Effects of changes in left ventricular contractility on indexes of contractility in mice

SHINTARO NEMOTO,1 GILBERTO DE FREITAS,1 DOUGLAS L. MANN,1,2 AND BLASE A. CARABELLO1
1Department of Medicine, Houston Veterans Affairs Medical Center, and 2Winters Center for Heart Failure Research, Baylor College of Medicine, Houston, Texas 77030

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Nemoto, Shintaro, Gilberto DeFreitas, Douglas L. Mann, and Blase A. Carabello. Effects of changes in left ventricular contractility on indexes of contractility in mice. Am J Physiol Heart Circ Physiol 283: H2504–H2510, 2002; 10.1152/ajpheart.00765.2001.—Measurement of left ventricular (LV) function is often overlooked in murine studies, which have been used to analyze the effects of genetic manipulation on cardiac phenotype. The goal of this study was to address the effects of changes in LV contractility on indexes of contractility in mice. LV function was assessed in vivo in closed-chest mice by echocardiography and by LV catheterization using a conductance pressure-volume (P-V) catheter with three different interventions that alter contractility by 1) atrial pacing to increase inotropy by augmentation of the force-frequency relation (modest increment of inotropy), 2) dobutamine to maximize inotropy, and 3) esmolol infusion to decrease contractility. Load-independent parameters derived from P-V relations, such as slope of end-systolic P-V relations (ESPVR) and slope of the first maximal pressure derivative over time (dP/dt max)-end-diastolic volume relation (dP/dt-EDV), and standard echocardiographic parameters were measured. The dP/dt-EDV changed the most among parameters after atrial pacing and dobutamine infusion (percent change, 162.8 ± 95.9% and 271.0 ± 44.0%, respectively). ESPVR was the most affected by a decrease in LV contractility during esmolol infusion (percent change, −49.8 ± 8.3%). However, fractional shortening failed to detect changes in contractility during atrial pacing and esmolol infusion and its percent change was <20%. This study demonstrated that contractile parameters derived from P-V relations change the most during a change in LV contractility and should therefore best detect a small change in contractility in mice. Heart rate has a modest but significant effect on P-V relationship-derived indexes and must be considered in the evaluation of murine cardiac physiology.

contractile indexes; echocardiography; catheterization; mouse heart

Genetically engineered murine models of heart failure have long been used to analyze the effects of a specific genetic manipulation on cardiac phenotype because they impute a cause-and-effect relationship between the candidate gene and the outcome of its manipulation. In phenotypes producing severe ventricular dysfunction, ejection performance is an adequate description of function, but when less severe contractile abnormalities are produced, more accurate measurements of cardiac performance are required, especially in the detection of early changes in contractility. Many approaches to measuring murine cardiac function have been reported. Echocardiography was established early in the history of transgenic models (19) and it has been a useful and convenient tool in evaluating left ventricular (LV) function (11, 21). Recently, the progress in microsurgery and biomedical engineering enabled measurement of LV pressure-volume (P-V) relations in murine models using a combined pressure and conductance transducers (4, 9). This method allows assessment of LV function more precisely by several load-independent indexes, such as maximum elastance (E max) and the slope of the end-systolic P-V relation (ESPVR). However, which parameters best represent cardiac performance is not clear, especially in detecting small changes in contractility. Although comparative studies examining the influence of various loading and inotropic conditions on indexes of LV contractility based on PV relationships were performed in dogs (13), there is no such study in a murine model, especially comparing contractility indexes derived from echocardiography to those from PV relationships.

The goal of this study was to address the effects of a change in contractility on indexes of LV contractility in mice. We assessed the sensitivity of contractile indexes to the following three interventions to alter cardiac contractility: 1) using an atrial pacing increase in heart rate (HR), which has a modest effect on contractility via the force-frequency relation (FFR) (8, 10, 12, 16); 2) using infusion of an inotropic agent, dobutamine (β-adrenergic agonist), which maximizes contractility; and 3) using an esmolol (β-adrenergic antagonist) infusion to decrease contractility.

Methods

Thirty-four male C57BL mice (12–24 wk) were used in this study. The experimental protocol was approved by the Ani-

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Address for reprint requests and other correspondence: S. Nemoto, 2002 Holcombe Blvd., Bldg. 110, Rm. 151C, Houston, TX 77030 (E-mail: snemoto@bcm.tmc.edu) and B. A. Carabello, Houston Veterans Affairs Medical Center, 2002 Holcombe Blvd., Houston, TX 77030 (E-mail: blaseanthony.carabello@med.va.gov).
nal Subjects Committee of Baylor College of Medicine and Houston Veterans Affairs Medical Center. All the animals in this study received humane care in compliance with the animal use principles of the American Physiological Society and the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the National Institutes of Health’s Guidelines for the Care and Use of Laboratory Animals (NIH Publication No. 55-23, Revised 1993).

**Animal Preparation and Procedures**

**Surgical procedures.** Mice were anesthetized with a mixture of ketamine (100 mg/kg ip), xylazine (2.5 mg/kg ip), and heparin (5,000 U/kg ip) and additional smaller doses were given as needed. Animals were placed in supine position on a temperature-controlled surgical table to maintain rectal temperature at 37°C and were allowed to breathe spontaneously with 1 l/min of oxygen via nasal cone. Under a dissecting microscope (model SZ40, Olympus; Tokyo, Japan), the right jugular vein was isolated, and a 1-Fr bipolar pacing catheter (model EP118-2, NuMED; Hopkinton, NY) or a polyethylene-10 tube for dobutamine or esmolol infusion was advanced into the right atrium and secured with 8-0 Prolene (model M8753, Ethicon; Somerville, NJ). Atrial pacing was established using a stimulator (model SD9E, Grass Medical Instruments; Quincy, MA). For LV catheterization, the right carotid artery was dissected and cannulated with a 1.4-Fr micro-tipped combined PV catheter (model SPR-719, Millar Instruments; Houston, TX). The conductance catheter was advanced retrograde into the LV and secured with the 8-0 Prolene. The conductance catheter was positioned in the LV chamber as the proximal electrodes of the catheter were positioned below the aortic valve by monitoring the two-dimensional (2-D) parasternal LV long axis image using echocardiography. The conductance signal was acquired online using data-acquisition software (BioBench version 1.0, National Instrument; Austin, TX) and analyzed off-line by analysis software (PVAN, version 2.7, 001-1031, Millar Instruments).

**Echocardiography**

The mice were placed in a slight left lateral decubitus position, and the transducer was placed on the chest while excessive pressure was carefully avoided. Transthoracic echocardiographic examinations were performed using a cardiac system equipped with a 15-MHz linear transducer (Sequoia C256 and 15L8, Acuson; Mountain View, CA). The 2-D parasternal LV long-axis images were obtained at the plane of the aorta and mitral valve with adequate visualization of the LV apex and the short-axis view was recorded at the level of the papillary muscles. After the short-axis view was ensured, 2-D targeted M-mode tracings were recorded through the anterior and posterior LV walls at a sweep speed of 200 mm/s. Pulsed-wave Doppler signals of LV outflow were obtained from apical four-chamber view and were recorded at a sweep speed of 200 mm/s. All images were digitally acquired and stored for off-line analysis.

**Protocols**

**Pacing study.** **DETERMINATION OF HR AT WHICH LV PRESSURE DERIVATIVE OVER TIME IS MAXIMAL IN FFR.** To achieve a modest increment of inotropy, the hearts were atrial paced to a HR (HR_{max}) at which the first derivative of LV pressure (LVPV) over time (dP/dt) was maximal by augmentation of the FFR. Although it has been reported that myocardial contraction and relaxation were enhanced modestly (13–15%) at HRs of 400–500 beats/min (8), five mice were used to define HR_{max} in this experimental setting because HR_{max} may differ significantly between strains of mice and between experimental conditions (11, 12, 16). When hemodynamic stability was ensured after the insertion of pacing and conductance catheters, baseline data were recorded. Atrial pacing was then initiated at just above the sinus rhythm (SR) HR and was increased at 1-Hz increments until maximum dP/dt (dP/dt_{max}) was visually decreased. The setting of the stimulator was 3 V with 2 ms pulse-width duration.

**Echocardiography at SR and HR_{max}.** Six mice were studied. Echocardiography was performed during the intrinsic SR and atrial pacing at HR_{max}.

**LV catheterization at SR and HR_{max}.** Another five mice were used in this LV catheterization protocol. After surgery and hemodynamic stabilization, baseline measurements were performed at SR. After atrial pacing was established at HR_{max} and hemodynamic stability was ensured, a second set of measurements were performed. A small transverse abdominal incision was then made on the upper abdominal wall and LV relations were obtained at HR_{max} by instrument occlusion of the inferior vena cava (IVC) with simple compression with the use of a cotton swab. Another set of P–V relation measurements were repeated at SR after termination of pacing and hemodynamic stabilization.

**Dobutamine infusion study.** Twelve mice were entered into this protocol. Echocardiography and catheterization were performed individually in two separate groups (6 mice in each group). After baseline measurements dobutamine was infused at a rate of 30 ng·g body wt·min⁻¹ through the right jugular vein. When hemodynamic stability was ensured during infusion, a second set of measurements were made.

**Esmolol infusion study.** Eleven mice (5 for echocardiography and 6 for catheterization individually) were used in this protocol. After baseline measurements, esmolol (ultrashort-acting β-adrenergic antagonist) was infused intravenously at a rate of 10 mg·kg body wt·min⁻¹. This dose was defined in a pilot study using five mice. The criteria to achieve a 10% decline in HR, a 30% decline in dP/dt, and reversibility of the effects after completion of the infusion.

**Data Analysis and Contractile Parameters**

**Echocardiography.** M-mode LV internal chamber dimension and posterior wall thickness were measured at end diastole (EDD) and end systole (ESD) using the leading-edge method of three consecutive cardiac cycles (6). End diastole was defined at the peak of the QRS complex of the electrocardiogram. End systole was defined as the peak of posterior wall thickening. LV fractional shortening (FS) was calculated as %FS = (EDD − ESD) × 100/EDD. LV volume at end diastole (EDV) and end systole (ESV) were calculated by the bullet method as EDV = 0.85 × CSA(d) × L(d) and ESV = 0.85 × CSA(s) × L(s), where CSA(d) and CSA(s) are endocardial cross-sectional areas in end diastole and end systole, respectively, obtained from short-axis view at the level of the papillary muscles and L(d) and L(s) are the LV length (apex to midmitral annulus plane) in end diastole and end systole, respectively, obtained from the parasternal long-axis view. LV ejection fraction (EF%) was then calculated as EF% = (EDV − ESV) × 100/EDV. Ejection time (ET) was determined from the actual pulsed-wave Doppler tracings of LV outflow by measuring the interval from the beginning of the acceleration to the end of the deceleration. The myocardial velocity of LV circumferential shortening (V_{cm}) was calculated as V_{cm} = (l[(EDD − ESD)/EDD])/ET, where
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V_{cf} is in circumferences per second and EjT is in seconds. Mean normalized systolic ejection rate (MNSER) was calculated as MNSER = [(EDV − ESV)/EDV]/EjT, where MNSER is in ejections per second. V_{cf} and MNSER were used to assess LV ejection performance.

Catheterization-derived indexes. The 1.4-Fr high-fidelity micromanometer catheter was calibrated with a mercury manometer at the beginning of each experiment. Baseline zero reference was obtained by placing the sensor in normal saline before insertion. LVP, HR, and ±dP/dt_{max} were determined with analysis software. Although dP/dt_{max} has been widely used as a cardiac contractile parameter in murine cardiac physiology, it is well recognized that dP/dt_{max} is load dependent, especially on changes in preload. On the other hand, previous studies (13, 14) have demonstrated that the slope of the dP/dt_{max}-EDV relation (dP/dt_{max}-EDV) represents a sensitive but less load-dependent parameter of chamber contractility. In previous studies, the slope of this relationship increased and shifted leftward with increased contractility, and it decreased and shifted rightward with depressed contractility. ESPVR and E_{max} also have been used to assess in vivo contractility. Although limited by dependence on cardiac size (if the heart increases in volume, ESPVR will decrease regardless of contractility) (2, 15, 18), ESPVR and E_{max} are thought to be ideal in acute studies such as this one because the heart size, mass, and volume do not change throughout the study. Therefore, ESPVR, E_{max}, and dP/dt_{max}-EDV were evaluated in vivo load independent LV contractile performance.

The relative volume unit (RVU) calibrator (Millar Instruments) was used in this study to convert acquired voltage to resistance or relative volume units. Because resistance is the inverse of conductance, conductance was available. However, we instead chose to leave our acquired signal in resistance or relative volume units. Because resistance is the frequency relationship. Incremental atrial pacing resulted in a significant increase and biphasic change in dP/dt_{max} (Fig. 1). HR_{max} was 505 ± 14 beats/min. We therefore used 505 beats/min as HR_{max} for the pacing study protocol.

Pacing Study

Hemodynamic parameters at both HRS were shown in Table 1. Figure 2 shows the percent change of the indexes in the mice. All parameters except for %FS showed a significant change. The dP/dt_{max}-EDV was the most affected (162.8 ± 95.9% increase) by a change in LV contractility, followed by E_{max} (67.9 ± 12.2% increase). The percent change in the dP/dt_{max}-EDV was significantly greatest among the parameters. Echocar-

\[
y = -0.074x^2 + 78.943x - 12619
\]

\[
R^2 = 0.5157, p = 0.718
\]

Fig. 1. Determination of heart rate (HR) at maximum of the first derivative of left ventricular (LV) pressure (dP/dt) from the force-frequency relationship. Incremental atrial pacing resulted in a significant increase and then a decrease in dP/dt_{max}. HR_{max} was 505 ± 14 beats/min. Regression line shows polynomial curve fitting. HR_{max}, HR at which dP/dt_{max} is maximal by augmentation of the force-frequency relationship.
diography-derived global systolic parameters, such as %FS and EF%, had very small percent changes (10.2 ± 6.3% and 16.6 ± 5.3%, respectively). The dP/dt at HRmax versus SR (8,549 ± 780 and 6,080 ± 715 mmHg/s, respectively, P < 0.05) also increased significantly. The percent change in ejection fraction performance expressed as Vcf and MNSER (42.2 ± 8.0% and 51.9 ± 5.6%, respectively) significantly increased after the pacing.

Dobutamine Study

Hemodynamic parameters at baseline and during dobutamine infusion were shown in Table 2. Figure 3 shows the percent change of the indexes. Dobutamine significantly increased contractile parameters with a large percentage of change except for ESPVR and Emax, where changes were inconsistent during dobutamine infusion. Again, dP/dt-EDV was the most affected parameter.

Table 1. Hemodynamic and contractile parameters in pacing protocol

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sinus</th>
<th>HRmax</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>290 ± 29</td>
<td>505 ± 14</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>%FS</td>
<td>27.9 ± 2.0</td>
<td>30.5 ± 2.4</td>
<td>0.38</td>
</tr>
<tr>
<td>EF%</td>
<td>40.0 ± 1.1</td>
<td>46.6 ± 2.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Vcf, cm/s</td>
<td>4.77 ± 0.32</td>
<td>6.75 ± 0.56</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>dP/dt-EDV, mmHg</td>
<td>6,080 ± 715</td>
<td>8,549 ± 780</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>-dP/dt-EDV, mmHg</td>
<td>-6,661 ± 711</td>
<td>-8,416 ± 754</td>
<td>0.1287</td>
</tr>
<tr>
<td>τ, ms</td>
<td>6.15 ± 0.6</td>
<td>5.3 ± 0.6</td>
<td>0.3812</td>
</tr>
<tr>
<td>ESPVR, mmHg/Lµl</td>
<td>11.6 ± 1.8</td>
<td>17.5 ± 2.5</td>
<td>0.0906</td>
</tr>
<tr>
<td>Emax, mmHg/Lµl</td>
<td>11.7 ± 1.7</td>
<td>21.6 ± 3.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>dP/dt-EDV, mmHg/s</td>
<td>590.6 ± 82.1</td>
<td>1,456.5 ± 231.7</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE. HR, heart rate; %FS, percent fractional shortening; EF%, percent ejection fraction; Vcf, myocardial velocity of LV circumferential shortening (cm/s); LVP, LV pressure; MNSER, mean normalized systolic ejection rate; dP/dt and -dP/dt, positive and negative peak rate of pressure development; τ, time constant of isovolumic relaxation by the Weiss method; ESPVR, end-systolic pressure-volume relationship; Emax, maximum chamber elasticity; dP/dt-EDV, slope of +dP/dtmax-EDV relation; HRmax, maximum HR at dP/dt.

Table 2. Hemodynamic and contractile parameters in dobutamine protocol

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Dobutamine</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>404 ± 14</td>
<td>634 ± 19</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>%FS</td>
<td>34.6 ± 2.4</td>
<td>46.5 ± 1.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>EF%</td>
<td>51.1 ± 2.0</td>
<td>60.2 ± 1.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Vcf, cm/s</td>
<td>6.39 ± 0.52</td>
<td>12.51 ± 0.70</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MNSER, s</td>
<td>9.44 ± 0.29</td>
<td>16.15 ± 0.55</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Peak LVP, mmHg</td>
<td>92.4 ± 0.7</td>
<td>121.3 ± 7.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>dP/dt-EDV, mmHg/s</td>
<td>5,666 ± 570</td>
<td>18,992 ± 1,575</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>ESPVR, mmHg/Lµl</td>
<td>23.0 ± 3.6</td>
<td>27.4 ± 5.0</td>
<td>0.5028</td>
</tr>
<tr>
<td>Emax, mmHg/Lµl</td>
<td>27.8 ± 4.3</td>
<td>31.9 ± 4.5</td>
<td>0.5343</td>
</tr>
<tr>
<td>dP/dt-EDV, mmHg/s</td>
<td>1,260.7 ± 139.9</td>
<td>3,923.1 ± 544.5</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE. HRmax, maximum HR.

Figure 4 shows a representative echocardiogram during dobutamine infusion and IVC occlusion, which explains the inconsistency in ESPVR. The proximal pair of electrodes was moved out from the small LV cavity during this maneuver. Figure 5 shows representative P-V loops during dobutamine infusion and IVC occlusion. All loops have a spike at the corner of end systole, which indicates interference between pressure manometer and the LV endocardial surface. The lowest P-V loops shift to the right of the ESPVR line because the proximal sensing electrodes were positioned above the aortic valve. These anatomic findings were observed only during dobutamine infusion, which caused tachycardia and small ventricular cavity in end systole.

Esmolol Study

Hemodynamic and LV functional parameters at baseline and during esmolol infusion are shown in Table 3. Figure 6 demonstrates the percent change of the indexes. Although esmolol significantly reduced LV contractility detected by catheterization-derived indexes, %FS and EF% failed to detect the changes in contractility. The percent change in %FS, EF%, Vcf, and MNSER were very small (%change: (271.0 ± 44.0%) increase) by changes in LV contractility among the parameters and was followed by Vcf and MNSER (103.5 ± 23.6% and 71.3 ± 4.8%, respectively).

Fig. 2. Percent change of contractile indexes in the pacing protocol. Percent change was calculated as [(value during dobutamine infusion) – (baseline value)]/(baseline value)] × 100. FS, fractional shortening; EF, ejection fraction; Vcf, myocardial velocity of LV circumferential shortening; MNSER, mean normalized systolic ejection rate; ESPVR, end-systolic pressure-volume relation; Emax, maximum elastance; EDV, end-diastolic volume. P < 0.001 by ANOVA.

*P < 0.05 vs. FS, EF, Vcf, MNSER, ESPVR, and Emax.

Fig. 3. Percent change of contractile indexes in the dobutamine protocol. P < 0.001 by ANOVA. *P < 0.05 vs. FS, EF, Vcf, MNSER, ESPVR, and Emax.
Fig. 4. Representative echocardiogram during inferior vena cava (IVC) occlusion at baseline (left) and under dobutamine infusion in end systole (right) [two-dimensional (2-D) parasternal long-axis view]. The proximal pair of electrodes (left) was moved out the LV cavity in end systole during dobutamine infusion, whereas all electrodes were below the aortic valve inside the LV cavity at baseline. The size of the LV cavity was very small due to IVC occlusion and tachycardia with hypercontractility caused by dobutamine. This phenomenon was not observed in the other protocols.

−8.7 ± 3.9, −11.4 ± 4.9, −14.5 ± 3.0, and −14.8 ± 3.9, respectively) and consistent with those in the pacing protocol. Interestingly, ESPVR was the most affected among the indexes (%change: −49.8 ± 8.3) but was not significantly greater than the change in $E_{\text{max}}$ and $dP/dt-EDV$ (%change: −41.9 ± 10.9 and −40.8 ± 10.7, respectively).

**DISCUSSION**

There were four major findings of this study. First, load-independent parameters derived from P-V relationships were the most affected by a change in LV contractility. Second, %FS failed to detect the changes in contractility produced by pacing and esmolol infusion. Third, HR has a modest but significant effect on murine LV contractility measured by P-V relation-derived parameters at relatively lower HR in this study. Fourth, the catheter used in this study caused artifactual malpositioning of the electrodes outside the small LV cavity caused by dobutamine infusion.

**Echocardiographic Indexes**

FS has been used in many murine studies because it is convenient and easy to understand. %FS is most useful in studies of transgenes, which have caused dramatic changes in cardiac phenotype. In our study, dobutamine, a strong inotrope, caused a significant change in %FS. However, %FS could not detect the difference in contractility increased by pacing at $HR_{\text{max}}$ or decreased during esmolol infusion. These results suggest that %FS would be ineffective in detecting small changes in contractility in murine models. In contrast, both mean $V_{\text{ef}}$ and MNSER consistently showed a significant change in all three settings of this study. However, because both indexes are also afterload sensitive (10, 21), the relation with end-systolic stress needs to be assessed when a significant difference in the wall stress among groups is expected, such as in severe hypertrophy and hypertension.

<table>
<thead>
<tr>
<th>Table 3. Hemodynamic and contractile parameters in esmolol protocol</th>
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<tr>
<td>HR, beats/min</td>
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<tr>
<td>%FS</td>
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<tr>
<td>EFc</td>
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<tr>
<td>$V_{\text{ef}}, \text{circ/s}$</td>
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<tr>
<td>MNSER, s</td>
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<tr>
<td>Peak LVP, mmHg</td>
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<td>$dP/dt, \text{mmHg/s}$</td>
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<td>$\tau, \text{ms}$</td>
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<tr>
<td>ESPVR, mmHg/µl</td>
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<tr>
<td>$E_{\text{max}}, \text{mmHg/µl}$</td>
</tr>
<tr>
<td>$dP/dt-EDV, \text{mmHg-s}^{-1} \cdot \text{µl}^{-1}$</td>
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Values are means ± SE.
Pacing at HR_{max} or dobutamine significantly increased ΔP/Δt_{max} compared with baseline in this study. On the other hand, ΔP/Δt_{max} was significantly decreased during esmolol infusion. However, caution must be used in assessing LV contractility using ΔP/Δt_{max} because of substantial load dependence (9). Georgakopoulos et al. (7–9) showed that reducing EDP by only 1 mmHg from resting baseline was sufficient to lower ΔP/Δt_{max} by 18 ± 5.2%. Thus parameters of LV contractile performance independent from or corrected for preload, such as ESPVR and E_{max} (18) and ΔP/Δt_{max}-EDV (14) established in larger animals, are also needed in making murine measurements of contractility. Whereas these parameters can be defined only by simultaneous measurement of pressure and volume, technical advances of microtipped combined PV catheters in the mouse have made it possible to obtain these parameters in vivo. These parameters increased significantly at HR_{max} and decreased significantly during esmolol infusion. The slope of ΔP/Δt_{max}-EDV changed the most among the parameters we studied in the pacing and the dobutamine studies. This finding was supported by a previous report using an isolated canine heart model with carefully controlled alterations in preload and afterload. The ΔP/Δt-EDV appeared an excellent choice to address contractility because of its marked sensitivity to inotropic change, its lack of preload dependence, and its minimal afterload dependence in the physiological range (13). On the other hand, in the current study, ΔP/Δt-EDV changed similarly to ESPVR and E_{max} during esmolol infusion. The possible explanation in the differential response of ΔP/Δt-EDV between positive and negative inotropy remains unclear.

In the dobutamine infusion protocol, the end-systolic parameter E_{max} failed to show significant increase and consistent percent change. As seen in Fig. 4, the small LV cavity caused malpositioning of the catheter electrodes outside the LV cavity, which led to an artifact on the volume signal (Fig. 5). Therefore, caution must be used in assessing ESPVR parameters in small LV cavities using the P-V catheter that we used in this study. In future catheter redesign, spacing electrodes for small LV size will obviate this problem.

In this study, the lack of the offset calibration had the disadvantage of failing to give a precise estimation of volume intercepts (V_o) of PV relations. Because V_o varies under different loading conditions (e.g., afterload shifts V_o to the left, whereas preload shifts it to the right), it is valuable to determine V_o in the P-V plane analysis. Therefore, the results of P-V relation-derived parameters in this study will need to be verified when dual frequency conductance system is available.

**Effects of HR on Cardiac Performance in Mice**

In the present study, HR was used to assess the effect of a modest inotropic stimulus on performance based on the known effects on force of increasing frequency. Georgakopoulos and Kass (8) reported that contraction (ΔP/Δt_{max}) and relaxation (time constant of isovolumetric relaxation, τ) were enhanced modestly (13–15%) at HRs of between 400 and 500 beats/min and ESPVR rose 35% at HRs from 400 to 600 beats/min. We noted similar changes in FFR and ESPVR. Georgakopoulos and Kass (8) noted that within the broad physiological HR (500–850 beats/min), by normalizing ΔP/Δt_{max} by instantaneous developed pressure to diminish preload dependence, the biphasic HR-ΔP/Δt relationship, especially the descending limb of the regular FFR, become flat. Accordingly, there was very little change in ESPVR at HRs >500 beats/min (8). They concluded that the normal mouse has a very limited force-frequency reserve at physiological HRs unlike larger mammals. Interestingly, however, although we did not test the effects of a wide range of physiological HRs on contractile function, the HR at which the normalized FFR plateaued (~500 beats/min) in the previous study (8) was similar to HR_{max} in this study.

The positive inotropic response in the upslope of FFR is possibly explained by an increase in Ca^{2+} availability to the contractile filaments as a result of increased Ca^{2+} storage in the sarcoplasmic reticulum (SR) with augmentation of subsequent Ca^{2+} transients (20). Interestingly, it is demonstrated that the recirculation fraction of Ca^{2+} was 93 ± 1% in intact mice (much higher than the 50% in larger animals), which implies a more dominant role of SR buffering compared with other source of Ca^{2+} (8). It is also widely known that depressed FFR with abnormal Ca^{2+} handling by the sarcoplasmic reticulum and the Na^{+}/Ca^{2+} exchanger pump play a major role in human (17) and experimental heart failure (22). Therefore, it is not surprising that FFR and HR_{max} can vary in different settings of heart failure and in various transgenic mice in which gene modification affects intracellular Ca^{2+} homeostasis and SR function.

In conclusion, contractile parameters derived from P-V relations are most sensitive to change in LV contractility and should be used especially to detect a small change in contractility. This study also identified limitations of echocardiographically derived %FS in the response to a modest change in contractility. However, caution must be exercised for assessment of ESPVR and E_{max} in small LV cavities because of possible malpositioning of catheter electrodes above the
aortic valve. Moreover, HR has a substantial effect on murine LV performance at subphysiological HRs and HR$_{max}$ must be used in the evaluation of cardiac function.

Limitations

All measurements in this study are performed during acute changes in contractility. Whereas it is well known that end-systolic indexes derived from P-V relations are changed by chronic changes in volume and mass regardless of contractility in larger animals (15, 18), how this concept applies to genetically altered mice with chronic cardiovascular changes awaits further study. Alternatively, to eliminate the load dependency and geometrical effect on the end-systolic parameters (chamber physiology), it might be better to define myocardial tissue contractile properties, such as end-systolic stiffness, by transforming the P-V relationship into the stress-strain relationship (3, 15).

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REFERENCES


