Invasive hemodynamics and force-frequency relationships in open- versus closed-chest mice

BRIAN D. HOIT, NANCY BALL, AND RICHARD A. WALSH
Division of Cardiology, University of Cincinnati Medical Center, Cincinnati, Ohio 45267-0542

Hoit, Brian D., Nancy Ball, and Richard A. Walsh. Invasive hemodynamics and force-frequency relationships in open- versus closed-chest mice. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2528–H2533, 1997.—We compared hemodynamics, ventricular function, and force-frequency relationships in six open-chest and six closed-chest anesthetized mice (FVB/N strain). Left ventricular (LV) pressure was measured with a 1.8- or 1.4-Fr Millar catheter placed via the right carotid artery and the LV apex in the closed- and open-chest state, respectively. Pacing was performed with electrodes placed either directly on atrial appendages (open chest) or with a 1-Fr bipolar catheter via the jugular vein (closed chest). Closed-chest animals had greater spontaneous heart rate (267 ± 106 vs. 147 ± 27 beats/min), LV systolic (81 ± 14 vs. 48 ± 9 mmHg) and diastolic pressures (11.2 ± 4.8 vs. 5.6 ± 2.4 mmHg), and maximal rise (+dP/dt\textsubscript{max}: 6,208 ± 2,519 vs. 3,682 ± 671 mmHg/s) and fall in pressure development (−dP/dt\textsubscript{max}: −6,094 ± 2,386 vs. −3,001 ± 399 mmHg/s). LV systolic pressure (98 ± 18 vs. 52 ± 11 mmHg), +dP/dt\textsubscript{max} (9,240 ± 2,459 vs. 5,777 ± 2,473 mmHg/s), and −dP/dt\textsubscript{max} (−8,375 ± 2,551 vs. −3,753 ± 1,170 mmHg/s) were significantly higher when animals were matched at a heart rate of 420 beats/min in closed-chest versus open-chest animals. Biphasic force-frequency relationships were seen in all animals, but the critical heart rate was greater in the closed- than open-chest animals (432 ± 42 vs. 318 ± 42 beats/min). We conclude that 1) there are significant differences between invasive indexes of systolic and diastolic function between the closed- and open-chest preparations, 2) there is a biphasic force-frequency relationship in the anesthetized mouse, and 3) dP/dt\textsubscript{max} can be used to assess the cardiovascular phenotype.

ventricular function; Starling relationship; transgenic mice

TRANSGENIC and gene-targeted manipulations of the murine genome are widely employed to understand mechanisms of cardiovascular physiology and disease (4–7, 14, 20, 21). A major limitation of such studies is that the small size of the mouse hamper the ability to characterize the cardiovascular functional phenotype. Although echocardiographic techniques have been used increasingly, a more complete (and complimentary) characterization requires invasive catheter-based approaches. Microsurgical techniques and miniaturization of catheter-transducer systems permit measurement of ventricular pressure; nevertheless, studies are limited largely to measurements of basal and catecholamine-stimulated isovolumic pressure indexes (20, 21). More sophisticated measurements, such as cardiac function curves derived by altering either load (aortic systolic pressure and left ventricular (LV) end-diastolic pressure (EDP)) or heart rate have not been reported. Moreover, both open- and closed-chest anesthetized animals are studied without an understanding of their intrinsic independent effects on measurements of ventricular function. Therefore, we compared hemodynamics and pressure-derived indexes of systolic and diastolic function in the open- versus closed-chest normal mouse.

Recent data in our laboratory indicate that there is a biphasic force-frequency relationship in the sedated primate, and the heart rate at which maximal pressure change over time (dP/dt\textsubscript{max}) reaches a maximum (critical heart rate) is a sensitive measure of ventricular systolic function (9). Therefore, the second objective of our study was to determine whether there is a biphasic force-frequency relationship in the anesthetized mouse, and whether it is altered by the type of surgical preparation employed. Finally, we wanted to determine the feasibility of steady-state alterations in LV systolic and diastolic pressure using volume infusions, because this technique has been used extensively in cardiovascular physiological studies in larger mammals.

METHODS

A total of 16 male and female mice (FVB/N strain) weighing 21.8–35 g were used for these experiments. Animals were studied either in the open-chest or closed-chest state. A dissecting microscope (Olympus) was used for all procedures. A three-lead electrocardiogram was used for timing purposes and to verify atrial capture.

Open-Chest Preparation

Mice (n = 6, 27.8 ± 5.0 g) were anesthetized with a mixture of ketamine (100 mg/kg), xylazine (5 mg/kg), and morphine (2.5 mg/kg) given at 0.25 ml ip and were then taped to the operating table in the supine position. The trachea was exposed and ligated with 6–0 silk. An airway was established by puncturing between the tracheal rings with a 24-gauge J elko iv catheter; the sheath was advanced 6–7 mm and secured with a 6–0 silk ligature. The animal was ventilated with a tidal volume of 500 µl at 100 cycles/min using a Harvard Respirator (model 863) and room air.

The chest was widely opened via a subxiphoid incision and bilateral thoracotomies, and the sternum was reflected and secured with a hemostat. Cautery was used to minimize bleeding. An 1.8-Fr Millar catheter was placed in the LV via the apical dimple and secured with a purse-string suture using 10–0 Dermalon. Custom-made platinum wire (0.127
mm diameter) was insulated with polyethylene tubing except for the unexposed tips, which were attached to the right and left atrial appendages for pacing, and the heart was covered gently with saline-moistened cotton. The jugular vein was cannulated with flame-stretched Nalgene polyethylene tubing (1/8” ID) for intravenous access, and the catheter was secured with a 6–0 silk suture.

**Closed-Chest Preparation**

Mice (n = 6, 28.9 ± 4.5 g) were anesthetized with a ketamine, xylazine, and morphine mixture as described in Open-Chest Preparation. The trachea was intubated with a 24-gauge Joco iv catheter, and the animal was allowed to breathe room air spontaneously. The right jugular vein was isolated and ligated cranially with 6–0 silk. Flame-stretched Nalgene polyethylene tubing was advanced into the vein and secured with 6–0 silk. A custom-made 1-Fr bipolar pacemaker (Numed) was positioned in the right atrium via the right internal jugular vein. The right carotid artery was isolated and ligated cranially with 6–0 silk. Flame-stretched Nalgene polyethylene tubing was advanced into the vein and secured with 6–0 silk. A custom-made 1-Fr bipolar pacemaker (Numed) was positioned in the right atrium via the right internal jugular vein. The right carotid artery was isolated and ligated cranially with 6–0 silk. Flame-stretched Nalgene polyethylene tubing was advanced into the vein and secured with 6–0 silk. A custom-made 1-Fr bipolar pacemaker (Numed) was positioned in the right atrium via the right internal jugular vein. The right carotid artery was isolated and ligated cranially with 6–0 silk. Flame-stretched Nalgene polyethylene tubing was advanced into the vein and secured with 6–0 silk. A custom-made 1-Fr bipolar pacemaker (Numed) was positioned in the right atrium via the right internal jugular vein. The right carotid artery was isolated and ligated cranially with 6–0 silk. Flame-stretched Nalgene polyethylene tubing was advanced into the vein and secured with 6–0 silk. A custom-made 1-Fr bipolar pacemaker (Numed) was positioned in the right atrium via the right internal jugular vein.

**Data Analysis**

The micromanometer was electronically calibrated in vitro by submerging the tip of the catheter in warmed saline with the reference zero level taken at midchest. The analog LV dp/dt signal was obtained online by electronic differentiation of the high-fidelity LV pressure signal. Analog signals for high-fidelity LV pressure, LV dp/dt, and the electrocardiogram were recorded online on a Gould WindowGraf four-channel recorder (Gould) at 25 and 100 mm/s paper speed. A constant infusion minipump (Baxter Autosyringe AS20A) was used for volume infusion.

**Experimental Protocols**

Incremental pacing. A Grass stimulator (model S88) was set at 3–4 V, 2 ms pulse-width duration, and the desired frequency. After hemodynamic stability was ensured and baseline data were recorded, pacing was initiated just above the intrinsic heart rate to avoid competing rhythms and was increased at 12 beats/min increments until the LV dp/dt max was visually decreased or until atrioventricular block unresponsive to 0.04–0.08 ng/g atropine supervened. The animals were allowed to recover between protocols.

After hemodynamics returned to baseline, LV pressure was increased in open-chest mice (see Volume Infusion) by incremental steady-state infusion of Hespan (6% hetastarch in 9% NaCl).

**Volume Infusion.** Open-chest animals (n = 8) were paced at a constant heart rate of 300 beats/min. Hespan was used to raise EDP and infused at a rate of 10 ml/h using a microinfusion pump, with “steady-state” data taken at 0.1-ml increments until +1.4 ml were infused.

Experiments were conducted in accordance with institutional guidelines and the Guide for the Care and Use of Laboratory Animals put forth by the United States Department of Health and Human Services, NIH publication No. 86–23. The experimental protocol was approved by the Institutional Animal Care and Use Committee at the University of Cincinnati.

**Table 1. Hemodynamics at intrinsic heart rates**

<table>
<thead>
<tr>
<th>Heart rate, beats/min</th>
<th>Open Chest</th>
<th>Closed Chest</th>
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<tbody>
<tr>
<td>LV systolic pressure, mmHg</td>
<td>48 ± 9</td>
<td>81 ± 14*</td>
</tr>
<tr>
<td>LV end-diastolic pressure, mmHg</td>
<td>56 ± 2.4</td>
<td>11.2 ± 4.8*</td>
</tr>
<tr>
<td>LV +dp/dt max, mmHg/g</td>
<td>3,682 ± 67</td>
<td>6,208 ± 2,519*</td>
</tr>
<tr>
<td>LV −dp/dt max, mmHg/g</td>
<td>3,001 ± 399</td>
<td>6,094 ± 2,386*</td>
</tr>
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</table>

Data are means ± SD; n = 6 mice for both groups. LV, left ventricular; +dp/dt max, maximal pressure rise (+) or decrease (−) over time. *P < 0.05 vs. open chest (unpaired t-test).

**Statistics**

Data from open- and closed-chest animals were compared with unpaired t-tests. Analysis of variance was used to test means within groups for the pacing and volume infusion studies, and Bonferroni-corrected t-tests were used to identify differences. Unless otherwise stated, data are expressed as means ± SD. A value of P < 0.05 was considered statistically significant.

**RESULTS**

Hemodynamics in the open- and closed-chest preparations at the intrinsic heart rate are shown in Table 1. The intrinsic heart rate, LV systolic and EDP, and increased (+dp/dt max) and decreased dp/dt max (−dp/dt max) were significantly greater in the closed-chest than open-chest animals. At a matched heart rate of 300 beats/min, the LV systolic pressure, LVEDP, and −dp/dt max were significantly greater in closed-chest than open-chest mice; the greater +dp/dt max in closed-than open-chest animals did not achieve statistical significance (Table 2). In contrast, at a matched heart rate of 420 beats/min, the LV systolic pressure, +dp/dt max and −dp/dt max were significantly greater in the closed-chest than open-chest animals (Table 2). Although the LVEDP was greater in the closed-chest preparations, this difference was not statistically significant.

The effects of incremental pacing on LV dp/dt max (biphasic force-frequency response) in closed- and open-chest animals are shown in Fig. 1. Incremental atrial pacing resulted in significant increases in dp/dt max in both open- and closed-chest animals. However, compared with the response in open-chest animals, the force-frequency curve in closed-chest animals was shifted upward and to the right, resulting in a delayed descending limb of the force-frequency relationship. Thus the critical heart rate for dp/dt was significantly greater in the closed- than open-chest preparation (432 ± 42 vs. 318 ± 42 beats/min, P < 0.05).

In both open- and closed-chest animals, incremental pacing produced significant increases in LV systolic pressure and decreases in LVEDP (Fig. 2). As pacing progressed beyond the critical heart rate, both LV systolic pressure and EDP decreased. Moreover, in the closed-chest state there was an upward and rightward shift of the LV pressure-heart rate relationship compared with the open-chest state.

The effect of Hespan infusion is shown in Fig. 3. Representative hemodynamic records are shown in Fig.
For the entire group, LV $\frac{dP}{dt}$ increased with LVEDP until an EDP of $\sim 15$ mmHg was reached and was significantly greater at high versus low LVEDP (Fig. 3B); additional increases in LVEDP could not be consistently achieved. In contrast, saline was ineffective in raising LVEDP in pilot studies (data not shown).

LV systolic pressure increased significantly with volume infusion (Fig. 3C); it should be recognized that the LV systolic pressure at a paced heart rate of 300 beats/min (near the critical HR) was greater than that observed at the intrinsic heart rate and the paced rate of 420 beats/min.

**DISCUSSION**

The principal findings of this study are that in the normal mouse left ventricle there is a biphasic force-frequency relationship in both the open- and closed-chest anesthetized state, hemodynamics and force-frequency relationships obtained in the closed-chest state are significantly different (i.e., less depressed) than those observed in the open-chest preparation, and steady-state alterations in LVEDP (and presumably LV end-diastolic volume) can be obtained with Hespan infusion.

Our data indicate that LV performance measurements vary depending on the particular state in which they are acquired, and emphasize the need for appropriate controls. The anesthetic we employed includes morphine, which may be responsible for the “nonphysiological” heart rates and systemic blood pressures, which were lower than those reported by other investigators (12, 15, 19). However, at the critical heart rate, the LV systolic pressure in the open-chest mice was similar to that previously reported (15, 19). Surgical trauma and the potential blood loss were greater in the

**Table 2. Hemodynamics at matched heart rates in open- and closed-chest mice**

<table>
<thead>
<tr>
<th>Heart rate, beats/min</th>
<th>Open chest</th>
<th>Closed chest</th>
<th>Open chest</th>
<th>Closed chest</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV systolic pressure, mmHg</td>
<td>300 ± 0</td>
<td>300 ± 0</td>
<td>410 ± 12</td>
<td>420 ± 0</td>
</tr>
<tr>
<td>LV end-diastolic pressure, mmHg</td>
<td>60 ± 12</td>
<td>95 ± 20*</td>
<td>52 ± 11</td>
<td>98 ± 18*</td>
</tr>
<tr>
<td>LV $+\frac{dP}{dt}$, mmHg/g</td>
<td>4.5 ± 1.5</td>
<td>10.5 ± 5.4*</td>
<td>3.3 ± 2.1</td>
<td>5.5 ± 3.0</td>
</tr>
<tr>
<td>LV $-\frac{dP}{dt}$, mmHg/g</td>
<td>6,994 ± 1,720</td>
<td>8,775 ± 2,559</td>
<td>5,777 ± 2,473</td>
<td>9,240 ± 2,459*</td>
</tr>
<tr>
<td>LV $\frac{dP}{dt}$max, mmHg/s</td>
<td>4,399 ± 1,171</td>
<td>7,250 ± 2,827*</td>
<td>3,753 ± 1,170</td>
<td>8,375 ± 2,551*</td>
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Data are means ± SD; n = 6 mice for all groups. *P < 0.05 vs. open chest (unpaired t-test).
open- than the closed-chest preparation; thus, we cannot exclude the possibility that the low dP/dtmax and the altered force-frequency relationships in open-chest mice were influenced by diastolic hypoperfusion and myocardial ischemia. However, in the current study, LVEDP decreased and LV systolic pressure increased as heart rate increased from the intrinsic to the critical heart rate in both groups of animals; these results suggest that myocardial ischemia is unlikely to be primarily responsible for the differences we observed. Irrespective of the cause(s) of the different hemodynamics, our findings argue strongly for the use of an experimental preparation appropriate for the anticipated cardiovascular phenotype. For example, a phenotype characterized by loss of function may be easier to identify in the context of a preparation with increased function (such as the open chest).

Incremental pacing has been used to assess myocardial contractility in isolated muscle preparations and has recently been described to assess LV function. A biphasic force-frequency relationship with ascending and descending limbs has been demonstrated in both isolated muscle strips (3, 16) and the sedated baboon (9). The effects of increased heart rate are greater in the anesthetized than conscious animal preparation; indeed, both the 44 and 40% increases in dP/dtmax we observed in the open and closed chest, respectively, are greater than the 26% increase in sedated baboons (9) and the 28% increase reported in conscious dogs (2).

The biphasic force-frequency relationship we observed differs from a report in the isolated blood-perfused mouse heart (1); in that study, incremental pacing from 3 to 10 Hz produced a negative developed pressure-heart rate relationship. However, our results are consistent with a recent report that demonstrated biphasic force-frequency relationships using a sinus node-slowing pharmacological agent and atrial pacing in the

Fig. 3. A: hemodynamic records showing LV pressure and dP/dt at 3 levels of EDP from a representative animal. B: LV dP/dtmax-EDP relationship generated by Hespan infusion in 8 open-chest animals. C: LV systolic pressure-EDP relationship generated by Hespan infusion in 8 open-chest animals. Pressures >20 mmHg could not consistently be obtained. Data are means ± SE.
open-chest mouse and the recovery from anesthesia in the closed-chest mouse (17). Interestingly, dP[/dt]max values at baseline and in response to changes in heart rate that were observed in both of their preparations are similar to our findings; however, it should be noted that a descending limb of the force-frequency was not seen in unpaced animals recovering from anesthesia (17).

The potential causes of the descending force-frequency limb in our preparations include 1) qualitative alterations in the contractile and calcium-cycling proteins that limit the ability of the myocyte to release, interact with, and resequester calcium; 2) arterial hypotension with resultant myocardial ischemia; 3) tachycardia-induced reduction of LV preload (LVEDP or volume); and 4) rapid-pacing-induced atrioventricular conduction delay causing a poorly timed atrial contraction. Previous studies have shown that both chronic thyroid hormone administration (9) and acute β-adrenergic stimulation (17), conditions that alter calcium homeostasis, delay the descending limb of the force-frequency relationship. Similar to our earlier results (9), it is likely that the decrease in preload produced by increased heart rates underestimated the magnitude of the force-frequency effect; moreover, the continued fall of LVEDP beyond the critical heart rate suggests that myocardial ischemia does not account for the descending limb. Finally, although atrioventricular conduction was not rigorously assessed in this study, atrioventricular nodal Wenckebach occurred at, or shortly after, the critical heart rate in the majority of animals (data not shown).

An important finding of our study is that dP[/dt]max increases with a volume-induced increase in LVEDP in the mouse. A linear relationship between LV end-diastolic volume and dP[/dt]max is predicted by the time-varying elastance model (11), although earlier studies employing LVEDP as a measure of LV preload yielded similar results (13, 24). It should be emphasized that we cannot extrapolate to a LVEDP >15 mmHg, where curvilinearity of the LV pressure-volume relationship might alter the EDP-dP[/dt]max relationship. Nevertheless, these data demonstrate the feasibility of altering load in the intact mouse and confirm that dP[/dt]max is preload dependent in the mouse as well as in other species. Volume and pressure loading can be used to assess cardiovascular function and cardiac reserve and combined with measures of ventricular size (for example with either miniaturized sonomicrometers in the open-chest or echocardiography in the closed-chest preparation) suggest that generation of pressure-dimension indexes of systolic and diastolic function are possible.

An important limitation of these studies is that they were performed in the anesthetized animal. Thus both of these preparations are confounded by the effects of general anesthesia. Although ketamine is a central sympathetic stimulant, most general anesthetics induce vasodilation and hypotension when given in sufficient doses. In addition, all anesthetics cause myocardial depression (18), affect cardiac control mechanisms (e.g., the Frank-Starling mechanism, the Anrep effect, and the treppe or Bowditch effect), and influence the mechanisms involved in the baroreceptor response and reflex control. It should be recognized that many of these control mechanisms are not considered to play a significant role in conscious animals at a physiological heart rate (23). Another potential limitation relates to the low LV systolic blood pressures (especially at the intrinsic heart rate in the open-chest animals), the absence of aortic diastolic pressures, and the resultant inability to be certain that LV dP[/dt]max occurred during isovolumic systole at these low pressures. Finally, it is assumed that the trauma of surgery and anesthesia does not alter the cardiovascular response to a specific experimental perturbation. Nevertheless, conscious data in the mouse should be interpreted with a healthy degree of caution; unless the experimental animals are acclimatized by a period of training, the associated stress leads to high circulating catecholamines and may alter the chronotropic and inotropic state of the preparation. In this regard, telemetered systems should prove useful in conscious studies (22).

Despite these limitations we have shown that a variety of techniques used to evaluate cardiovascular function in larger animals can be applied to evaluate the phenotypic expression and complex physiological traits in the intact transgenic or gene-targeted mouse. Insofar as all animal models have difficulties and limitations, understanding the influence of the preparation will minimize interpretative errors and facilitate recognition of true physiological or pathological differences.

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