Murine Cardiac Function
A Cautionary Tail

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The capacity to selectively mutate genes or create excessive or deleted gene expression in mice has yielded a powerful new approach to structure-function studies of cardiac proteins and their role in heart disease. As it happened, the molecular techniques required to generate such animals developed more rapidly than did the methods for studying the chamber physiological phenotype. Mainly because of these methodological limitations, studies to date have often presented hemodynamic data that would fail the standards usually applied in larger species. In particular, heart rates (HRs) and basal levels of systolic contraction are frequently depressed to a substantial degree. It has required a major leap of faith to assume that the physiological differences measured between genetically modified and wild-type animals under such conditions translate to the healthy heart or intact animal. Furthermore, in the understandable rush to assess the impact of molecular manipulations, the careful assessment of normal murine cardiac physiology has been left shortchanged. Only now are we beginning to see the results of such analysis, with molecular techniques required to generate such animals developed more rapidly than did the methods for studying the chamber physiological phenotype. Mainly because of these methodological limitations, studies to date have often presented hemodynamic data that would fail the standards usually applied in larger species. Such data may be very important for properly interpreting the physiological consequences of targeted genetic manipulations, as reviewed by James et al in this issue of Circulation Research.

As with larger animals, compromises can and often must be made to balance the need for adequate control to precisely assess cardiac mechanics and physiological intactness to maintain near-normal physiology. Although conscious animal data are often considered the gold standard in larger species, they are not necessarily required to provide relevant or valuable hemodynamic insights. However, awareness of more intact normal physiology has always been critical to properly interpret data obtained in more invasive or isolated heart studies. Rarely has the discrepancy between conscious animal and anesthetized or isolated heart data been as extreme as it appears to be in the mouse. By highlighting these disparities and their potential causes, we hope to bring the target range for normal murine cardiac function into better focus and stimulate efforts to define basal states in various strains in greater detail.

What Is Normal for the Resting Mouse Heart?
The most direct sign of abnormal chamber function in murine cardiac function assessments has been profound basal bradycardia. Many studies in closed- and open-chest preparations or isolated ventricles have presented data with basal HRs near 300 bpm. Such rates are well below the physiological range for the mouse. Basal HRs in conscious mice are within the range of 550 to 620 bpm. Thus, most murine studies are reporting cardiac mechanics at resting rates that would correspond to 20 to 40 bpm in a human, and features of cardiac physiology are greatly modified at such slow rates.

Equally important is corresponding evidence of baseline cardiac depression. The three most commonly reported systolic parameters are mean arterial pressure, left ventricular systolic pressure, and the first derivative of left ventricular pressure (dP/dtmax). Mean pressure is reported at 100 to 115 mm Hg in conscious mice, with peak systolic pressures near 120 mm Hg. Yet the majority of murine studies in anesthetized animals report mean pressures of ≤80 mm Hg, with peak systolic pressures in the same range. Values for baseline dP/dtmax are typically near 4000 mm Hg/s, with only a few studies reporting values as high as 9000 mm Hg/s.

The Table summarizes published data for HR and dP/dtmax in normal hearts from several mammalian species in the conscious state. The ratio of dP/dtmax to HR is seen to be well conserved between 25 and 30. Thus, mice with resting rates of 550 bpm would be expected to have a value of dP/dtmax near 16 000 mm Hg/s. Although these data have yet to be reported in fully conscious animals, studies have found such values in mice awakening from anesthesia, and our laboratory has similarly obtained values near 17 000 mm Hg/s under such conditions. Importantly, the often reported depressed dP/dtmax values are not just a reflection of bradycardia, since pacing such hearts even to subphysiological rates leads to further cardiodepression.

To date, very few alternative systolic function parameters to dP/dtmax have been reported for mice. We recently reported several such parameters derived from pressure-volume relation analysis, including the end-systolic elastance and preload-recruitable stroke work. The latter parameter (slope of relations between cardiac stroke work [ie, pressure · volume] and end-diastolic volume) is particularly useful since it has force (pressure) units and is chamber scaling-size independent. This parameter was measured in C3H/HeJ control mice under α-chloralose/urethane anesthesia and yielded values in the range of 80 to 100 mm Hg, very similar to values reported in conscious humans and dogs. dP/dtmax was ≈12 000 mm Hg/s and HRs were

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The corresponding equation: \( \text{HR}_{\text{rest}} = \frac{550}{\text{M}_b} \), with \( \text{M}_b \) being body mass in kilograms. Prior studies\(^{26}\) had reported that resting HR was similarly related to body mass by the equation: \( \text{HR}_{\text{rest}} = 1.87 \cdot \text{M}_b^{-0.1} \).

Maximal HR reserve as a percentage of baseline HR is known to differ among species. In 1992, Vornanen\(^{10}\) reported that maximal HR induced by exercise was very similar to that observed with maximal isoproterenol stimulation. This was well defined by an allometric equation: \( \text{HR}_{\text{max}} = 450 \cdot \text{M}_b^{-0.15} \), with \( \text{M}_b \) being body mass in kilograms. Prior studies\(^{26}\) had reported that resting HR was similarly related to body mass by the corresponding equation: \( \text{HR}_{\text{rest}} = 241 \cdot \text{M}_b^{-0.25} \). Thus, the ratio of maximal to resting HR is as follows:

\[
\frac{\text{HR}_{\text{max}}}{\text{HR}_{\text{rest}}} = 1.87 \cdot \text{M}_b^{0.1}
\]

Figure 1 displays this last relation. For species at body masses of \( >1 \text{ kg} \), maximal HR is 2- to 3-fold above baseline. However, in substantially smaller animals, such as the mouse,\(^{10}\) maximal HR is \( \approx 30\% \) above resting rates. This is consistent with upper rate limits of actin-myosin crossbridge kinetics that predict maximal rates of \( \approx 1000 \text{ bpm} \) and with experimental data reporting maximal HRs of 720 to 800 bpm in mice during exercise or with maximal-dose isoproterenol.\(^{10,15}\) Continuous telemetric monitoring in conscious mice has yielded HRs of 550 to 600 bpm, with peak rates observed at night consistent with nocturnal behavior.\(^{11}\) These results raise doubts about the role of the force-frequency reserve mechanism in the intact mouse. On the other hand, the influence of HR on cardiac function is considerable if one starts at greatly reduced rates, such as those lowered by anesthesia or by direct pharmacological sinus node inhibition.\(^{18}\)

A similar word of caution is appropriate when evaluating the role of adrenergic reserve. In hearts with acutely reduced basal HRs and contractility, adrenergic reserve often appears enhanced. Resting vagal tone appears to play a minimal role in modulating HR in mice,\(^{16}\) but sympathetic withdrawal with heart isolation or anesthesia could greatly contribute to resting cardiodepression and bradycardia. The resultant adrenergic reserve would likely be very different if compared in animals with normal basal dP/dt\(\max\) nearer 17 000 mm Hg/s. Cardiac effects from molecular manipulations of adrenergic signaling or excitation-contraction coupling proteins could also be amplified in preparations with reduced sympathetic stimulation. This may underlie discrepancies between the lack of long-term morbidity or mortality found in a number of genetically engineered models influencing excitation-contraction coupling and adrenergic signaling and more pronounced physiological differences in the isolated and anesthetized hearts from these same animals.

### Chamber Loading

Although dP/dt\(\max\) is the most widely used measure of murine cardiac systolic function, this parameter has well-recognized loading sensitivities, primarily from preload change.\(^{27,28}\) At low levels of mean arterial pressure, dP/dt\(\max\) also has afterload dependence.\(^{27}\) Interventions that alter cardiac chamber filling volumes (such as vasodilators and HR changes) may thereby alter dD/dt\(\max\). This has been well characterized in the canine heart, but there has been, as yet, little study of dP/dt\(\max\) load dependence in the murine heart.

Recent data suggest that such dependence is considerable.\(^{23,24}\) For example, in C3H/He and C57BL6 strains, reducing end-diastolic pressure (EDP) by only 1 mm Hg from resting baseline is sufficient to lower dP/dt\(\max\) by 18±5.2%. Thus, unless EDP can be accurately and reliably measured to a precision of <1 mm Hg, it may be difficult to ensure that dP/dt\(\max\) changes were not influenced by preload on the basis of the EDP.

Figure 2 displays an example of the load sensitivity of dP/dt\(\max\) in a mouse under control conditions and during intravenous
infusion of propranolol. There is substantial dP/dt_{\text{max}} load sensitivity, with a 23% decline (~3300 mm Hg/s, dotted line) corresponding to a <1 mm Hg EDP change in the control condition. Furthermore, as shown by the change in the relation slope with propranolol, this load sensitivity varies with inotropic state. Thus, an intervention that enhances contractility also increases the preload sensitivity of dP/dt_{\text{max}}. If this intervention also leads to a decline in EDP of only 1.5 mm Hg (close to the noise level), this contractile change could be greatly underestimated (arrow). Alternatives, such as the slope of the dP/dt_{\text{max}}-EDP relation as shown in Fig 2, may be valuable in this regard.

**Anesthesia and Ventilation**

Undoubtedly, major contributors to basal cardiac depression in the in vivo murine ventricle are both 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-tribromo-ethanol (avertin). Many studies using the former have yielded data with low systolic pressures and bradycardia, so it appears not recommendable for mice. 2,2,2-tribromo-ethanol has been reported to raise postanesthesia mortality to 35% within 3 months and is also a cardiodepressant. There is also considerable interstrain differences in anesthesia sensitivity, and careful adjustments are required for each strain. This can complicate studies in which strains have been combined to a varying and often unknown extent, such as the very commonly used C57BL/6×SV129 strain.

Several forms of anesthesia appear to be better tolerated and yet are infrequently used. One is α-chloralose/urethane, which has minimal effects on cardiovascular reflexes and appears to be well tolerated in mice.\(^6\)\(^2\) Our experience with this agent supports its use even in open-chest preparations, yielding physiological HRs of 550 to 600 bpm, systolic pressures at or above 100 mm Hg, and dP/dt_{\text{max}} values of 12,000 mm Hg/s or more.\(^6\)\(^2\) One disadvantage is that this agent is not generally used for survival procedures. Alternatives such as inhaled methoxyflurane\(^20\) or etomidate given at a dose of 20 to 30 mg/kg IP are both well tolerated, yield physiological HRs and blood pressures, and are fully compatible with recovery. More attention to optimizing the use of anesthetics in murine studies is clearly needed.

Close attention also needs to be paid to methods and patterns of artificial ventilation. Ventilation is not provided at all in some studies but is administered by a volume respirator in others, and there is remarkable variability with respect to the ventilation rate and tidal volumes used, with little to no assessment of its adequacy. Mice spontaneously breath at rates between 160 and 280 bpm, with tidal volumes of 160 µL, yielding a minute ventilation of \(\approx 1.2\) mL \cdot min\(^{-1}\) \cdot g\(^{-1}\).\(^{33}\) These values also vary between strains. Conscious murine ventilation occurs with an elevated functional residual capacity (FRC) that is due to expiratory braking from persistent inspiratory muscle activity and/or increased glottal resistance.\(^32\) In anesthetized animals, loss of this braking mechanism along with reduced tidal volume and frequency can lower the FRC to near relaxation volumes, with a resultant risk of atelectasis. The latter is further assisted by very high chest wall compliance,\(^33\) which, although useful for enabling the animals to squeeze under doorways, likely contributes to airway collapse under anesthesia. Furthermore, the hemoglobin-oxygen dissociation curve in mice is shifted substantially rightward (the partial pressure at 50% hemoglobin saturation \([P_{50}]\) is 27 mm Hg for humans versus 65 mm Hg for mice) compared with other mammalian species.\(^34\) Thus, room air ventilation may not be ideal and could contribute to cardiovascular depression. More attention should be paid to inflation pressures, making sure that overinflation and underinflation are not occurring. Normal airway flow pressures should be between 5 and 10 cm/H\(_2\)O. One should carefully assess pulmonary ventilatory mechanics in each preparation and take efforts to optimize ventilation.

**The Challenge**

Only a decade ago, few if any investigators could have imagined the intense interest that would develop around assessing cardiovascular function of mice. New techniques are evolving, and better methods will undoubtedly appear in the near future. At present, however, one too often comes across hemodynamic data far removed from physiological operating ranges, with control data falling short of this claim. The concern is that such abnormal baselines bias assessments of genetic manipulations to overestimate or underestimate their real influence. It is certainly possible that studying murine hearts under these conditions indeed yields data relevant to intact larger mammalian physiology and pathophysiology, but this remains an assumption that needs testing. It is hoped that greater attention to these issues and to characterizing truly normal murine cardiac function, potentially in intact unanesthetized mice, will clarify these assumptions.

**References**

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