Regulatory effects of phospholamban on cardiac function in intact mice

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Lorenz, John N., and Evangelia G. Kranias. Regulatory effects of phospholamban on cardiac function in intact mice. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2826–H2831, 1997.—Phospholamban (PLB) regulates Ca2+-adenosinetriphosphatase activity in cardiac sarcoplasmic reticulum and participates in the regulation of myocardial performance. Animal models with altered levels of PLB permit in vivo evaluation of the physiological role of PLB. This study examined left ventricular (LV) performance in intact PLB heterozygous and homozygous mice under basal and stimulated conditions. A Millar Mikro-Tip transducer was inserted into the right carotid artery and advanced into the LV for direct measurement of ventricular pressure and the first derivative of intraventricular pressure (dP/dt). Baseline blood pressures were increased in PLB heterozygotes and even more so in PLB homozygotes compared with wild types (WT), and there were no differences in heart rate or LV end-diastolic pressure. The increase in pressure was primarily caused by an increase in systolic pressure. Baseline values for positive and negative dP/dt were linearly correlated with PLB levels. In PLB heterozygotes, contractile response to isoproterenol (Iso) was blunted compared with WT, but maximum rates of contraction were similar between the two groups. Contractile performance in PLB homozygous mice, which under baseline conditions was similar to maximum levels seen in WT, showed a blunted response to Iso, and maximum rates of contraction were significantly greater than in either of the other groups, indicating an essential but perhaps not exclusive role for PLB in mediating the inotropic effects of β-adrenergic agonists. The effects of Iso on negative dP/dt were also blunted in both PLB heterozygous and PLB homozygous animals. Our results demonstrate that myocardial function is highly dependent on PLB level and suggest that the cardiovascular effects of PLB perturbations are largely uncompensated for in the intact mouse.

left ventricular pressure; first derivative of intraventricular pressure; heart; calcium adenosinetriphosphatase; gene targeting; gene knockout; cardiac contractility

THE PHOSPHOPROTEIN phospholamban (PLB) is a key regulator of Ca2+-adenosinetriphosphatase (ATPase) in cardiac sarcoplasmic reticulum (3, 5, 6, 14, 15) and has been shown to contribute to the regulation of myocardial contractility (4, 7, 10, 11, 16). The role of PLB in the control of myocardial performance has been investigated recently through the generation of animal models with targeted ablation of the PLB gene. The resulting heterozygous and homozygous PLB-mutant mice appear outwardly normal and express predicted levels of PLB protein (10, 11). Ex vivo studies utilizing isolated cardiac myocytes (16) and isolated, perfused, working hearts (10, 11) indicated that basal contractile parameters were directly correlated with PLB protein levels. In addition, the contractile response to β-adrenergic stimulation was blunted in PLB heterozygous hearts and myocytes and further attenuated in those from PLB homozygous animals. Importantly, although the β-adrenergic response was completely abolished in work-performing hearts from PLB homozygous mice, isolated myocytes from these animals retained a substantial response to β-adrenergic stimulation (10, 11, 16).

Although these ex vivo studies establish the importance of PLB in mediating basal and stimulated myocardial performance in isolated myocytes and hearts, the relative role of this protein in mediating in vivo contractility is not entirely clear. Neuronal and/or humoral inputs to the heart, not present in isolated preparations, may be able to access other cardiac regulatory pathways and influence cardiac function even in the absence of PLB. It is further possible that the PLB knockout mice might develop various compensatory mechanisms that allow them to regulate cardiac performance in the complete absence of PLB. In fact, previous M-mode echocardiographic studies (4) have indicated that although some indexes of baseline cardiac performance, such as the velocity of circumferential shortening, were moderately enhanced in PLB knockout mice, other indexes, such as fractional shortening (a commonly used ejection-phase index of myocardial performance), were unaltered. Furthermore, unlike the isolated heart preparation, PLB knockout mice retained a substantial inotropic response to β-adrenergic stimulation as determined by M-mode echocardiography. Thus some of the echocardiographic findings in the intact mice resembled those obtained in ex vivo preparations, whereas others were different from the observations in isolated myocytes and hearts. The present study was therefore undertaken to quantitatively examine left ventricular (LV) performance in intact, anesthetized PLB heterozygous and PLB homozygous mice, using a direct and sensitive method for evaluating isovolumic indexes of myocardial performance. Closed-chest mice were instrumented with a high-fidelity Millar micromanometer for the measurement of LV pressure (LVP) and first derivative of intraventricular pressure (dP/dt) under basal and β-stimulated conditions. These studies sought to clarify three important issues. 1) What is the relative level of contractility in the intact animal under basal conditions, maximally stimulated (10, 11) or partially stimulated (4, 16)? 2) What is the dose-response relationship to β-adrenergic stimulation in the intact PLB-deficient animals compared with wild types? 3) Are there mechanisms independent of PLB that can produce an inotropic response in the intact PLB knockout mouse?
Methods

Mouse colony, PLB homozygous, PLB heterozygous, and wild-type mice were obtained from an established colony generated at the University of Cincinnati as previously described (10). Genotype was determined by polymerase chain reaction analysis of tail biopsies and by Southern blot analysis.

Surgery and experimental protocols. Age-matched mice weighing between 25 and 35 g were allowed free access to food and water up to the time of surgery. Assessment of LV function was performed as previously described (9). Briefly, mice were anesthetized with intraperitoneal injections of 50 µg/g body weight (BW) of ketamine and 100 µg/g BW thiobutabarbital (Inactin, Research Biochemicals International, Natick, MA) and placed on a thermally controlled surgical table. After tracheostomy, the right femoral artery and vein were cannulated with polyethylene tubing (OD 0.3–0.5 mm). The arterial catheter was connected to a COBE CDXIII fixed-dome pressure transducer (COBE Cardiovascular, Arvada, CO) for measurement of arterial blood pressure, and the venous catheter was connected to a syringe pump for the infusion of experimental drugs. The right carotid artery was then cannulated with a high-fidelity, 1.8-F Millar Mikro-Tip transducer (model SPR-612, Millar Instruments, Houston, TX). During continual monitoring of the blood pressure wave, the tip of the transducer was carefully advanced through the ascending aorta and into the LV. When a stable waveform, characteristic of the ventricular pressure profile, was achieved, the transducer was anchored in place using 7–0 silk sutures. After surgery was completed, animals were allowed to stabilize for 30–45 min.

LV measurements. Cardiovascular responses to increasing doses of isoproterenol (Iso) administered as a constant infusion (0.01, 0.02, 0.04, 0.08, 0.16, and 0.32 ng·min⁻¹·g BW⁻¹) were determined. Each dose was delivered at a rate of 0.1 µl/g BW for 3 min, and animals were allowed to recover to baseline for 10–15 min between doses. After completion of the dose-response protocol, a bolus dose of propranolol (100 ng/g BW) was administered to evaluate the baseline cardiac function in the absence of endogenous β-adrenergic activity.

Aortic measurements. In a separate protocol, systolic and diastolic pressures in the ascending aorta were determined to evaluate the differences in mean arterial pressure (MAP). Surgery was performed as described, except that the tip of the Millar catheter was positioned just at the entrance of the right carotid artery.

Analytical and statistical procedures. Pressure signals were recorded using a MacLab 4/s data acquisition system at a sampling rate of 1,000 samples·s⁻¹·channel⁻¹. Average values for MAP, heart rate (HR), systolic and diastolic LVP, and LV end-diastolic pressure (LVEDP) were determined for 20- to 30-s periods. Several indexes of ventricular performance were calculated from the ventricular dP/dt tracing: maximum and minimum dP/dt (dP/dtmax, dP/dtdmin), dP/dt at 40 mmHg of developed pressure (dP/dt40, an index that attempts to correct for differences in afterload), and dP/dtmax divided by the developed pressure at dP/dtmax (dP/dtmax/DP, an index that attempts to correct for differences in preload).

Data were analyzed by one-factor (within) or mixed, two-factor analysis of variance using SUPERANOVA software by Abacus. Differences between individual groups were further analyzed using single degree-of-freedom contrasts. Differences were regarded as significant at the P < 0.05 probability level.

Results

Basal cardiac function. To evaluate cardiac function in the absence of endogenous β-adrenergic activity, animals were treated with the β-adrenergic blocker propranolol. These measurements were performed at the end of each experiment to avoid interference with the Iso dose-response relationship. Baseline values for whole animal cardiovascular function are shown in Table 1, and these data demonstrate that, compared with wild-type animals, myocardial performance (dP/dtmax, dP/dtdmin, and dP/dtmax/DP) is increased in the heterozygous and even more so in the homozygous knockout mice. There were no differences in HR and LVEDP among any of the three groups of animals. MAP and LV systolic pressure were elevated in PLB heterozygous animals compared with wild types, and pressures in the PLB homozygous animals were significantly greater than in both wild-type and PLB heterozygous animals. This increased blood pressure in PLB homozygous mice, which has not been reported previously, was further investigated in a separate series of experiments (see Ascending aortic pressure measurements). All three indexes of myocardial contractility, dP/dtmax, dP/dtdmin, and dP/dtmax/DP, were significantly elevated in the PLB heterozygous animals compared with wild types; they were also elevated in the PLB homozygous animals compared with both wild-type and PLB heterozygous animals. The index for myocardial relaxation, dP/dtdmin,

Table 1. Baseline cardiovascular variables during treatment with propranolol

<table>
<thead>
<tr>
<th></th>
<th>Wild Type</th>
<th>P Value</th>
<th>Heterozygote</th>
<th>P Value</th>
<th>Homozygote</th>
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<tr>
<td>n</td>
<td>7</td>
<td>7</td>
<td>8</td>
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<tr>
<td>Body weight, g</td>
<td>31 ± 1</td>
<td>0.332</td>
<td>33 ± 2</td>
<td>0.959</td>
<td>33 ± 2</td>
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<td>HR, beats/min</td>
<td>528 ± 21</td>
<td>0.151</td>
<td>486 ± 19</td>
<td>0.941</td>
<td>488 ± 11</td>
<td>0.231</td>
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<tr>
<td>MAP, mmHg</td>
<td>72 ± 6</td>
<td>0.054</td>
<td>82 ± 2</td>
<td>0.014</td>
<td>95 ± 1</td>
<td>0.0009</td>
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<tr>
<td>Systolic LVP, mmHg</td>
<td>97 ± 5</td>
<td>0.03</td>
<td>110 ± 3</td>
<td>0.0067</td>
<td>127 ± 2</td>
<td>0.0003</td>
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<tr>
<td>LVEDP, mmHg</td>
<td>7.0 ± 1.1</td>
<td>0.430</td>
<td>8.7 ± 1.2</td>
<td>0.585</td>
<td>7.4 ± 2.6</td>
<td>0.863</td>
</tr>
<tr>
<td>dP/dtmax, mmHg</td>
<td>7.181 ± 754</td>
<td>0.0001</td>
<td>13.407 ± 487</td>
<td>0.0001</td>
<td>18.351 ± 640</td>
<td>0.0001</td>
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<tr>
<td>dP/dtdmin, mmHg</td>
<td>-8.426 ± 944</td>
<td>0.0017</td>
<td>-11.576 ± 436</td>
<td>0.0003</td>
<td>-15.541 ± 243</td>
<td>0.0001</td>
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<td>dP/dtmax/DP</td>
<td>6.160 ± 466</td>
<td>0.0001</td>
<td>10.400 ± 292</td>
<td>0.0019</td>
<td>12.625 ± 544</td>
<td>0.0001</td>
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<tr>
<td>dP/dtdmin/DP</td>
<td>135 ± 16</td>
<td>0.0001</td>
<td>221 ± 4</td>
<td>0.054</td>
<td>250 ± 10</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of mice. MAP, mean arterial pressure; LVP, left ventricular pressure; LVEDP, left ventricular end-diastolic pressure; dP/dtmax, dP/dtdmin, average maximum and minimum values of first derivative of intraventricular pressure; dP/dt40, average dP/dt at 40 mmHg of developed pressure; dP/dtmax/DP, dP/dtdmin divided by instantaneous ventricular pressure at dP/dtmax; P values compare adjacent groups except for rightmost column, which compares homozygotes to wild types.
was significantly increased (more negative) in the PLB heterozygous animals compared with wild types (P = 0.0017) and significantly increased in the PLB homozygous animals compared with both wild-type (P = 0.0001) and PLB heterozygous animals (P = 0.0003).

The relationship between myocardial PLB protein levels and dP/dt<sub>max</sub> and dP/dt<sub>min</sub> during basal conditions is illustrated in Fig. 1. It has been previously reported that hearts from the PLB heterozygous mice have 40% of the PLB levels present in wild-type hearts (11). Thus, as shown in Fig. 1, there is a direct relationship between myocardial performance and the relative amount of PLB protein in the PLB homozygous (0%), PLB heterozygous (40%), and wild-type (100%) animals, and these data demonstrate that the quantitative relationship between the level of PLB protein and myocardial contractility persists in the intact animal.

Responses to β-adrenergic stimulation. To compare the responsiveness of the three groups of animals to β-adrenergic stimulation, the dose-response relationships for intravenous infusions of Iso were determined in each group of animals. As shown in Fig. 2, increasing doses of Iso resulted in significant increases in HR throughout the dose-response relationship, and there were no differences among the three groups of animals at any dose of Iso. Similarly, LVEDP decreased significantly in response to Iso in all three groups of animals, and there were no differences among the groups at any dose of Iso. Although MAP and LV systolic pressure were largely stable throughout the dose-response relationship, there were modest but statistically significant responses to Iso in some groups. MAP decreased slightly in both the PLB heterozygous (P < 0.0001) and PLB homozygous (P < 0.0001) animals in response to Iso but remained unchanged in the wild types. In a similar pattern of response, LV systolic pressure remained unchanged in PLB heterozygous and PLB homozygous animals, but increased slightly (P < 0.01) in wild-type animals in response to Iso.

Contractile responses to β-adrenergic stimulation are illustrated in Fig. 3, which shows the Iso dose-response relationships for dP/dt<sub>max</sub>, dP/dt<sub>40</sub>, and dP/dt<sub>max</sub>/DP in the three groups of animals. Similar to the
findings in propranolol-treated animals, the untreated baseline values for $dP/dt_{\text{max}}$ were significantly greater in PLB heterozygotes compared with wild types and greater yet in PLB homozygous animals ($P < 0.0001$ for all comparisons). However, when these data are related with those in Table 1, it is interesting to note that compared with untreated animals, treatment with propranolol caused a significant decrease in myocardial performance in the wild-type animals ($7,181 \pm 6754$ vs. $9,426 \pm 6539$ mmHg/s) but not in the PLB heterozygous and PLB homozygous animals ($13,407 \pm 487$ vs. $13,039 \pm 432$ and $18,154 \pm 640$ vs. $18,372 \pm 313$ mmHg/s, respectively). In response to ISO, $dP/dt_{\text{max}}$ increased in a dose-dependent manner in all three groups of animals, but the magnitude of this response was blunted in the PLB heterozygous animals compared with the wild types, such that the maximum values of $dP/dt_{\text{max}}$ were not different from each other. In the PLB homozygous animals, the magnitude of the ISO response was further blunted compared with PLB heterozygotes, but the maximum value of $dP/dt_{\text{max}}$ was actually greater than in the PLB heterozygous ($P < 0.0023$) or wild-type ($P < 0.046$) animals. This pattern of response was also observed for the other two indexes of myocardial performance, $dP/dt_{40}$ and $dP/dt_{\text{max}}/DP$ (Fig. 3). The only difference was that at the highest dose of ISO, $dP/dt_{\text{max}}/DP$ was greater in the PLB heterozygotes than in the wild types ($P < 0.0007$) and greater still in the PLB homozygotes ($P < 0.0001$ compared with wild types and $P < 0.0014$ compared with PLB heterozygotes).

The ISO dose-response relationship for $dP/dt_{\text{min}}$, an index of myocardial relaxation, is shown in Fig. 4. As expected, $dP/dt_{\text{min}}$ increased in response to increasing doses of ISO in the wild-type animals. Although baseline $dP/dt_{\text{min}}$ was marginally elevated in PLB heterozygous animals compared with wild types ($P < 0.07$), there was no significant stimulation by ISO, and at the highest dose of ISO the values in PLB heterozygous mice were significantly less than in wild types ($P < 0.0001$). In the PLB homozygous animals, the baseline values of $dP/dt_{\text{min}}$ were significantly elevated compared with wild types ($P < 0.0001$), and there was no significant stimulation by ISO. It is important to note that the maximally stimulated values of $dP/dt_{\text{min}}$ in the wild-type animals were not significantly different from the baseline values in the PLB homozygous mice.

Ascending aortic pressure measurements. The differences in blood pressure described in Basal cardiac function contrast with previously reported findings using tail cuff measurements (10). To further evaluate these differences, we examined mean, systolic, and diastolic pressures in wild-type and PLB homozygous mice by placing the Millar transducer in the ascending aorta, at the origin of the carotid artery. We confirmed that MAP was higher in the PLB homozygous mice than in wild types: $114 \pm 68$ vs. $93.4 \pm 43$ mmHg ($P < 0.05$). This difference was largely reflective of an increase in systolic pressure and owed less to an increase in diastolic pressure. Systolic pressure was $107 \pm 4$
DISCUSSION

The findings of the present study are the first to quantitatively demonstrate that, in the intact animal, cardiac performance is critically dependent on the level of PLB gene expression in the murine myocardium. Furthermore, the present results demonstrate that 1) the cardiovascular effects of PLB perturbations are, to a large extent, uncompensated for in the intact mouse and 2) PLB deficiency is associated with increased MAP, largely owing to an increase in systolic pressure. These PLB "gene-dosage" effects on blood pressure and myocardial performance dramatically illustrate the relative role and importance of PLB in regulating cardiovascular function in the fully intact animal. PLB deficiency was also associated with a markedly blunted, but not abolished, β-adrenergic dose-response relationship. However, because PLB homozygous animals did respond to β-adrenergic stimulation, albeit slightly, these data suggest that the role of PLB in mediating the inotropic response to β-stimulation is not exclusive.

The dependence of MAP on PLB gene expression illustrated in this study is intriguing. It was originally reported that MAP, as assessed by tail-cuff sphygmomanometry (10), was not different in PLB homozygous animals compared with wild types. We report here, in two separate series of experiments (aortic and LV), that MAP measured intra-arterially under anesthesia is significantly increased in PLB-deficient animals. The reason for these different findings is not entirely clear but may be that tail-cuff measurements cannot discern subtle differences in pressure or alternatively that actual differences exist between awake and anesthetized animals. Use of the Millar catheter to measure pressure in the ascending aorta permitted precise determination of systolic and diastolic pressure that would not be possible using a fluid-filled catheter. The resulting data demonstrate that the increased MAP in PLB-deficient mice is largely reflective of an increased systolic pressure, suggesting that the observed hypertension is caused more by a difference in cardiac performance than by a difference in vascular reactivity. However, because PLB is expressed in vascular smooth muscle (8), a vascular component to the observed difference in blood pressure cannot be ruled out. It is interesting to note that, although PLB deficiency was consistently associated with increased blood pressure in both aortic and LV experiments, the actual blood pressure values were substantially higher in the aortic experiments than in the LV experiments, a difference that may be related to the ages of the animals. Mice used in the LV experiments were ~12 wk of age, whereas those in the aortic experiments were ~16 wk of age. This possible age dependence of blood pressure will require further investigation.

Previous studies using the isolated heart preparation (10, 11) showed that contractile parameters (dP/dt max and time to peak pressure) in PLB homozygous hearts under baseline conditions were elevated to levels that were not different from the maximal levels observed with β-adrenergic stimulation in wild-type hearts. In addition, these earlier studies showed that β-stimulation of the PLB homozygous hearts with Iso produced no further increments in contractility. In contrast, studies using M-mode echocardiography reported that ejection-phase indexes of cardiac performance (fractional shortening and velocity of circumferential shortening) in PLB homozygous mice under baseline conditions were either not different from or moderately increased compared with wild-type animals (suggesting significant compensation in the intact animal) and that β-adrenergic stimulation resulted in increases in cardiac performance to similar levels in the two groups of animals (4). It is known, however, that ejection-phase indexes of ventricular function can be highly dependent on peripheral vascular function (2), which may be altered in PLB homozygous mice as suggested by the blood pressure data discussed in RESULTS. Thus, based on the data from these previous studies, it is not clear whether the effects of PLB deficiency are compensated for in the intact animal or whether there are PLB-independent mechanisms available to the intact animal that are able to mediate an inotropic response to β-adrenergic stimulation. To address these issues, the present study sought to examine isovolumic indexes of cardiac performance. Values for dP/dt max, dP/dt50, and dP/dt max/DP were greatly enhanced in PLB homozygous animals under baseline conditions, and the inotropic response to β-stimulation was severely but not completely blunted. It appears, therefore, that in the intact animal, changes in myocardial contractility induced by PLB perturbations are largely uncompensated. That is, presumed neurogenic or hormonal systemic mechanisms attempting to slow the heart are largely unable to decrease cardiac contractility in the face of PLB deficiency. Furthermore, although PLB deficiency dramatically blunted the response to β-adrenergic stimulation, there seemed to be residual mechanisms available to the intact animal to further increase contractility. It may be reasonable to speculate that because the chronotropic effects of β-adrenergic stimulation are fully intact in the PLB-deficient animal, the increase in cardiac performance may be related to the tetrode phenomenon (1). In this regard, in separate experiments using an atrial pacing electrode to directly increase HR, we have observed that HR increases within the range observed here have only mild effects on dP/dt (i.e., <1,000 mmHg/s; data not shown). Other possible regulatory mechanisms include phosphorylation of other proteins, such as sarcolemmal reticulum Ca2+/ATPase (15), troponin I (7), ryanodine receptor (18), and phospholemman (13).

Several indexes of myocardial performance, which attempt to correct for differences in loading conditions, were determined in the present study in addition to dP/dt max. We calculated dP/dt50, an index that attempts to correct for differences in preload (12), and dP/dt max/DP, which attempts to correct for afterload differences. These PLB "gene-dosage" effects on blood pressure and cardiovascular function in the fully intact animal. PLB deficiency was also associated with a markedly blunted, but not abolished, β-adrenergic dose-response relationship. However, because PLB homozygous animals did respond to β-adrenergic stimulation, albeit slightly, these data suggest that the role of PLB in mediating the inotropic response to β-stimulation is not exclusive. The dependence of MAP on PLB gene expression illustrated in this study is intriguing. It was originally reported that MAP, as assessed by tail-cuff sphygmomanometry (10), was not different in PLB homozygous animals compared with wild types. We report here, in two separate series of experiments (aortic and LV), that MAP measured intra-arterially under anesthesia is significantly increased in PLB-deficient animals. The reason for these different findings is not entirely clear but may be that tail-cuff measurements cannot discern subtle differences in pressure or alternatively that actual differences exist between awake and anesthetized animals. Use of the Millar catheter to measure pressure in the ascending aorta permitted precise determination of systolic and diastolic pressure that would not be possible using a fluid-filled catheter. The resulting data demonstrate that the increased MAP in PLB-deficient mice is largely reflective of an increased systolic pressure, suggesting that the observed hypertension is caused more by a difference in cardiac performance than by a difference in vascular reactivity. However, because PLB is expressed in vascular smooth muscle (8), a vascular component to the observed difference in blood pressure cannot be ruled out. It is interesting to note that, although PLB deficiency was consistently associated with increased blood pressure in both aortic and LV experiments, the actual blood pressure values were substantially higher in the aortic experiments than in the LV experiments, a difference that may be related to the ages of the animals. Mice used in the LV experiments were ~12 wk of age, whereas those in the aortic experiments were ~16 wk of age. This possible age dependence of blood pressure will require further investigation.

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contractile performance in the PLB homozygotes was uncompensated in vivo. The dramatically elevated basal vascular effects of PLB perturbations appear to be largely manifest in the intact mouse, the cardiovascular function in the intact animal is highly dependent on the relative level of PLB. Under basal conditions, cardiac relaxation in these experiments and, consistent with previous reports (10, 11), found that dP/dt max was elevated by ~40% in the PLB heterozygotes and nearly doubled in the PLB homozygous animals compared with wild types under basal conditions.

In summary, the results of the present study demonstrate that both basal and stimulated myocardial function in the intact animal is highly dependent on the relative level of PLB. Under basal conditions, cardiac contractile parameters were significantly elevated in the PLB heterozygotes and even more so in the PLB homozygous animals compared with wild types. Furthermore, because these differences in myocardial performance persisted in the fully intact mouse, the cardiovascular effects of PLB perturbations appear to be largely uncompensated in vivo. The dramatically elevated basal contractile performance in the PLB homozygotes was associated with a blunted response to β-adrenergic stimulation. However, because the response to β-adrenergic agonists was not completely abolished, the data suggest that the role for PLB in mediating the inotropic effects of β-agonists is not exclusive.

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