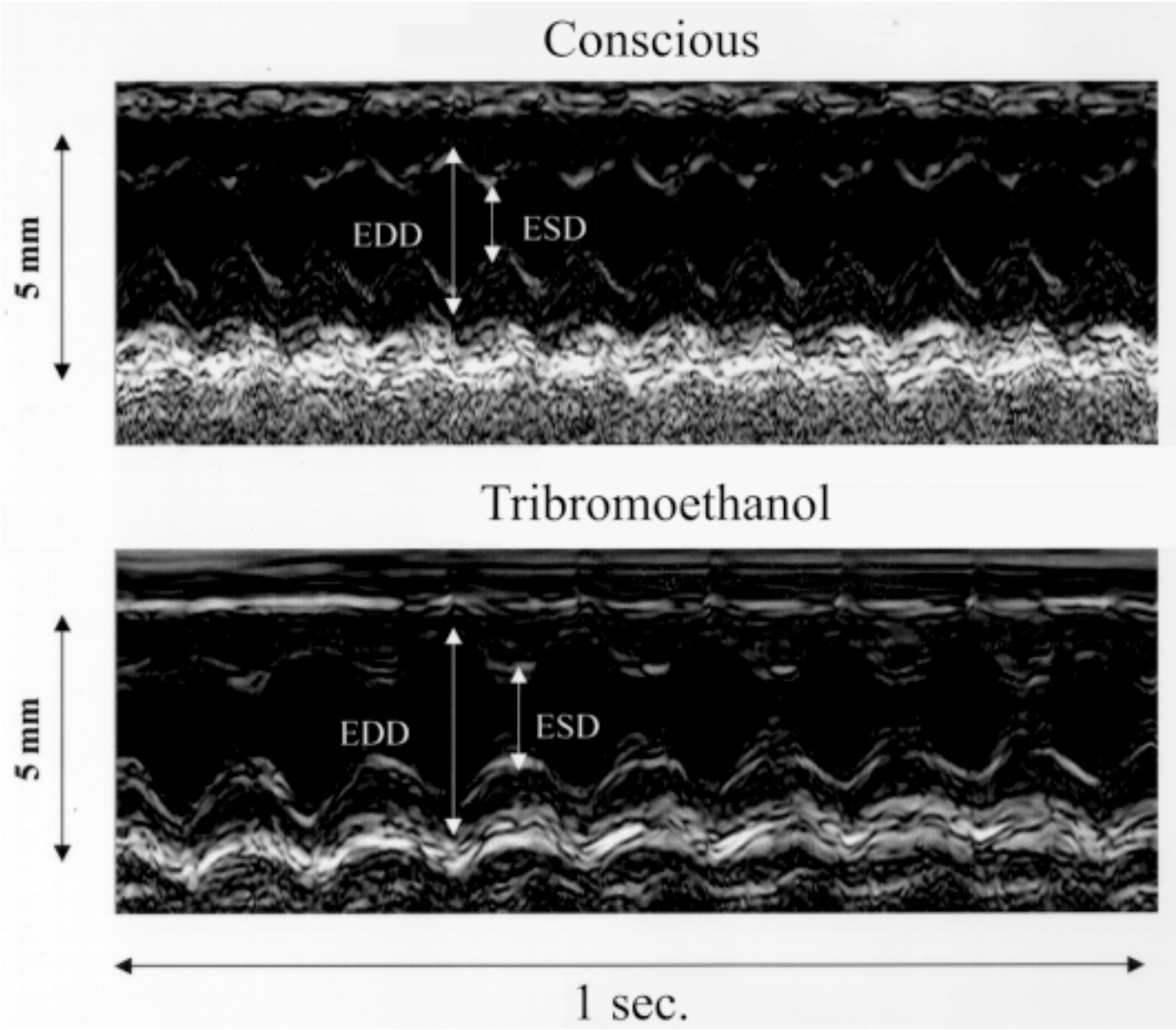

Figure 1. Representative M-mode echocardiograms from a conscious (top) and a tribromoethanol-anesthetized (bottom) mouse. EDD = end-diastolic dimension, and ESD = end-systolic dimension.

of the left ventricle were obtained by angulation and rotation of the transducer. M-Mode tracings were generally obtained from the short-axis view at the level of the largest left ventricular diameter, but occasionally the best M-mode tracings were obtained from the long-axis view. Tracings were recorded in digital format on a magneto-optical disk for subsequent review and analysis.

Data analysis: Left ventricular end-diastolic (LVEDD) and end-systolic (LVESD) dimensions were measured from the M-mode tracing at the time of maximal and minimal chamber dimensions, respectively. Interventricular septum and caudal wall thicknesses were measured at end-diastole. All measurements were made, using leading edge to leading edge, according to American Society of Echocardiography guidelines (8).

Left ventricular fractional shortening (LV FS) was calculated from the equation:

$$\text{LV FS} = ([\text{LVEDD}–\text{LVESD}]/\text{LVEDD}) \times 100.$$

Stroke volume (SV) and cardiac output (CO) were calculated as follows:

$$\text{SV} = (\text{LVEDD})^3 – (\text{LVESD})^3, \text{ and } \text{CO} = \text{SV} \times \text{HR}.$$

Interpretative variability: A total of 15 beats (5 from each strain) were selected at random from 15 animals in the conscious and anesthetized state, and were analyzed for left ventricular dimensions and fractional shortening by two observers. A single observer repeated M-mode echocardiographic measurements from 15 beats on a separate day to determine intra-observer variability. Interobserver and intra-observer differences were calculated as the absolute value of the difference between two observations divided by the mean of the observations, and were expressed as percentages. Variability was also quantified by use of the Bland-Altman method, a technique for assessing

the agreement between two methods of clinical measurement (9). It is also a useful statistical test for examining repeatability of a method and interpretative variability between two observers. Thus, if two measurements (or observers) have "good agreement," the differences between them should be close to zero, and the 95% confidence limits of the mean should be narrow.

Statistical analysis: All data are presented as mean ± SD. M-Mode echocardiographic parameters and heart rate were compared in conscious and anesthetized mice by use of paired *t*-tests. One-way analysis of variance (ANOVA) was used to compare the differences between conscious and tribromoethanol studies among the various mouse strains. A value $P < 0.05$ was considered significant.

Results

Effect of tribromoethanol anesthesia on left ventricular function: The effects of tribromoethanol on left ventricular function, heart rate and cardiac output are summarized in Table 1. Tribromoethanol anesthesia caused a small (12%), but significant reduction of fractional shortening; however, the depressant effects of tribromoethanol on heart rate were greater (29%). The heart rate-slowing effect of tribromoethanol induced left ventricular chamber dilatation, and as a result, calculated cardiac output was not significantly changed. The increased left ventricular end-diastolic dimension was (as anticipated) accompanied by thinning of the septal and caudal walls. Adequate Doppler waveforms were not available in all animals of this study and accordingly, were not anal0yzed.

Effect of mouse strain: Except for a slightly higher fractional shortening (51.6 ± 3.0% versus 46.7 ± 2.0%) and heart rate (732 ± 26 beats/min [bpm] versus 612 ± 78 bpm) in the FVB/N, compared with the A/J strain, baseline values in conscious animals were not significantly different among the three mice strains.

The effects of anesthesia on left ventricular fractional shortening, cardiac output, and heart rate were statistically similar among the three mouse strains. However, the effects of tribromoethanol on left ventricular end-diastolic and end-systolic diameters were slightly, but significantly different in the C57Bl/6J, compared with the A/J strain (Table 2).

Interpretative variability: Interpretative variability is summarized in Table 3. Technically adequate M-mode tracings were obtained from all animals. Intra- and interobserver variability for LVEDD, LVESD, and LV FS were acceptable and were similar during the anesthetized and conscious states.

Discussion

The principle findings of this study were: tribromoethanol anesthesia induces only modest effects on M-mode echocardiographic estimates of basal cardiac function and does not influence cardiac output; cardiovascular functional effects of tribromoethanol anesthesia are similar among FVB/N, A/J, and C57Bl/6J mouse strains; and interpretative variability of M-mode chamber dimension is similar and acceptable between conscious and anesthetized mice. Thus, "good agreement between observations was observed in conscious and anesthetized states."

Transgenic and gene-targeting techniques are powerful methods for identifying the role of various genes in regulation of hemodynamics and cardiac function during physiologic and pathologic conditions. Echocardiography is a simple technique that allows non-invasive, repetitive, and accurate assessment of left ventricular ejection performance (1-3). However, anesthesia is generally used for murine echocardiography; importantly, most anesthetics have cardiodepressant effects that potentially influence the study results. For example, diazepam, pentobarbital, and halothane are potent direct negative inotropic drugs (4). Recently, it was documented that pentobarbital and ketamine-xylazine anesthesia markedly decrease cardiac hemodynamics and performance significantly in mice, as was indicated by decreased mean arterial blood pressure, heart rate, echocardiographic fractional shortening, and cardiac output (5). Although we did not directly examine the effects of these anesthetics, the magnitude of cardiac depression those investigators observed was greater than that seen by us. Thus, the decreases in cardiac output and fractional shortening associated with pentobarbital were 34 and 35%, respectively, and similar decreases were reported for ketamine and xylazine. In contrast, we found a 12% reduction in fractional shortening, and no change in cardiac output.

Tribromoethanol, a short-acting, alcohol-based anesthetic has received widespread acceptance in many murine laboratories. Although some serious side effects have been reported (10-12), the safety and efficacy of tribromoethanol in mice are well documented (6). The lack of details regarding storage conditions and age of the anesthetic and presence of decomposition products (due to improper storage) make interpretation of reported adverse effects of tribromeoethanol difficult (6). Although our study was not designed to evaluate the safety of tribromoethanol, we did not observe associated morbidity or mortality.

Tribromoethanol may induce CNS depression, involving the respiratory and the cardiovascular centers (13). In the mouse embryo, tribromoethanol caused significant, but transient cardiac arrhythmia (14). To the authors' knowledge, the effect of this anesthetic on cardiac performance in adult mice has not been evaluated. Our study indicated that tribromoethanol had only modest effects on left ventricular fractional shortening and heart rate, and did not significantly alter cardiac output. Moreover, we observed similar effects in three widely used strains. However, the small differences owing to anesthesia in the A/J strain (significant only for left ventricular dimensions) suggests the potential for strain dependency, and warrants caution when comparing cardiovascular parameters among tribromoethanol-anesthetized strains of mice.

There are several limitations of this study that merit discussion. First, Doppler echocardiographic studies were not analyzed. Despite a brief training period and manual restraint, animal movement remained an important obstacle for obtaining a good Doppler signal, particularly aortic velocity with a narrow inter-

Table 1. M-Mode echocardiographic data from conscious (n = 15) and tribromoethanol (TriBE)-anesthetized (n = 15) mice

Variable	IVST (mm)	CWT (mm)	LVEDD (mm)	LVESD (mm)	LV FS (%)	HR (bpm)	CO (ml/min)
Conscious	0.47 ± 0.02	0.47 ± 0.01	3.12 ± 0.25	1.59 ± 0.15	49.3 ± 3.0	680 ± 70	18.27 ± 4.5
TriBE	0.40 ± 0.0*	0.40 ± 0.0*	3.74 ± 0.3*	2.13 ± 0.2*	43.1 ± 3.9*	480 ± 3*	20.83 ± 5.64

Values are means ± SD; IVST = interventricular septal thickness; CWT = caudal wall thickness; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; LV FS, left ventricular fractional shortening; HR, heart rate; CO, cardiac output. *$P < 0.05$.

Table 2. Effect of tribromoethanol anesthesia on echocardiographic variables among three mice strains

Strain	IVST (mm)	PWT (mm)	LVEDD (mm)	LVESD (mm)	LVFS (%)	HR (bpm)	CO (ml/min)
A/J	0.06 ± 0.02	0.07 ± 0.02	-0.33 ± 0.08	-0.31 ± 0.06	3.9 ± 1.8	144 ± 80	0.8 ± 4.4
FVB/N	0.07 ± 0.02	0.07 ± 0.01	-0.65 ± 0.35	-0.56 ± 0.28	6.5 ± 4.9	240 ± 60	-2.6 ± 6.3
C57Bl/6J	0.07 ± 0.03	0.07 ± 0.03	-0.86 ± 0.23*	-0.75 ± 0.18*	8.0 ± 4.3	216 ± 33	-5.3 ± 3.6

Values are mean ± SD differences between conscious state minus tribromoethanol anesthesia. *$P < 0.05$.
See Table 1 for key.

Table 3. Interpretative variability of M-mode echocardiographic measurements

	Interobserver error	
Variable	Conscious % (mean of difference ± SD)	Tribromoethanol % (mean of difference ± SD)
LV EDD	3.9 ± 2.7 (0.17 ± 0.08 mm)	5.4 ± 2.6 (0.14 ± 0.09 mm)
LV ESD	5.6 ± 4.8 (0.14 ± 0.08 mm)	8.5 ± 4.7 (.125 ± 0.12 mm)
LV FS	6.3 ± 5.3 (2.75 ± 2.41%)	5.4 ± 4.7 (2.68 ± 2.12 %)
LVEDD	2.4 ± 2.1 (0.29 ± 0.260 mm)	3.4 ± 2.1 (0.51 ± 0.30 mm)
LVESD	3.2 ± 2.2 (0.19 ± 0.13 mm)	4.4 ± 3.7 (0.19 ± 0.13 mm)
LV FS	2.2 ± 1.7 (4.49 ± 3.63 %)	2.2 ± 1.3 (3.91 ± 2.50%)

Values are mean ± SD.
See Table 1 for key.

cept angle. In addition, the rapid heart rate during the conscious state resulted in fusion of transmitral diastolic velocities. Second, stroke volume was calculated from single left ventricular dimensions, which requires assumptions of uniform ventricular wall motion and spherical cardiac geometry. However, regional wall motion abnormalities were neither anticipated nor observed on the 2D-directed echocardiographic study. A related problem is that, although mice were "trained," it is likely that the need for manual restraint precluded true baseline measurements. In this regard, it is interesting that our baseline cardiac output determinations (indexed for body weight) were similar to those reported using radioactive microspheres in conscious mice (15). Third, although cardiac depression in our study was less than that reported for pentobarbital and ketamine/xylazine anesthesia (5), it is difficult to evaluate anesthetic effects in different studies; a side-by-side comparison of multiple anesthetic agents is warranted. Finally, the effect of tribromoethanol on arterial blood pressure (i.e., the left ventricular afterload), which is a critical determinant of left ventricular ejection performance was not assessed. Thus, the influence of tribromoethanol on intrinsic myocardial contractile state cannot be determined with confidence. Nevertheless, we conclude that tribromoethanol anesthesia has minimal effects on M-mode echocardiographic estimates of left ventricular fractional shortening and cardiac output.

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