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Reports

Comparing Isoflurane with Tribromoethanol Anesthesia for Echocardiographic Phenotyping of Transgenic Mice

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Cardiac phenotyping of transgenic mice typically requires anesthesia. Chemical-grade tribromoethanol (TBE) is commonly used for this purpose due to its relatively short duration of action, modest cardiodepressive effects, and its noncontrolled status. In the present study, we used both genders of C57BL/6;C3H-Tg(Slc8a1)hKdp transgenic (TG) mice and C57BL/6;C3H wild-type (WT) mice to evaluate isoflurane (ISF) as a pharmaceutical-grade alternative to TBE for echocardiography and electrocardiography. Baseline target physiologic heart rates (beats per minute) were established by use of telemetry as 544 ± 10 in WT mice and 580 ± 21 in TG mice. TG and WT animals were anesthetized with either 0.8% to 1% inhalational ISF or 250 mg/kg intraperitoneal TBE. The following parameters were measured or calculated according to the previously defined physiologic heart rates: end diastolic and systolic dimensions; posterior wall and ventricular septal thicknesses; left ventricular mass, aortic ejection times; left ventricular fractional shortening; velocity of circumferential fiber shortening; and left ventricular ejection fraction. No significant difference between anesthetics was found for any measured cardiac parameters. However, the time required for data acquisition was significantly shorter for ISF (10 min) than for TBE (14 min). This study demonstrates that comparable echocardiographic results can be obtained at higher throughput by use of pharmaceutical-grade ISF than with chemical-grade TBE.

Abbreviations: bpm, beats per minute; CO, cardiac output; ECG, electrocardiogram; EDD, end diastolic dimension; ESD, end systolic dimension; ET, ejection time; ISF, isoflurane; LVFS, left ventricular fractional shortening; NCX1, the product of the cardiac sodium–calcium exchanger transgene Slc8a1; PWT, posterior wall thickness; TBE, tribromothanol; TG, transgenic; USDA, United States Department of Agriculture; Vcf, velocity of circumferential fiber shortening; VST, ventricular septal thickness; WT, wild-type

The evaluation of cardiovascular function in mice under physiologic conditions can be problematic due to their small size and relatively high heart rates. This difficulty is particularly true for some transgenic models with marginal heart function that might be compromised further during investigative procedures. Hoit[12] recently reviewed the wide spectrum of methods used to evaluate the cardiovascular phenotype in mice. These techniques include both invasive and noninvasive screening procedures such as echocardiography, telemetry, and catheterizations for blood pressures and flows. The overwhelming majority of these approaches involve anesthesia that typically depresses contractile function and heart rate, leading to nonphysiologic conditions that can mask data of interest.[12,14]

For the past decade, echocardiography has become a particularly useful noninvasive tool for the rapid screening of cardiac morphometry and function in mice.[5,6,10,11,13,15,31] Unlike with humans and compliant veterinary patients, fractious small mammals such as mice must typically be anesthetized to obtain sufficient immobility and reduce distress during echocardiographic procedures. Numerous anesthetics have been used for echocardiography with mice, including ketamine–xylazine, ketamine–midazolam, chloral hydrate, pentobarbital, halothane, isoflurane (ISF), and tribromoethanol (TBE).[4,6,9,14,17,26,28,35,37] Data from these studies clearly showed that ketamine–xylazine, ketamine–midazolam, chloral hydrate, and pentobarbital anesthesia depressed the heart rate and altered measured echocardiographic results compared with those obtained at more physiologic levels. In contrast, some echocardiographic studies have reported data obtained from conscious, habituated mice.[5,11,29,30] In these cases, heart rates were at the high end of the presumed physiologic range, suggesting a stressful experience that could alter the level of adrenergic stimulation and thus enhance the measured functional parameters in unknown ways.

In murine echocardiography, TBE generally has been the anesthetic of choice because of its short duration and modest cardiodepressive effects compared with those of other injectable anesthetics. However TBE is a chemical-grade compound and United States Department of Agriculture (USDA) Policy 3 states, "investigators are expected to use pharmaceutical-grade medications whenever they are available" and "cost savings alone are not an adequate justification for using non-pharmaceutical grade compounds."[33] Although mice of the genus *Mus* are not presently regulated by the USDA, many institutional animal care and use committees, as part of their commitment to quality and humane care of laboratory animals, extend animal welfare federal regulations to all animals.

There is also concern that the injection of chemical-grade TBE

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may result in unnecessary morbidity and mortality.[3,16,18,20,24,32,36] For example, peritonitis has been reported in mice 24 h after injection with freshly reconstituted[36] or stored[3] TBE. In addition, a recent study reported evidence of pneumonia and septicemia secondary to generalized ileus after intraperitoneal injection of fresh TBE.[18] Peritonitis was also reported in female Sprague Dawley rats (*Rattus norvegicus*)[34] and Mongolian gerbils (*Meriones unguiculatus*).[20] In another study, significant increases in aspartate transaminase levels and hepatosplenic apoptosis in male Hsd:ICR mice were noted just 3 h after injection of TBE ketamine–xylazine but not ISF.[32] Developmental limb abnormalities (syndactyly) and poor embryonic survival have been documented in the progeny of mice anesthetized with TBE as opposed to halothane at the end of pregnancy.[16] Contrary to these reports, some investigators have found TBE to be safe in rodents as long as proper preparation and storage practices are used.[7,8,22]

Our laboratory has used TBE for echocardiographic studies for 6 y without any documented problems. However, in our constant effort to obtain the highest quality data and to comply with the initiative of our institutional animal care and use committee, we undertook this study to compare TBE with a commonly used gas anesthetic, ISF. Although several recent studies have compared the gas anesthetics ISF and halothane with injectable TBE and ketamine–xylazine,[4,14,26] none were performed with potentially unstable transgenic mice, nor did any of these studies establish true physiologic heart rates for the mice prior to the study to ensure repeatable and reliable cardiac function data. In this study we sought to verify that regulatory-compliant ISF can substitute for the widely used TBE in echocardiographic studies in transgenic mice overexpressing the cardiac sodium–calcium exchanger (NCX1)[1,23] without causing anesthesia-induced alterations of measured cardiac indices.

Materials and Methods

Experimental animals. Twenty-five male and female mice (*Mus musculus*) with C57BL/6 and C3H mixed backgrounds were evaluated in this study at 2 to 3 mo of age; 14 of these mice were transgenic and overexpressed the cardiac sodium–calcium exchanger transgene *Slc8a1*, the product of which also is known as NCX1.[1,23,25] All animals used were generated intramurally. These mixed, inbred B6;C3-Tg(Slc8a1)hKdp (designated in this manuscript as NCX1) transgenic (TG) mice and their wild-type (WT) counterparts were housed 3 or 4 mice per cage in polycarbonate static isolator cages with hardwood bedding (Sani-Chips, PJ Murphy Forrest Products, Montville, NJ) in a conventional mouse room within a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International. The mice were fed ad libitum with unsterilized rodent chow (7013, Harlan Teklad, Madison, WI) and provided with tap water ad libitum in standard glass bottles. The animal room was set to a 12:12-h light:dark cycle and maintained within 21.1 to 24.4 °C and 30% to 70% humidity. The mice were tested negative for Sendai virus, pneumonia virus of mice, mouse hepatitis virus, minute virus of mice, Theiler murine encephalomyelitis virus, reovirus 3, lymphocytic choriomeningitis virus, ectromelia virus, epizootic diarrhea of infant mice virus, mouse parvovirus, *Mycoplasma pulmonis*, *Syphacia obvelata*, *Aspiculuris tetraptera*, *Syphacia muris*, *Myobia musculi*, *Radforia affinis*, and *Myocoptes musculinus*, as determined by soiled-bedding sentinels. All animal handling and care procedures conformed to the Public Health Service Policy[21] and the standards set forth in the *Guide for the Care and Use of Laboratory Animals*[19] in accordance with the University of California–Los Angeles Office for Protection of Research Subjects. This study was approved by the University of California–Los Angeles Animal Research Committee and performed under the supervision of the Division of Laboratory Animal Medicine.

Study design. The study was divided into 2 parts using separate groups of mice from the same breeding colony. First, normal physiologic heart rates were established via telemetry in a group of 12 awake, free roaming mice (4 male WT, 4 female WT, 2 male TG, and 2 female TG). Subsequent echocardiographic evaluation was performed using a separate group of 13 age-matched mice (2 male WT, 1 female WT, 6 male TG, and 4 female TG). The echocardiographic evaluation was performed when this 2nd group of mice reached an anesthetic level at which their heart rates were similar to the heart rates obtained via telemetry from their unanesthetized cohorts. The amount of time from anesthetic induction to completion of an echocardiographic scan of a mouse (including time to reach the targeted normal physiologic heart rate) also was measured to evaluate potential for high throughput. These procedures were performed on all animals using TBE anesthesia for trials 1 and 2 and ISF for trials 3 and 4 (2 trials per anesthetic regimen). Each trial was performed on separate days between 1300 and 1600, a time corresponding to the normal diurnal resting period in mice.

Establishing physiologic heart rates. As detailed in the study design section, physiologic heart rates first were established from a group of 12 unanesthetized mice with telemetry. The mice were implanted with telemetry devices (model TA10ETA-F20, Transoma-Data Sciences, St Paul, MN) to monitor their electrocardiograms (ECG), body temperature, cage activity, and heart rates. Transmitter units were implanted in the peritoneal cavity of mice anesthetized with ISF (1.5% to 2.0%) vaporized in oxygen under sterile surgical conditions, as previously described.[15,34] The mouse was placed on a water-circulating heating pad on the surgical table to maintain body temperature during the procedure. The 2 electrical leads used for ECG were tunneled through the subcutaneous space and secured near the apex of the heart and the right acromion. After surgery, mice were given 6 mg/kg subcutaneous carprofen (Rimadyl, Pfizer Animal Health, Exton, PA) once daily for 2 d for analgesia and enrofloxacin antibiotic (Baytril, Bayer Animal Health, Shawnee Mission, KS) at 2 ml of a 3.23% solution in 125 ml drinking water (changed every 5 d), approximately equivalent to 85 mg/kg, for 10 d. We recorded 10 s of data from the mice every 5 min, 24 h daily, for 2 to 6 wk via an antenna receiver under the cage in the vivarium. ECG waveforms, body temperature, and cage activity data were collected with a computer system and analyzed and displayed with the telemetry software (ART 3.1, Transoma-Data Sciences). Heart rates were determined from the R–R intervals of the ECG during both the light (resting) and dark (active) diurnal phases. The range of physiologic heart rates from these mice obtained during the normal diurnal resting period (lights on) was used as the standard (targeted physiologic heart rate) to set the depth of anesthesia for the subsequent echocardiograpic and physiology studies.

Anesthesia. Two anesthetic regimens were used and compared during the echocardiographic evaluation: TBE and ISF. 2.5% TBE (Avertin, Sigma–Aldrich Chemical, St Louis, MO) solution was prepared fresh daily by mixing 0.625 g of 97% crystalline TBE powder with 25 ml sterile 0.9% saline (Baxter Healthcare, Deerfield, IL). The solution was warmed over a magnetic mixer until all powder had dissolved, followed by cooling to room temperature. No organic solvent was used as diluent, and the container was protected from light with aluminum foil. The TBE solution was injected intraperitoneally at 0.01 ml/g body

mass (250 mg/kg).[6]

Isoflurane (Abbott Laboratories, North Chicago, IL) was administered by means of a precision vaporizer (Ohio Medical Products, Madison, WI) with a modified nose cone constructed from a gas sampling elbow with gas port (Portex, Keene, NH) equipped with a gasket made of a finger glove (Paean Veritas, Colorado Springs, CO). Anesthetic induction was achieved via the nose cone at 2.5% to 3% ISF for 2.5 to 3 min, and anesthesia was maintained at 0.8% to 1.0% ISF; oxygen flow rate was set at 500 ml per min. Waste anesthetic gas was scavenged via an activated charcoal canister (F/Air, Engler Engineering, Hialeah, FL).

Echocardiography. Cardiac parameters from echocardiography were measured twice from the 2nd group of 13 animals, once with each anesthetic method, as described in the study design section. After anesthetic induction, the mouse was placed on a water-circulating heating pad. The ventral thorax of the mice was carefully shaved with a #40 electric clipper, and ultrasound acoustic gel was applied. Platinum needle electrodes (Grass Instruments, Warwick, RI) were inserted subcutaneously in the standard lead II arrangement, with 1 electrode attached to the right thoracic limb and the other attached to the left pelvic limb. The ECG signal was amplified (Grass Instruments), digitally acquired, filtered, displayed, and analyzed (HEM version 3.3 software, Notocord Systems, Croissy sur Seine, France) on a computer system. The heart rate was determined from the R–R interval of the ECG. In addition, heart rates were confirmed from the echocardiographic images by calculating the peak systolic intervals and Doppler flow intervals. The data were recorded continuously for the entire duration of each study to ensure that the echocardiographic imaging occurred within the baseline physiologic heart rates.

The depth of anesthesia for echocardiography was adjusted until the heart rate of the anesthetized animal approximately matched the predetermined standards (physiologic range, 544 ± 10 beats per min (bpm) in WT mice and 580 ± 21 bpm in TG mice; see Results). With TBE anesthesia, if the heart rate exceeded 700 bpm, another 25% of the initial TBE dose for that animal was administered intraperitoneally in order to lower the heart rate. If the heart rate was less than 500 bpm, we simply waited until the anesthetic depth decreased and heart rate increased. With ISF anesthesia, the depth of anesthesia was controlled by adjusting the concentration of vaporized anesthetic until the heart rate was within the predetermined physiologic range. The depth of anesthesia was not necessarily the same from mouse to mouse during these echocardiographic studies, because the TG mice tended to require less anesthetic than the WT mice did to be within their physiologic range.

When the target heart rate was reached, echocardiographic imaging was performed with an Acuson Sequoia C256 (Siemens Medical Solutions USA, Malvern, PA) equipped with a 15-MHz (15L8) probe. Long-axis, short-axis, and apical views were used to obtain 2-dimensional, M-mode, and spectral Doppler images. Specifically, the 2-dimensional guided M-mode images of the left ventricle were obtained in the short-axis view at the papillary level with the mouse in left lateral recumbency. All echocardiographic imaging and measurements were performed by the same person. Measurements and calculations were made from M-mode images using the proprietary Acuson and AccessPoint software (Freeland Systems, Westfield, IN) by a single observer and were reviewed independently by 2 additional observers. End diastolic dimension (EDD), end systolic dimension (ESD), posterior wall thickness (PWT), and ventricular septal thickness (VST) were measured during systole and diastole. Left ventricular mass was calculated as $1.055 \times [(EDD + PWT + VST)^3 - EDD^3]$.[31] Aortic ejection time (ET) was determined from Doppler images. Left ventricular fractional shortening (LVFS = 100% × (EDD – ESV)/EDD] and the velocity of circumferential fiber shortening (Vcf = [LVFS/100] / [ET/1000]) were calculated as indices of contractility.[31] In addition, the 3-dimensional left ventricular ejection fraction was estimated by means of proprietary software using Simpson's rule on the echocardiography machine.

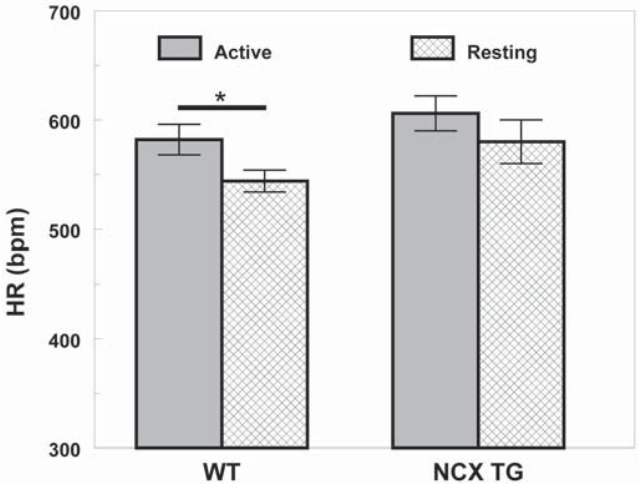

Figure 1. Mean heart rates (HR) from telemetry of 8 wild-type (WT) and 4 transgenic (TG) mice during their active (dark) and resting (light) diurnal periods over several weeks. Heart rates were significantly greater (*, $P < 0.044$ 2-tailed t test) when the WT mice were active. Error bars, standard error of the mean.

Statistical methods. Data were compiled and are shown as mean ± standard error. Significant difference between 2 groups of data was determined by means of 2-tailed t tests. Significant differences among more than 2 groups of data were determined with analysis of variance. We used Instat statistical software (version 3.05, GraphPad, San Diego, CA), and $P < 0.05$ was deemed statistically significant for all analyses.

Results

Baseline physiologic data. One of the goals of this study was to evaluate cardiac function from mice under physiologic conditions. Therefore, heart rates from the ECG, body temperature, and cage activity were determined from 12 awake, free-roaming mice by use of radiotelemetry to establish standard physiologic values prior to echocardiography. Mice, being nocturnal, are substantially more active during the dark period than during the light period, when they tend to sleep, groom, or rest quietly. In WT mice, the activity index from telemetry rose significantly ($P < 0.001$, n = 8, 2-tailed t test) from 4.5 ± 0.5 (mean ± standard error, arbitrary units) during the light diurnal cycle to 28.4 ± 6.2 during the dark cycle, while their body temperatures rose significantly ($P < 0.001$) from 35.5 ± 0.1 °C to 37.1 ± 0.2 °C from the resting to active cycles. In TG mice, activity rose significantly ($P < 0.043$, n = 4, 2-tailed t test) from 3.8 ± 0.7 to 7.8 ± 1.4, and body temperature rose significantly ($P < 0.001$) from 35.8 ± 0.1 °C to 36.7 ± 0.1 °C from the resting to active cycles. There were no significant differences in body temperature and resting activity between the WT and TG during the same part of the diurnal cycle. The TG mice were significantly ($P < 0.046$, n = 4 and 8, 2-tailed t test) less active than the WT mice during the active (dark) period.

Heart rates averaged for the 2 diurnal periods show a significant difference between the active and resting periods in WT mice as determined by 2-tailed t test (Figure 1). Although the TG mice exhibited faster heart rates at this age, only the

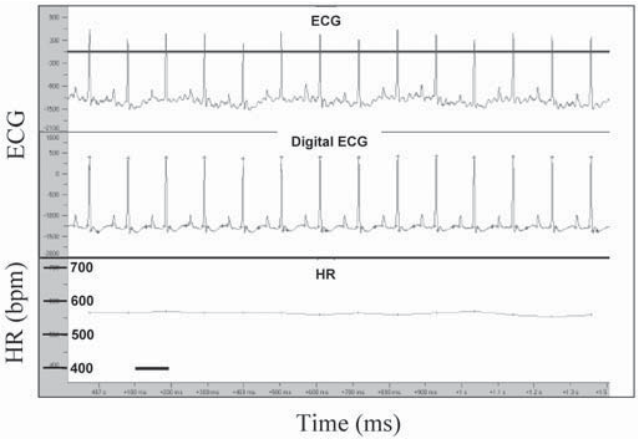

Figure 2. Electrocardiogram (ECG) traces and calculated heart rates from an anesthetized NCX1 TG mouse during an echo procedure. The top trace is the unfiltered ECG, and the middle trace is the digitally filtered ECG in arbitrary voltage units. The heart rate (in beats per minute) is calculated from the R–R interval of the middle trace. The horizontal bar time scale is 100 ms.

active–resting difference in the WT was significantly ($P < 0.044$, n = 8, 2-tailed t test) different. Because activity can vary from time to time, we defined the physiologic heart rate for subsequent cardiac evaluations as the resting value (during the light cycle) from these telemetry data. Therefore the target physiologic heart rate (in bpm) was 544 ± 10 in WT mice and 580 ± 21 in TG mice.

Measurement of heart rate under anesthesia. Heart rates can vary enormously during a cardiac evaluation procedure, such as echocardiography, depending upon the study conditions, particular anesthetics, and restraint methods.[4,5,7,9,1726-30,35] Therefore, we continuously monitored the heart rates of the 13 mice being evaluated to maintain the rates previously established by telemetry. Figure 2 shows a sample ECG and heart rate recording during an echocardiographic study of an NCX1 TG mouse. The top trace and middle traces are the unfiltered and digitally filtered ECG, respectively. R-wave peaks are indicated by the + signs, and the heart rates derived from the R–R intervals are displayed in the bottom trace. In this example, the heart rate is about 560 bpm, which is within the target physiologic value determined from telemetry for these mice.

Echocardiographic parameters under anesthesia. Figure 3 shows a representative echocardiographic M-mode image acquired from an NCX1 TG mouse during a typical evaluation under ISF anesthesia at physiologic heart rates. Left ventricular PWT, VST, EDD, and ESD were determined from M-mode images like that in Figure 3.

Table 1 shows the results from the multiple cardiac echocardiographic evaluations of 13 TG (n = 10) and WT (n = 3) mice under ISF and TBE anesthesia. Mice were evaluated twice with each anesthetic, and there was a week between each trial and the next. The echocardiographic image data were obtained when the heart rate for each mouse was within our targeted physiologic value. There are no significant differences in heart rate between the echocardiographic study groups or the target rates determined from telemetry. Within these tightly controlled physiologic conditions, we found no significant differences in any echocardiographic parameter between any groups or between trials in a given mouse. There were no deaths or clinical morbidity associated with either anesthetic, and all mice recovered uneventfully from anesthesia.

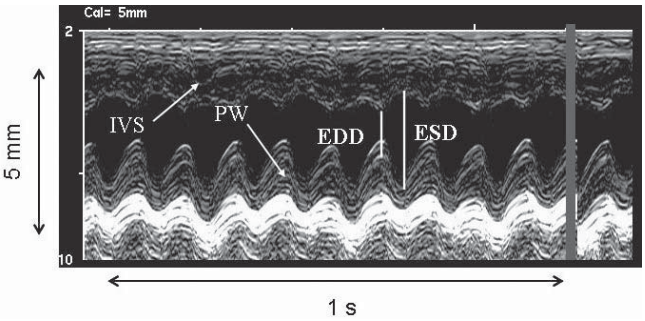

Figure 3. Representative 2-dimensional directed M-mode echocardiograms from an NCX1 transgenic mouse with a physiologic heart rate. IVS, intraventricular septum; PW, posterior wall; EDD, end diastolic dimension; ESD, end systolic dimension.

Although there were no differences in cardiovascular parameters measured when mice were under either anesthetic, there were 2 main differences in the experimental procedures. First, it took significantly ($P < 0.039$, n = 13, 2-tailed t test) longer to reach the physiologic target heart rate and complete the study when the injectable TBE anesthetic was used (14 min) as opposed to the more rapidly acting and titratable inhalation anesthetic, ISF (10 min, data not shown). Second, TG mice were more sensitive to the anesthetic dose than were the WT mice. The ISF induction time and dose were 2.5 min at 2.5% for the TG mice but 3 min at 3% for the WT mice to achieve the targeted physiologic heart rates. Heart rate was highly sensitive to the maintenance ISF level, in that small changes within the 0.8% to 1.0% range could trigger large changes in heart rate. It was substantially more difficult to maintain a physiologic heart rate when using the injectable TBE anesthetic.

Discussion

A pharmaceutical-grade drug or product is manufactured according to standards set forth by independent organizations, such as the United States Pharmacopeia and the Food and Drug Administration. A pharmaceutical-grade product is ensured to be free of contaminants, and its purity is superior to that of technical- or chemical-grade reagents. Research animals that are regulated by the USDA are required to receive pharmaceutical-grade compounds unless there is scientific justification and approval from the institutional animal care and use committee to use a nonpharmaceutical product.[33] Despite the current unregulated status of the mouse (genus *Mus*), the well-being of a single mouse warrants as much consideration as that of any other laboratory animal. Because TBE has been reported to cause peritonitis,[3,18,36] an alternative to TBE was desirable. To this end, we demonstrated that a pharmaceutical-grade drug, in this case ISF, yields comparable echocardiographic results to those from chemical-grade TBE for both the NCX1 TG mice and their wild-type counterparts under physiologic conditions.

Precision-vapor halogenated gas anesthetics are an excellent alternative to TBE because they are short-acting, titratable, stable, and of pharmaceutical grade. ISF was our 1st choice because it is less cardiodepressive and arrhythmogenic than halothane,[2] minimally metabolized, and is currently more prevalent and much less expensive than sevoflurane. Although there can be some cardiovascular depression with these agents, it can be minimized by appropriate titration and monitoring.[28] We found that ISF was easily titrated to maintain a physiologic heart rate during echocardiographic imaging. With TBE, the study was often delayed until the anesthetic level decreased

Table 1. Summary of echocardiographic evaluation of cardiac function at physiologic heart rates under isoflurane and tribromoethanol anesthesia

	Isoflurane		Tribromoethanol	
	Wild-type	Transgenic	Wild-type	Transgenic
No. of evaluations	6	19	6	18
Heart rate (bpm)	550 ± 9	543 ± 9	541 ± 21	524 ± 13
Ventricular septal thickness (mm)	0.69 ± 0.02	0.68 ± 0.01	0.62 ± 0.02	0.68 ± 0.02
Posterior wall thickness (mm)	0.64 ± 0.03	0.65 ± 0.01	0.58 ± 0.02	0.61 ± 0.02
End diastolic dimension (mm)	3.60 ± 0.15	3.75 ± 0.09	3.75 ± 0.09	3.64 ± 0.07
End systolic dimension (mm)	2.44 ± 0.21	2.41 ± 0.11	2.25 ± 0.15	2.46 ± 0.10
Ejection time (ms)	51 ± 1	52 ± 1	51 ± 1	54 ± 1
Left ventricular fractional shortening (%)	32.8 ± 3.5	36.2 ± 1.6	40.1 ± 3.2	32.8 ± 1.6
Velocity of circumferential fiber shortening (no. of circumferential fibers/s)	6.41 ± 0.59	6.89 ± 0.26	7.96 ± 0.79	6.12 ± 0.36
Left ventricular ejection fraction (%)	69.2 ± 5.6	71.8 ± 2.1	75.9 ± 4.0	66.6 ± 2.4

No significant differences were noted across each row of data via analysis of variance.

enough to achieve a stable target heart rate. The ease of ISF use reduced the total study time, thereby not only increasing screening throughput but also improving animal welfare by minimizing anesthetic exposure. Furthermore, because of careful maintenance of a target heart rate within a documented physiologic range, data were highly reproducible and more easily compared from animal to animal. Finally, ISF, unlike TBE, is a pharmaceutical-grade anesthetic that meets all regulatory guidelines and is not associated with noteworthy morbidity and mortality.[3,16,32,36]

The importance of performing echocardiography at a predetermined physiologic heart rate cannot be overemphasized. The baseline telemetric data established the physiologic average heart rate for the line of mice used in this study. When these animals are phenotyped echocardiographically, the heart rate should be maintained at or near the baseline to avoid physiologic alterations in cardiac output (CO) and performance. For example, if the depth of anesthesia is too great, heart rate would fall below what is considered normal at rest, and CO and other measured parameters would decrease because CO = heart rate × stroke volume (volume of blood pumped out of the left ventricle per contractile event). Both TBE and ISF maintained the physiologic heart rate sufficiently for echocardiography. However, depth of anesthesia, and therefore heart rate, can be controlled when using ISF, resulting in a 4-min reduction ($P < 0.05$) in study time per mouse compared with that for TBE.

Previous studies have compared ISF with TBE and other injectable anesthetics,[14,26] but none rigorously examined both normal and transgenic animals, assessed female mice, or verified the baseline physiologic heart rate of their mice. Because resting heart rate can be highly variable from mouse strain to strain[37] or between TG and WT mice,[5] we carefully obtained resting heart rates in the TG and WT mice under awake, free-roaming conditions via radio telemetry (Figure 1). We then monitored the mice during the echocardiographic studies (Figure 2) to maintain their resting physiologic heart rate for optimal data acquisition and ease of comparison. Similar heart rates were reported by others using 1% to 1.25% ISF[14,26,29] and 0.75% halothane.[4] However, prolonged exposure to ISF or higher levels of ISF depressed heart rate.[26] TBE typically induced somewhat lower heart rate,[17,26] whereas ketamine–xyline, ketamine–medetomindine, pentobarbital, and other injectables initiated severe reductions from physiologic levels.[9,14,30,35,37] Echocardiographic studies on conscious mice were associated with elevated heart rates often exceeding 700 bpm,[5,17,30,35] likely due to sympathetic stimulation, a value well beyond our average active period baseline heart rate obtained from telemetry. Such high heart rates are indicative of stress or excitement, which may confound data interpretation and limit comparison. By first establishing the normal heart rate for our animals, we avoided the confounding results due to the animals' sympathetic activation during live handling.

Transgenic mice examined in echocardiographic studies often have cardiac targeted gene alterations that can compromise their heart function. In our experience, this characteristic is often accompanied by anesthetic intolerance or magnified cardiodepression at the same dose as used in WT mice. Over the past 6 y, we have noted that our TG mice have been less tolerant of anesthetics regardless of the procedure or specific agent used (unpublished observations). Esposito and colleagues[5] also reported lower heart rates in MLP/βARKct TG mice than in WT littermates at the same TBE anesthetic dose. Although none were noted here, there also could be gender-associated differences in anesthetic tolerance.

Finally, it should be noted that our maintenance ISF dose of 0.8% to 1.0% is lower than that necessary for invasive procedures. Because echocardiographic imaging is noninvasive and unlikely to induce any pain, it is necessary only to immobilize the animal for a short duration to obtain data from a quiet, unstressed animal. Our induction levels were somewhat lower than typical, too. We found that more typical induction levels of 4% to 5% ISF caused clinically significant cardiodepression for long periods of time, unnecessarily prolonging the total anesthetic exposure of the animal and decreasing throughput.

In summary, we have demonstrated that isoflurane is a safe and effective anesthetic for echocardiographic imaging of a transgenic mouse model. Although TBE has been used effectively for years in many labs, ISF is a suitable alternative to TBE, with no alterations to data acquisition. Measured cardiac parameters were comparable under ISF versus TBE chemical immobilization. In addition, ISF is a pharmaceutical-grade compound, affording mice the same standard of care as that of USDA-regulated species.

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