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Anesthetic inhibition in ischemic and nonischemic murine heart: comparison with conscious echocardiographic approach

SHIN TAKUMA, KOTARO SUEHIRO, CAROL CARDINALE, TAKESHI HOZUMI, HIDEAKI YANO, JUICHIRO SHIMIZU, SAMANTHA MULLIS-JANSSON, ROBERT SCIACCA, JIE WANG, DANIEL BURKHOFF, MARCO R. DI TULLIO, AND SHUNICHI HOMMA
Division of Cardiology, Department of Medicine, Columbia University, New York, New York 10032

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Takuma, Shin, Kotaro Suehiro, Carol Cardinale, Takeshi Hozumi, Hideaki Yano, Juichiro Shimizu, Samantha Mullis-Jansson, Robert Sciacca, Jie Wang, Daniel Burkhoff, Marco R. Di Tullio, and Shunichi Homma. Anesthetic inhibition in ischemic and nonischemic murine heart: comparison with conscious echocardiographic approach. *Am J Physiol Heart Circ Physiol* 280: H2364–H2370, 2001.—It is well known that the level of anesthesia obtained by intraperitoneal injection is variable and may alter cardiac function. In this study, we compared the effects of different anesthetics on cardiac function with the conscious state using high-resolution two-dimensional echocardiography in nonischemic and ischemic mice. Eighty-four mice were tested before and after surgery with ligation of the coronary artery. All 84 mice were studied in the conscious state and under high-dose intraperitoneal anesthesia. Twenty-two of 84 mice were studied under low-dose intraperitoneal anesthesia. Another 22 mice were also studied under gas anesthesia and spontaneous breathing. Experiments in the conscious state were performed by two investigators before the administration of anesthesia: one investigator held the animal and the transducer and the other operated the ultrasound equipment. Left ventricular systolic function was measured, and measurements obtained after surgery were compared with infarcted areas assessed by histological staining. Results showed that both high- and low-dose intraperitoneal anesthesia significantly reduced heart rates and left ventricular contractility in both pre- and postsurgical mice as opposed to conscious mice ($P < 0.01$). There were significantly higher correlation coefficients between mean fractional area change (FAC) and infarcted area in conscious state compared with high-dose intraperitoneal anesthesia ($P < 0.05$). The correlation coefficient between FAC and infarcted area during gas anesthesia was also significantly higher compared with high-dose intraperitoneal anesthesia ($P < 0.05$). In conclusion, conscious experiments or the use of gas anesthesia is preferred for echocardiographic assessment of cardiac function in mice because intraperitoneal injection significantly induces a significant reduction in heart rate and left ventricular systolic function.

cardiac function; intraperitoneal; M-mode; noninvasive

TWO-DIMENSIONAL (2-D) echocardiography is an established tool for the investigation of circulatory physiology in large animals (4, 35, 44); however, limited spatial resolution and insufficient framing rates for image capture have prevented adequate echocardiographic assessment in small animals such as mice. M-mode echocardiography has been used for measurement of left ventricle (LV) dimensions in mice because M-mode data acquisition allows investigators to obtain LV dimensional changes even at heart rates (HR) as high as 1,000 Hz (11, 14, 24, 25). However, the M-mode echocardiographic approach is based on one-dimensional (1-D) measurements of the LV at midpapillary level and does not take into account variations in LV chamber and wall dimensions along other anatomic levels (6, 10).

With the advent of techniques for the manipulation of the mammalian genome, it has become possible to generate animal models for studying cardiovascular function and disease while the trait responsible for the perturbation is precisely defined at the genetic level (5, 8, 17). Because of technical and economic considerations, mice are currently almost always the model of choice for carrying out these genetic modifications. The availability of high-frequency transducers with high framing rates has created a means for obtaining 2-D echocardiographic images in various transgenic and microsurgical murine models. 2-D echocardiography provides more extensive tomographic sampling and enhanced spatial orientation, thereby eliminating many of the limitations of the M-mode technique (37, 43).

The model of transmural myocardial infarction in mice represents a particular challenge (23, 27) because it initiates a cascade of structural and functional changes in the LV that may need to be assessed by geometric findings using 2-D echocardiography. Also, the need to assess intact normal physiology has always been critical for proper interpretation of data obtained in more noninvasive cardiac studies (18). Of importance is the observation that the anesthesia needed for imaging may lead to depressed cardiac function and possibly masks the functional changes resulting from ischemia. As a result of these difficulties, investigators

Address for reprint requests and other correspondence: S. Homma, Dept. of Medicine, Division of Cardiology, Columbia Univ., PH 3–342, 630 West 168th St., New York, NY 10032 (E-mail: HOMMASH@medicine1.cpmc.columbia.edu).

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often resort to the use of larger rodents such as rats for cardiac research. The ability to image the murine heart without anesthesia would constitute an innovative step toward investigations in the mouse ischemic model.

In this study, we compared 2-D echocardiographic assessment of cardiac contractility in nonischemic and ischemic mice while the animals were conscious, under high- and low-dose intraperitoneal anesthesia, and under gas anesthesia using isoflurane. Echocardiographic measurements were followed by histological determination of the infarcted area.

METHODS

Experimental protocol. Animals were handled according to the "Care and Use of Laboratory Animals" and the Animal Welfare Act regulations administered by the US Department of Agriculture. The institutional Animal Care and Use Committee of Columbia University approved the experimental protocol. A total of 84 mice (mean body wt 36.2 ± 6.4 g; age 8–24 wk) that survived 2 days after the surgery were included in this study. Mice were obtained from Swiss-Webster (Germantown, NY). 2-D targeted M-mode echocardiography and 2-D echocardiography were performed 2 days before the surgery according to the methods described in *Echocardiography*. All 84 mice were studied while conscious and under high-dose intraperitoneal anesthesia. Of these 84 mice, 22 were randomly selected to be studied under low-dose intraperitoneal anesthesia, and another group of 22 were chosen to be studied under gas anesthesia. Two days after surgery, the same echocardiographic examination was repeated in these mice. The presence and extent of myocardial infarction were determined via Evans blue staining immediately after the echocardiographic studies.

Surgery. Mice were anesthetized with a combination of ketamine hydrochloride (50 mg/kg; Parke Davis; Morris Plains, NJ) and xylazine (6 mg/kg; Miles Laboratory; Shawnee, MO) via intraperitoneal injection. Endothoracheal intubation was performed while the mice were in the supine position, and they were ventilated with room air [tidal volume was 1.0 ml; rate was 150 breaths/min; and positive end-expiratory pressure (PEEP) was 5 cmH_2O] using an animal respirator (Harvard Apparatus). All surgical procedures were performed under sterile conditions using an operating microscope (Leica; Deerfield, IL) at ×5–24 magnification. The mice were placed in the right lateral position, and a left thoracotomy was performed. An 8-0 polypropylene suture was placed halfway down the left coronary artery and tied. Occlusion was confirmed by pallor of the LV wall. The lungs were inflated to reduce the pneumothorax, and the chest cavity was closed in layers with 3-0 silk. Once spontaneous respiration had resumed, the endothoracheal tube was removed, and the animal was placed on a heating pad and warmed with heat lamps.

Echocardiography. A high-frequency transducer (12 MHz) with frequency fusion technology (SONOS 5500, Hewlett-Packard, Andover, MA) was used in both conscious and anesthetic studies. The index finger of a latex grove (Allegiance Healthcare, McGaw Park, IL) was filled with acoustic coupling gel (Aquasonic 100, Parker Laboratories, Orange, NJ) and attached to the convex head of the transducer. The distance between the top of the transducer and the thoracic wall was ~10 mm. All images were acquired at a depth setting of 2 cm. With zoomed imaging, 2-D image acquisition was performed at a frame rate of 120 Hz and recorded on an optical disc. 2-D and M-mode echocardiography were performed for each mouse and were repeated eight times during 15 min of examination. HR during each scanning was determined by counting total beats during 2-s M-mode intervals; e.g., if 20 beats were counted during 2 s, HR would be represented as 600 beats/min.

Conscious animal experiments. Conscious animal experiments were performed by two investigators (S. Takuma and K. Suehiro) before the administration of anesthesia. The mice were carefully held with the left hand by grasping the mouse's skin on the back of the neck and wrapping the tail to prevent movement during the echocardiographic studies. Care was taken to avoid excessive pressure on the animal, which could result in death from suffocation or vagal reflex. The transducer (with a standoff) was placed on the left hemithorax using the right hand with the right elbow fixed on the table. Particular attention was also given to avoiding excessive pressure on the thorax, which might induce cardiac depression.

Intraperitoneal anesthesia. Thirty minutes after the conscious echocardiographic experiments, the mice were anesthetized with a mixture of ketamine hydrochloride (50 mg/kg ip) and xylazine (6 mg/kg ip) for high-dose intraperitoneal anesthetic studies. Five minutes after the high-dose intraperitoneal injection, the mice were placed in a shallow left-lateral decubitus position with the limbs fixed. The transducer (with the same standoff) was placed on the left hemithorax to facilitate ultrasonic imaging. A warming pad was used to maintain normothermia. As with the conscious animal experiments, care was taken to avoid excessive pressure on the thorax. Echocardiographic studies were performed for 15 min in the same manner as for the conscious animal experiments.

The mice had completely recovered within 2 h after the high-dose intraperitoneal anesthetic studies, and 22 mice were then randomly selected for low-dose intraperitoneal anesthetic studies. These mice were anesthetized with a mixture of ketamine hydrochloride (50 mg/kg ip) and xylazine (0.5 mg/kg ip) for low-dose intraperitoneal anesthetic studies. Echocardiographic studies were performed for 15 min in the same manner as the high-dose intraperitoneal anesthetic studies.

Gas anesthesia. Another 22 mice were randomly selected for the studies with gas anesthesia. Anesthesia in the mice was induced via a facial mask and was maintained by a minimum dose of isoflurane (0.6–2.2%, Abbott; Chicago, IL). After induction of anesthesia, the mice were positioned in the same manner as described for the intraperitoneal study. Spontaneous breathing was maintained throughout the echocardiographic studies via isoflurane blowing. Echocardiographic studies were performed for 15 min in a similar fashion as the previous studies. Echocardiographic studies were performed in ~15 min. After the studies were completed, the mask was removed, and mice were allowed to recover over a period of 30–40 min.

2-D echocardiography. 2-D echocardiographic short-axis images of the LV were obtained at papillary muscle level (Fig. 1). According to the recommendations of the American Society of Echocardiography for 2-D echocardiography, the endocardium was traced by covering the innermost edge of that surface (34). This was performed from the data that had been recorded on an optical disk (video cassette recorder tape would only be able to store 30 frames/s compared with 120 frames/s, as used in this study). The gain setting was optimized to enable imaging of the endocardial boundary. Each study was analyzed by one investigator who was unaware of the pathology findings. The endocardial borders were traced frame by frame throughout the entire cardiac cycle. End-systolic area (ESA) and end-diastolic area (EDA) were deter-

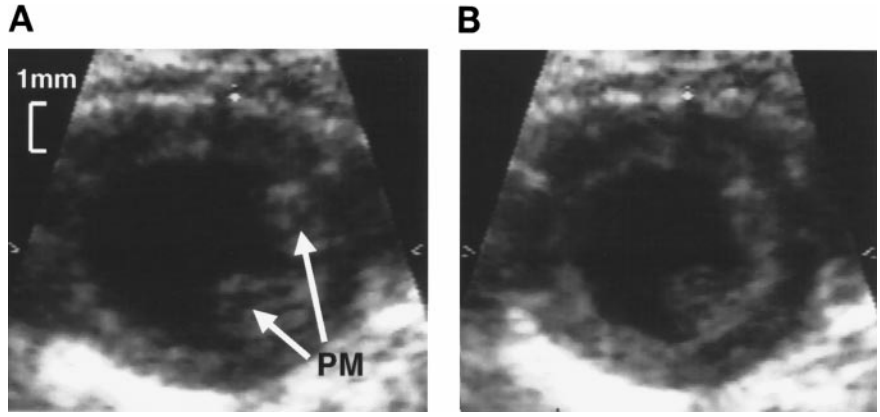

Fig. 1. Two-dimensional echocardiographic short-axis images of the left ventricle at end diastole (*A*) and end systole (*B*). PM, papillary muscle.

mined as the minimum and maximum values for these tracings, respectively. Systolic function was evaluated as the fractional area change (FAC). The percentage of FAC was calculated as

$$\text{FAC}\ (\%) = [(\text{EDA} - \text{ESA})/\text{EDA}] \times 100$$

Eight measurements were averaged during each examination to calculate EDA, ESA, and FAC.

Histology. After all echocardiographic examinations were completed, the mice were administered ketamine hydrochloride (50 mg/kg ip) and xylazine (6 mg/kg ip), and the right carotid artery was canulated. To achieve diastolic arrest, potassium chloride in saline solution (40 meq/l) was injected through the catheter. Immediately after cardiac arrest, 5% Evans blue dye was injected through the same catheter and the heart was excised. A cross section of the LV at the papillary muscle level, which corresponded to the 2-D echocardiographic view, was obtained. The myocardial infarction area (region at risk) was determined by the absence of blue dye. The stained section was photographed in digital format. Each digitized file was computerized using a video tracing system (Adobe Photoshop 4.0, Adobe Systems; Mountain View, CA). The infarct size was calculated as a percentage of the area at the midpapillary level. Pathology findings were analyzed by an investigator unaware of the echocardiographic findings.

Statistical analysis. Data obtained from 2-D echocardiography with and without anesthesia as well as the percentage of infarcted area were reported as means ± SD. A two-way ANOVA for repeated measures was used to analyze the data to compare results before and after surgery with each different type of restraint. The significance of differences in HR and FAC among conscious, high-dose intraperitoneal anesthesized, and gas-anesthesized mice was assessed for pre- and postsurgery states by ANOVA. The significance of differences in HR and FAC among conscious, high-dose intraperitoneal anesthesized, and low-dose intraperitoneal anesthesized animals was also assessed for both pre- and postsurgery states by ANOVA. Additionally, the relationship between FAC obtained after surgery and the corresponding region at risk obtained by histological staining was assessed by linear regression. The significance of differences in correlation coefficients was tested using the *Z* transformation (44).

RESULTS

Values for EDA and ESA for each condition before and after surgery are shown in Table 1. Values for HR and FAC among conscious, gas anesthesized, and high-dose intraperitoneal anesthesized animals before and after surgery are shown in Table 2. Values for HR and FAC among conscious, low-dose intraperitoneal anesthesized, and high-dose intraperitoneal anesthesized animals before and after surgery are shown in Table 3. All before- and after-surgery comparisons were significant at $P < 0.01$ by two-way repeated measures ANOVA. In contrast to the findings observed in the conscious study, both high-dose and low-dose intraperitoneal anesthesia significantly reduced mean HR and mean FAC ($P < 0.01$). A significant difference in HR was also observed between low- and high-dose intraperitoneal anesthesia ($P < 0.01$).

The mean percentage of infarcted area indicated by histological staining in this study was 52.3 (16.2%; range of 16.3–78.3%). Figures 2 and 3 illustrate the relationship between the percentage of infarcted area and FAC after surgery under each condition. Low correlations between mean FAC and infarcted area were observed with the use of both high- and low-dose intraperitoneal anesthesia ($r = -0.52$ and -0.53). By the *Z* transformation there were significantly better correlation coefficients between mean FAC and infarcted area in both conscious and gas-anesthesized animals compared with high-dose intraperitoneal anesthesized animals ($P < 0.05$).

Figure 3*A* demonstrates the relationship between the percentage of infarcted area and FAC after surgery in the conscious state and during gas anesthesia in 22 mice. A significantly higher correlation coefficient be-

Table 1. *Echocardiographic values before and after surgery*

	Conscious Mice (n = 84)	High-Dose Intraperitoneal Anesthesia (n = 84)	Gas Anesthesia (n = 22)	Low-Dose Intraperitoneal Anesthesia (n = 22)
Before surgery				
EDA, mm^2	10.1 ± 1.3	10.5 ± 1.1	10.4 ± 1.9	10.3 ± 1.8
ESA, mm^2	4.5 ± 0.8	6.8 ± 0.9†	4.6 ± 0.4	5.1 ± 0.6*
After surgery				
EDA, mm^2	11.1 ± 1.4	11.0 ± 1.1	11.4 ± 1.2	11.3 ± 1.5
ESA, mm^2	7.2 ± 0.9	8.3 ± 0.8†	6.8 ± 0.7	8.4 ± 0.5†

Values are means ± SD; n, no. of mice. EDA, end-diastolic area; ESA, end-systolic area. *$P < 0.05$ vs. conscious mice; †$P < 0.01$ vs. conscious mice.

Table 2. *Comparisons among conscious state, gas anesthesia, and high-dose intraperitoneal anesthesia before and after surgery*

	Conscious Mice (n = 84)	Range	High-Dose Intraperitoneal Anesthesia (n = 84)	Range	Gas Anesthesia (n = 22)	Range
Before surgery						
Mean HR, beats/min	523.5 ± 20.8	450–600	306.3 ± 29.3†	165–480	507.1 ± 13.6*	450–570
Mean FAC, %	54.7 ± 3.4	48.0–68.3	35.1 ± 3.8†	21.0–54.2	55.2 ± 8.4	46.3–65.3
After surgery						
Mean HR, beats/min	476.7 ± 37.8	360–555	227.9 ± 30.1†	150–405	454.1 ± 22.6*	330–525
Mean FAC, %	34.5 ± 8.4	17.8–63.2	24.2 ± 5.0†	14.3–46.6	39.0 ± 8.5*	24.5–51.2

Values are means ± SD; n, no. of mice. HR, heart rate; FAC, functional area change. All comparisons were significant at $P < 0.01$; *$P < 0.05$ vs. conscious mice; †$P < 0.01$ vs. conscious mice.

tween mean FAC and infarcted area in conscious state was obtained compared to gas anesthesia ($r < 0.05$). Figure 3*B* shows the relationship between the percentage of infarcted area and FAC after surgery in the conscious state and during low-dose intraperitoneal anesthesia in another series of 22 mice. Correlation coefficients between FAC and infarcted area in the conscious state were also significantly higher compared with low-dose intraperitoneal anesthesia ($r < 0.05$).

DISCUSSION

Although larger mammals (e.g., rabbits, pigs, or dogs) could be used to develop transgenic models and create surgical ischemic models, the cost and time required to prepare these models would make their use impossible or very impractical. Mice have been used because of their small size, low cost, early and frequent reproductive cycles, and the availability of an established technology for the development of transgenic and knockout models. In mice, an in vivo open-chest model was developed and first used to study the ventricular expression of a fusion gene (16). To study cardiac function in vivo, the mouse was anesthetized, the chest was opened, and a bilateral vagotomy was performed. However, the interrelated forces of cardiac contractility, intrathoracic pressures, and pericardial constraints are obviously affected in such open-chest models. In anticipation of the expanded use of transgenic manipulation for studying cardiac pathophysiology, electrophysiological measurements have also been developed in mice under anesthesia using relatively complex experimental apparatuses (3).

Recently, morphological and functional characterization of the cardiac phenotype in transgenic and surgical mice models was obtained using high-resolution echocardiography (1, 13, 32, 33, 37, 42, 43). However, even when using high-resolution images with dedicated hardware and software in these echocardiographic studies, the data have often shown homodynamic results that would fail to meet the standards usually applied to larger animals particularly those for HR and basal level of systolic function (18). In large part the alternations result from the necessity for anesthetic that significantly depresses these parameters (9, 11, 14, 15, 25, 26, 29, 37).

There has been no study to assess the effect of routinely used anesthesia in mice. Our echocardiographic methodology for conscious mice was developed to provide a more physiologically reasonable alternative to commonly used intraperitoneal anesthesia. The present study showed lower correlation between FAC and infarcted area in both high-dose and low-dose intraperitoneal animals compared with conscious ani-

Table 3. *Comparisons among conscious state and low- and high-dose intraperitoneal anesthesia before and after surgery*

	Conscious Mice (n = 22)	Range	High-Dose Intraperitoneal Anesthesia (n = 22)	Range	Low-Dose Intraperitoneal Anesthesia (n = 22)	Range
				*		
Before surgery						
Mean HR, beats/min	534.1 ± 15.3	450–585	295.4 ± 17.8*	180–480	468.2 ± 34.0*	420–555
Mean FAC, %	56.0 ± 2.6	49.2–65.2	33.4 ± 3.3*	23.1–53.8	50.0 ± 2.9*	39.8–61.0
				*		
				*		
After surgery						
Mean HR, beats/min	484.1 ± 28.6	360–540	210.8 ± 19.8*	165–405	239.7 ± 25.4*	180–450
Mean FAC, %	31.9 ± 6.3	20.3–62.3	22.9 ± 5.1*	14.3–45.3	26.2 ± 3.7*	19.3–51.0
				*		

Values are means ± SD; n, no. of mice. All comparisons significant at $P < 0.01$; *$P < 0.01$ vs. conscious mice.

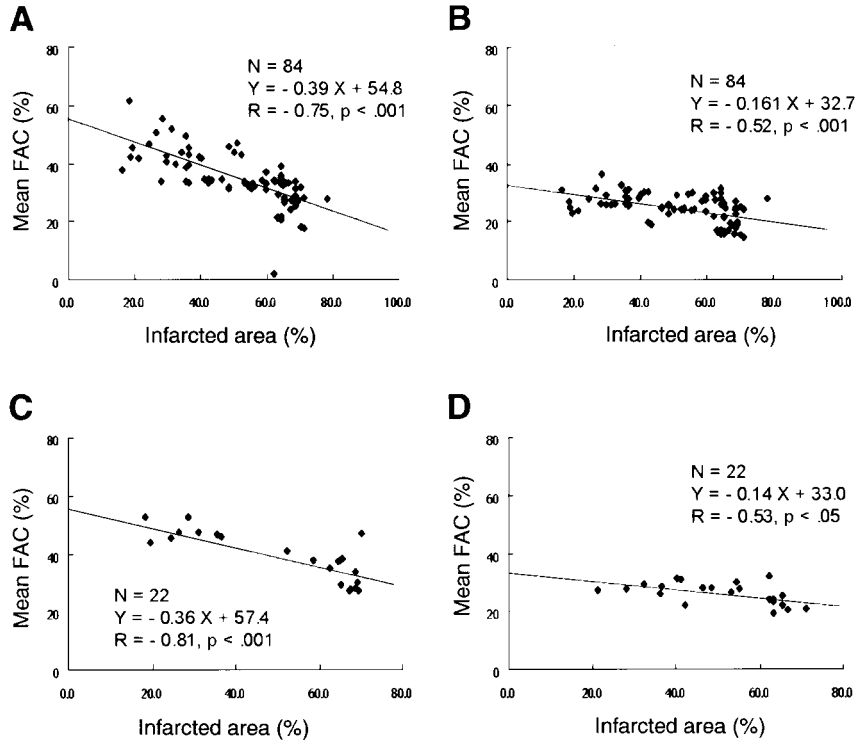

Fig. 2. Regression equations (*Y*) and correlation coefficients (*r*) between infarcted area and fractional area change (FAC) in each condition after surgery. *A*: conscious animals. *B*: high-dose intraperitoneal anesthesia administration. *C*: gas anesthesia administration. *D*: low-dose intraperitoneal anesthesia administration. *N*, number of mice.

mals. An intraperitoneal anesthesia-induced reduction in FAC appears to have especially contributed to the discrepancy between FAC and infarcted area noted in animals with relatively small infarcted area and preserved function. Our data also demonstrated a significantly decreased HR after intraperitoneal anesthesia in mice. Thus the present data suggest that HR and LV systolic function determined during conscious studies closely resemble the physiological values as opposed to those obtained under intraperitoneal anesthesia. Gas anesthesia also provided more physiological values when compared with high-dose intraperitoneal anesthesia. However, the correlation between FAC and infarcted area during gas anesthesia was significantly lower than that observed in conscious state, possibly due to mild reduction of LV systolic function. The use of intraperitoneal anesthesia in the intact murine model clearly induced a depression in cardiac function independent of other experimental manipulations. This finding needs to be considered in any experiments using intraperitoneal anesthesia.

Heart rate. It is known that all anesthetic agents depress myocardial function to some degree and that xylazine is a very potent α-adrenergic blocking agent (9, 15, 26). Such rates are well below the physiological range for mice. In conscious mice, normal HR is within the range of 550–620 beats/min (18, 29). Thus most murine studies report cardiac mechanics at rest rates that would correspond to 30–40 beats/min in a human. Features of cardiac physiology are likely to be severely modified at such slow rates.

Our results for HR under intraperitoneal anesthesia before and after coronary ligation were in agreement with previous studies using intraperitoneal anesthesia. Conscious HR in our study is slightly lower than previously reported basal HR. Hoit and colleagues (15) reported that there was a biphasic force-frequency relationship during anesthesia in mice. Palakodeti and

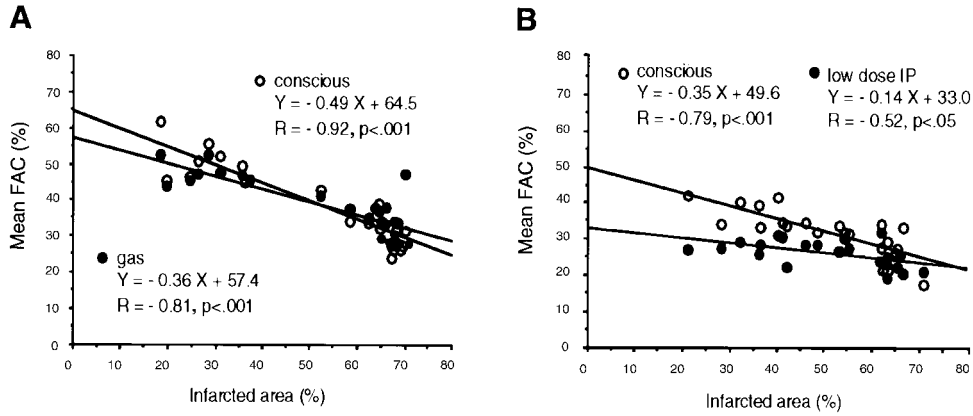

Fig. 3. *A*: correlation between infracted area and FAC before and during gas anesthesia administration after surgery. *B*: correlation between infracted area and FAC before and during low-dose intraperitoneal anesthesia given after surgery. Data from animals in conscious state (○) and during gas or low-dose intraperitoneal (IP) anesthesia administration (●) are shown.

co-workers (29) suggested that the sinus-node rate remained a critical determinant of myocardial contractility while nonsurgically treated mice recovered from anesthesia. These studies indicate that the influence of HR on cardiac function is considerable at greatly reduced rates such as those lowered by anesthesia. Caution is also needed when evaluating the role of adrenergic stimulation for these echocardiographic measurements. For species with body masses >1 kg, maximum HR is two- to threefold above baseline values. However, in small animals such as mice, maximum HR is <30% above resting rates (40). Maximal HRs of 720–800 beats/min during exercise or with a maximal dose of isoproterenol have been reported (12, 40).

Cardiac parameters assessed by other methods. Besides echocardiographic assessment of systolic function, the three most commonly reported systolic parameters are mean arterial pressure, LV pressure, and the first derivative of LV pressure. Higher mean blood pressure values (100–115 mmHg) have been obtained in conscious mice than in anesthetized mice (<80 mmHg) (7, 12, 40). However, to acquire these pressure measurements, catheter-tip micromanometers must remain in the aorta or LV, which requires a surgical procedure for successful placement. Pressure measurements also have well-recognized loading sensitivities to preload changes (19, 22). Moreover, the invasive skin-cutting approach for pressure measurement induces cardiovascular changes by which inevitable changes in the hemodynamic parameters may occur.

Magnetic resonance imaging (MRI) has also been a valuable diagnostic tool for measuring compartmental volumes, wall thicknesses, and shapes of hearts at different anatomic levels in in vivo animals, but it does require anesthesia (22, 36). However, cardiovascular disease is a dynamic process characterized by periods of compensation and decompensation. In these circumstances, analytical methods that can be repeated over time would be especially attractive. Compared with MRI, our noninvasive method enables acquisition of physiological data on cardiac function in real time without anesthesia. Most importantly, repeat measurements in conscious mice are feasible; therefore, our methods allow for repeated studies without killing the animals.

Anesthesia and ventilation. Major factors related to cardiac inhibition in previous studies of in vivo murine hearts are the type of anesthesia and mode of ventilation. The anesthesia used in a majority of studies has either been a combination of xylazine with ketamine or 2,2,2-tribromoethanol (Avertin). Similar to our study, many studies using xylazine have yielded data with diminished systolic function and bradycardia. Close attention also must be paid to methods and patterns of artificial ventilation. Ventilation is not provided at all in some of the studies but is administrated by a volume respirator in others. There is remarkable variability with regard to the ventilation rate and tidal volumes used with little to no assessment of their adequacy (21, 28). Our study is also similar to a study using inhaled methoxyflurane, which was well tolerated physiologically with HR and blood pressures compatible with full recovery (38). Subchronic low-dose exposure to isoflurane in the same strain of mice (Swiss-Webstar) showed that there was no evidence of toxicity (31). There was no loss of mice during gas anesthesia in our studies; therefore, isoflurane anesthesia with spontaneous breathing could be used for studying mice to reduce depression of cardiac function.

Limitations. Even with current techniques it is only possible to image the heart at a rate of 120 frames/s. If HR is 600 beats/min, 1 cardiac cycle will consist of 12 images. In our study, there are also some technical considerations that lead to disagreements between pathological and echocardiographic findings. The pathological planes at the midpapillary level may not be identical to those recorded echocardiographically. Large transmural infarcts result in complex alterations in ventricular architecture involving both the infarcted and noninfarcted zones (30). When disproportionate thinning and dilation occur in the infarcted region, they are accompanied by a distortion in the shape of the entire heart including the remote normal myocardium. Thus these remodeling processes may affect the functional measurement of the LV in vivo. Additionally, myocardial stunning, local ischemia, and local adhesions could each cause regional wall-motion abnormalities that could be detected echocardiographically yet would not be detected by pathological assessments. Finally, the order of performance of echocardiographic studies (after different types of anesthesia) was not randomized. This may possibly have affected the cardiac parameters.

Additionally, the present conscious echocardiographic approach requires two operators: one to hold the animal and the transducer and the other to operate the ultrasound equipment. Finally, in the present study, echocardiography could not be performed under each condition in exactly the same position. Changes in the scanning position of mice may have affected the results.

In conclusion, intraperitoneal anesthetic administration significantly reduces HR and cardiac contractility as opposed to the use of the conscious approach or gas anesthesia in both baseline and infarcted mouse hearts. Gas anesthesia with spontaneous breathing is also suited for assessing systolic function. However, by virtue of its relative simplicity and noninvasive nature, conscious murine echocardiography may be the most suitable method for assessing systolic cardiac function in murine studies.

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