Superinhibition of Sarcoplasmic Reticulum Function by Phospholamban Induces Cardiac Contractile Failure*

Received for publication, March 16, 2001, and in revised form, April 26, 2001
Published, JBC Papers in Press, April 27, 2001, DOI 10.1074/jbc.M102403200

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To determine whether selective impairment of cardiac sarcoplasmic reticulum (SR) Ca\(^{2+}\) transport may drive the progressive functional deterioration leading to heart failure, transgenic mice, overexpressing a phospholamban Val49 \(\rightarrow\) Gly mutant (2-fold), which is a superinhibitor of SR Ca\(^{2+}\)-ATPase affinity for Ca\(^{2+}\), were generated, and their cardiac phenotype was examined longitudinally. At 3 months of age, the increased EC\(_{50}\) generated, and their cardiac phenotype was examined

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Cardiac hypertrophy and failure are highly complex disorders that arise as a result of a combination of mechanical, hemodynamic, hormonal, and pathological stimuli (1). In response to these effectors, the heart undergoes an adaptive response of compensatory hypertrophy (2) followed by decompensated heart failure that is characterized by defects in Ca\(^{2+}\) handling during excitation-contraction coupling. Studies of end-stage-failing hearts have shown that the disturbed calcium homeostasis is associated with alterations in the expression

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* This work was supported by National Institutes of Health Grants HL-26057, HL-64018, RR12358, and HL52318 (to E.G.K.) and by a Grant (to D.H.M.) from the Heart and Stroke Foundation of Ontario. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 The abbreviations used are: SR, sarcoplasmic reticulum; PLB, phospholamban; PLB-MT, mutant phospholamban; V49A, a mutant form of phospholamban in which Val49 is mutated to Ala; SERCA2a, cardiac sarcoplasmic reticulum, Ca\(^{2+}\)-ATPase; HEK, human embryonic kidney; \(\alpha\)-MHC and \(\beta\)-MHC, \(\alpha\)- and \(\beta\)-myosin heavy chain; ANF, atrial natriuretic peptide; kb, kilobase pair(s); I\(_{Ca}\), L-type Ca\(^{2+}\) current; LV, left ventricular; pF, picofarads.
was expressed in vivo, using recombinant adenoviruses. The V49A mutant acted dominantly to increase contractility in cardiac ventricular cells in the absence of catecholamines (23). This study pointed out the importance of the PLB carboxyl terminus in mediating its interaction with SERCA2a and proposed that interfering with this interaction may provide a novel therapeutic approach for the prevention of dilated cardiomyopathy. To further elucidate the functional significance of the PLB Val49 residue in vivo, we generated a mutant, Val49 → Gly (V49G), which acts as a potent inhibitor of the Ca2+ affinity of SERCA2a. Cardiac-specific overexpression of the V49G mutant PLB resulted in superinhibition of cardiac contractility and cardiac remodeling, which progressed to dilated cardiomyopathy and early mortality. Thus, enhanced inhibition of SERCA2a by PLB may serve as a prime candidate for driving the onset and progression of heart failure.

**EXPERIMENTAL PROCEDURES**

*In Vitro Co-expression of SERCA2a and Mutant Phospholamban*—Rabbit PLB wild type or V49G mutant and SERCA2a cDNAs were co-transfected into the human embryonic kidney cell line (HEK-293 cells), and microsomal Ca2+ transport activity was assayed according to a previous study (24).

*Creation of Mutant Phospholamban Mice*—The site-specific mutation of V49G (GTG to GGG) was introduced into PLB cDNA by polymerase chain reaction (24). The entire expression construct was composed of the cardiac-specific α-myosin heavy-chain promoter (α-MHC, 5.5 kb), a gift from Dr. J. Robbins, Children’s Hospital, Cincinnati, OH), the PLB coding region with the V49G mutation (0.65 kb), and the SV40 polyadenylation signal. Microinjection and identification of transgenic mice were performed (24).

*Biochemical Assays*—Quantitative immunoblotting of cardiac homogenates was used to determine the levels of PLB and SR calcium-handling proteins (25). Cardiac gene expression was assayed by dot blot analysis of total RNA (10 μg) using 32P-labeled oligonucleotide probes (25). Oxalate-supported Ca2+ uptake in cardiac homogenates was measured by a modified Millipore filtration technique (15).

*Functional Measurements*—Mouse left ventricular (LV) cardiomyocytes were isolated, and cardiomyocyte mechanical properties and Ca2+ transients were examined (24). Whole-cell L-type Ca2+ channel currents were recorded, using patch-clamp techniques (26). Briefly, Ca2+ channel currents were elicited by 300-ms depolarizing pulses from a holding potential of −50 mV, applied every 10 s. Cell capacitance was measured using voltage ramps of 0.8 V/6 from a holding potential of −50 mV for 0.5 s. Left ventricular M-mode and Doppler measurements (27) were performed at baseline and after the administration of isoproterenol (2 μM intraperitoneally) in mice, which were anesthetized with 2.5% isoflurane (0.01 ml/g).

*Histology*—Hearts from mutant PLB and control mice were excised, photographed, and subjected to histological analysis. Briefly, hearts were collected, fixed overnight in 10% formalin, buffered with phosphate-buffered saline for up to 24 h, dehydrated in 70% ethanol, and transferred to xylene and then to paraffin. Paraffin-embedded hearts were sectioned at 4 μm and stained with Masson’s trichrome.

*Statistical Analysis*—Data are presented as mean ± S.E. The number n indicates the number of mice unless otherwise stated. Statistical analysis was performed by one-way or two-way analysis of variance, followed by Student-Newman-Keuls’ test for multiple comparisons. Values of p < 0.05 were considered to be statistically significant.

**RESULTS**

*In Vitro Co-expression of Mutant Phospholamban and SERCA2a*—We identified a mutation (V49G) in the transmembrane domain of PLB that did not alter the monomer-to-pentamer ratio, but resulted in superinhibition of SERCA2a’s Ca2+ affinity in vitro. Co-expression of wild type PLB with SERCA2a in HEK-293 cells was associated with a significant shift in the Ca2+ dependence (EC50) of Ca2+ uptake activity from 0.25 ± 0.05 μM to 0.55 ± 0.05 μM (n = 9, p < 0.05). However, when the PLB V49G mutant was co-expressed with SERCA2a, there was a further significant (p < 0.05) shift in the EC50 value (1.29 ± 0.14 μM, n = 9), indicating a greater inhibitory effect compared with wild type PLB.

**Characterization of Transgenic Mice Expressing Mutant Phospholamban**—To assess the physiological significance of the superinhibitor mutant PLB in vivo, transgenic mice with cardiac-specific expression of mutant PLB (PLB-MT) were generated. Four founder (lines 1, 2, 3, and 4) mice, harboring the mutant PLB transgene were identified. The expression of a transgenic transcript migrating at −1.0 kb was only detected in the hearts of transgenics, using Northern blot analysis of total RNA (data not shown) (14).

Western blot analysis of cardiac homogenates from 3-month-old male or female transgenic and wild type mice revealed that the PLB protein levels were increased by 2-fold in lines 1, 3, and 4 and by 1.8-fold in line 2, compared with wild types. Assessment of the SERCA protein levels in transgenic mouse hearts showed no significant changes (Fig. 1, A and B). Lines 3 and 4 were then propagated for further characterization along with transgenic mice overexpressing similar (2-fold) levels of wild type PLB (14). To avoid gender differences, initial characterization studies were carried out using male mice at 3 months of age.

**Sarcoplasmic Reticulum Ca2+ Uptake Assays**—The initial rates of SR Ca2+ uptake, assessed over a wide range of [Ca2+]i (Fig. 1C), indicated that overexpression of mutant PLB (line 3) resulted in a significant increase in the EC50 value for Ca2+ (0.67 ± 0.01 μM), compared with wild type PLB overexpression (0.48 ± 0.04 μM) and control hearts (0.30 ± 0.02 μM). Similar results were obtained with line 4. These data suggest that the mutant PLB interacts with, and inhibits, SERCA2a to a greater extent than wild type PLB.

**Mechanical Properties and Ca2+ Transients in Isolated Cardiomyocytes**—To elucidate whether the decreased affinity of the SERCA2a for Ca2+ following mutant PLB overexpression was associated with alterations in cardiac function, the contractile parameters of LV cardiomyocytes were assessed. Fig. 2 indicates that the extent of cardiomyocyte shortening and the maximal rates of shortening (+dL/dt: 57%) and re-lengthening (−dL/dt: 31%) were depressed in transgenic (line 3) cardiomyocytes overexpressing mutant PLB, compared with wild type PLB overexpression (+dL/dt: 68% −dL/dt: 64%) cardiomyocytes (wild types: 100%). Furthermore, resting cell length was significantly increased (132%) in mutant, compared with wild type PLB overexpression or wild type cardiomyocytes (100%). The changes observed in contractile parameters suggested that alterations would be found in Ca2+ kinetics in transgenic myocytes. Thus, cardiomyocytes were loaded with 4 μM Fura-2-AM to permit detection of changes in free Ca2+ during stimulation. Peak amplitude was not significantly different in mutant relative to wild type PLB overexpression and wild type control myocytes (Fig. 2). The time to 80% decay of the Ca2+ signal (T50) was more prolonged (165%) in cardiomyocytes overexpressing the mutant PLB than in cardiomyocytes overexpressing wild type PLB (125%), compared with controls (100%).

To clarify whether the superinhibitory effects could be relieved by β-agonists, cardiomyocytes were subjected to maximal stimulation (100 nM) by isoproterenol. Administration of isoproterenol resulted in complete reversal of the inhibitory effects in cardiomyocytes overexpressing wild type PLB. However, in transgenic cardiomyocytes overexpressing mutant PLB, the maximally stimulated T50 of the Ca2+ transient parameter remained depressed, compared with maximally stimulated values in controls.

**Characterization of L-type Ca2+ Channel Current (Ica)**—Mutant PLB myocytes exhibited significantly larger membrane capacitance (198.2 ± 5.7 picofarads (pF), n = 64) compared...
with wild type controls (114.7 ± 3.6 pF, n = 64), suggesting increased myocytes size. Examination of the characteristics of ICa indicated that ICa activated rapidly around 230 mV, peaked around 110 mV, and reversed near 160 mV (Fig. 3, A and B). In wild type myocytes, ICa inactivated rapidly during maintained depolarization. In contrast, the current decay was significantly slower in mutant PLB myocytes (T1/2: 28.7 ± 1.4 ms, n = 34) compared with wild type controls (T1/2: 17.1 ± 1.0 ms, n = 64).

When peak ICa amplitude, normalized relative to cell capacitance (pA/pF) as a function of voltage (ICa - V relationship), was determined, the current density was significantly increased in mutant PLB myocytes (13.7 ± 0.6 pA/pF, n = 39) compared with wild type myocytes (8.7 ± 0.3 pA/pF, n = 64). Myocytes overexpressing wild type PLB exhibited no significant differences in L-type Ca2+ channel current compared with wild types (data not shown).

In Vivo Assessment of Cardiac Function—To determine whether the depressed function, observed in cardiomyocytes from mutant PLB mice, correlated with similar depression in LV systolic function in vivo, M-mode and Doppler echocardiography was performed in the three groups under basal (Fig. 4) and isoproterenol stimulation conditions. End diastolic and end systolic dimensions were significantly higher, whereas heart...
control mice (data not shown). The relative wall thickness (h/r) was also higher in mutant hearts (Table I), indicating the presence of concentric hypertrophy.

Isoproterenol administration enhanced ejection time, heart rate, fractional shortening, and velocity of circumferential fiber shortening, and the percent increase, relative to baseline values, was similar between all groups. However, with the exception of ejection time, the maximally stimulated parameters in mutant PLB mice were attenuated, which is in agreement with the findings in cardiomyocytes. Echocardiography was also performed in line 4 under basal and isoproterenol stimulation conditions. The results indicated similar alterations (data not shown) to those observed with line 3.

Cardiac Hypertrophy—Because mutant PLB cardiomyocytes exhibited increased cell capacitance and M-mode echocardiography indicated the presence of concentric hypertrophy in mutant hearts (Table I), gravimetric analysis of heart and body weights was pursued in 3-month-old males. Overexpression of the mutant PLB was associated with increases in heart-to-body weight ratios, which were 6.30 ± 0.13 (n = 12), compared with 3.88 ± 0.09 (n = 12) for wild type PLB overexpression, and 3.95 ± 0.18 (n = 12) for wild type control males. The lung-to-body weight and liver-to-body weight ratios were also significantly higher in mutant (5.44 ± 0.18 and 51.70 ± 0.09 mg/g, respectively) compared with wild type PLB overexpression (4.38 ± 0.20 and 37.98 ± 0.10 mg/g, respectively) and wild type controls (4.60 ± 0.18 and 39.5 ± 0.11 mg/g, respectively). Line 4 also showed increased heart-to-body weight ratios (6.89 ± 0.07 mg/g, n = 12).

The increases in heart-to-body weight ratios were associated with increased expression of a fetal cardiac gene program, particularly the β-myosin heavy chain isoform (8.25-fold), α-skeletal actin (8.5-fold), and ventricular expression of atrial natriuretic factor (ANF) (11-fold). The relative increases in β-myosin heavy chain and α-skeletal actin protein levels were also assessed, and they were 7.35- and 8-fold over wild types, respectively, when normalized to calnslequinrin protein levels (data not shown).

To further assess the hypertrophic alterations in the mutant PLB transgenic male hearts, histologic analysis was performed at 3 months of age. Every mutant PLB mouse analyzed showed a dramatic increase in heart size relative to wild type controls (Fig. 5A). Furthermore, histologic analysis revealed myocardial fibrosis in hearts of mutant PLB transgenics (Fig. 5B).

Effect of Aging on Cardiac Function—Male mutant PLB mice died by 6–7 months of age from congestive heart failure, which is supported by the increases in lung-to-body weight ratios (6.71 ± 0.4 mg/g at 6 months; n = 8). To evaluate LV systolic function at that time, M-mode and Doppler echocardiography were performed. The fractional shortening and velocity of circumferential fiber shortening were significantly depressed in mutant PLB (22.61 ± 0.80% and 4.02 ± 0.31 cm/s, respectively; n = 8) at 6 months of age compared with 3-month-old males (Table I). Furthermore, the end diastolic dimension, end systolic dimension, LV mass, and ejection time were significantly higher at 6 months (4.92 ± 0.33 mm, 3.82 ± 0.30 mm, 112.3 ± 14.1 mg, and 89.43 ± 4.05 ms, respectively; n = 8) compared with 3-month values. The wall thickness-to-cavity radius ratio (0.29 ± 0.03; n = 8) was also further decreased. In addition, gravimetric analysis revealed further increases in heart and liver-to-body weight ratios (7.38 ± 0.45 and 62.01 ± 0.23, respectively, n = 8). However, no significant differences were observed in wild type PLB overexpression or wild type control mice upon aging (data not shown). Thus, the mutant PLB mice exhibited LV concentric hypertrophy and contractile dysfunction at 3 months, which was followed by progressive LV
dilation, deterioration of LV systolic function, and development of overt congestive heart failure at 6 months.

Gender-dependent Survival Rate and Cardiac Function—A Kaplan-Meier analysis demonstrated that male mutant PLB mice died between 6 and 7 months of age, whereas female mutant PLB mice died between 15 and 18 months of age (Fig. 6A). There were no deaths in wild type male or female controls during this period. These findings suggested a gender-dependent progression in cardiac dysfunction. Thus, LV function and its time course of deterioration were further examined in female mutant PLB mice. M-mode and Doppler echocardiography indicated that end diastolic dimension, end systolic dimension, fractional shortening, velocity of circumferential fiber shortening, heart rate, and ejection time were not significantly different between female mutant and wild type PLB overexpression mice at 3 months (Table II). However, the LV mass and wall thickness-to-cavity radius ratio were higher in mutant than wild type PLB overexpression females, indicating a concentric hypertrophic response. Gravimetric analysis also revealed increases in heart-to-body weight ratios (6.18 ± 0.55, n = 6), compared with wild type PLB overexpression (3.90 ± 0.07, n = 6) and wild type control (3.79 ± 4.04, n = 5) males and females. Echocardiographic assessment of LV-to-body mass ratio (B), end diastolic dimension (EDD, C), fractional shortening (FS, D), and velocity of circumferential fiber shortening (Vcf, E) at 3 and 6 months of age in male (n = 14 at 3 and 8 at 6 months of age) and female (n = 3 at 3, 12 at 6, and 4 at 12 months of age) mutant PLB (PLM-MT) mice. The same group of mice was used for sequential echocardiographic measurements. *p < 0.05 versus wild type; **p < 0.05 versus PLB-MT.
Phospholamban and Contractile Failure

**TABLE II**

Echocardiographic measurements of LV function in female PLB-MT and PLB-WT control at 3, 6, and 12 months of age

|                | PLB-WT | PLB-MT |
|----------------|--------|--------|
| Age (months)   | 3      | 6      |
|                | 12     | 3      | 6      | 12     |
| HR (bpm)       | 310 ± 21 | 310 ± 21 |
|                | 356 ± 56.6 | 285 ± 28.6 |
|                | 279 ± 34.5 | 350 ± 14.0 |
| EDD (mm)       | 3.33 ± 0.08 | 3.46 ± 0.09 |
|                | 3.58 ± 0.04 | 3.59 ± 0.10 |
|                | 4.04 ± 0.13 | 4.05 ± 0.10 |
| ESD (mm)       | 2.08 ± 0.07 | 2.31 ± 0.14 |
|                | 2.26 ± 0.04 | 2.29 ± 0.08 |
|                | 2.51 ± 0.09 | 2.74 ± 0.10 |
| LV mass (mg)   | 48 ± 2.8 | 54 ± 5.0 |
|                | 56 ± 4.0 | 72 ± 6.0b |
|                | 77 ± 7.0b | 83 ± 6.0a |
| LV/body mass (mg/g) | 2.23 ± 0.17 | 2.01 ± 0.11 |
|                | 2.46 ± 0.14 | 3.12 ± 0.24a |
|                | 3.28 ± 0.24b | 3.38 ± 0.34b |
| h/r            | 0.37 ± 0.01 | 0.36 ± 0.01 |
|                | 0.35 ± 0.01 | 0.41 ± 0.01b |
|                | 0.35 ± 0.01c | 0.35 ± 0.02c |
| FS (%)         | 38 ± 1.14 | 39 ± 2.58 |
|                | 37 ± 1.0 | 36 ± 1.20 |
|                | 38 ± 0.61 | 32.62 ± 0.76b,c |
| ET (ms)        | 81 ± 1.14 | 76 ± 5.0 |
|                | 75 ± 10.0 | 79 ± 4.9 |
|                | 84 ± 6.0 | 79 ± 3.0 |
| Vcfc (circ/s)  | 6.54 ± 0.25 | 7.08 ± 0.68 |
|                | 6.61 ± 0.03 | 6.79 ± 0.21 |
|                | 6.82 ± 0.18 | 6.15 ± 0.12 |

*a* HR, heart rate; EDD, end diastolic dimension; ESD, end systolic dimension; h/r, wall thickness-to-cavity radius; FS, fractional shortening; ET, ejection time; Vcfc, velocity of circumferential fiber shortening corrected for HR.

*b* p < 0.05 versus PLB-WT at the same age.

*c* p < 0.05 versus PLB-MT at 3 months.

also significantly higher in mutant (7.12 ± 0.67 and 58.00 ± 0.67 mg/g, respectively) compared with wild type PLB overexpression (5.11 ± 0.67 and 49.00 ± 0.14 mg/g, respectively) and wild type controls (5.43 ± 0.14 and 50.00 ± 0.05 mg/g, respectively). Isoproterenol enhanced fractional shortening and velocity of circumferential fiber shortening, in mutant and wild type PLB overexpression mice, and the percentage increases, relative to baseline values, were similar between both groups. Further analysis of fractional shortening, velocity of circumferential fiber shortening, and ejection time at 6 months did not show any significant alterations in mutant females compared with wild type PLB overexpression females, indicating that the LV systolic function was preserved. However, end diastolic dimension was significantly increased in mutant females at 6 months compared with wild type PLB overexpression mice, indicating the onset of LV dilation in these hearts. Upon aging to 12 months, the mutant females exhibited no further alterations in end diastolic dimension and LV-to-body mass ratio. Interestingly, fractional shortening was decreased compared with 3-month-old mutant and age-matched wild type PLB overexpression female controls. Between 12 and 15 months of age, the LV function deteriorated rapidly and progressed to cardiac failure. These findings suggested that mutation of V49G in PLB resulted in preserved contractile function and concentric LV hypertrophy in females at 3 months of age, which consequently progressed to dilation and contractile dysfunction during the aging process. Gravimetric analysis also revealed increased heart-to-body weight ratios at 6 and 12 months of age (7.28 ± 0.45 and 7.89 ± 0.52, respectively), but these increases were not significantly different between 6 and 12 months.

To assess gender differences in LV remodeling, hypertrophy, and function in response to the mutant PLB superinhibitory effect, the LV and body mass, end diastolic dimension, and LV systolic function in male and female mutant PLB mice were compared at 3 and 6 months of age. Female mutant mice had significantly lower body weights (23 ± 0.74 and 27 ± 0.44 g, at 3 and 6 months, respectively) compared with male mutant (30 ± 1.0 and 34 ± 0.7 g, at 3 and 6 months, respectively) mice. However, body weights were similar between female mutant mice and their age-matched wild type controls, and this was also observed for male mice. At 3 months, both males and females developed the same degree of hypertrophy (Fig. 6B), measured by percent increases in their LV-to-body mass over their male or female wild type controls, which was also supported by similar gravimetric measurements described above. The fractional shortening and velocity of circumferential fiber shortening declined in males but not in females, which exhibited preserved LV systolic function at 3 and 6 months of age (Fig. 6, D and E), indicating differences in their adaptive hypertrophic responses (concentric versus eccentric). Upon aging to 6 months, the end diastolic dimension was increased in mutant PLB male mice, indicating decompensation with cavity dilation and transition to heart failure (Fig. 6C). Mutant PLB females also exhibited significant increases in end diastolic dimension, compared with their 3-month-old counterpart, but their LV function was preserved at 6 months.

**DISCUSSION**

This is the first study to demonstrate that a mutation in PLB, which is associated with superinhibition of SERCA2a and contractile parameters, may lead to dilated cardiomyopathy, overt heart failure, and early mortality, which are modified by gender. Previous studies in animal models or human with end stage heart failure showed that the increases in diastolic calcium and impaired relaxation were linked to inhibition of SR Ca<sup>2+</sup> transport (3, 29). However, the depressed SR function was suggested to be secondary to insults arising from extracellular factors and may act as a modifier for the progression of cardiac deterioration (30). Thus, it is not currently clear whether inhibition of SR Ca<sup>2+</sup> transport is sufficient to cause heart failure or it is a contributing factor to cardiomyocyte dysfunction in the context of pre-existing heart disease. To better address the role of depressed SR Ca<sup>2+</sup> transport activity in the onset and progression of heart failure, we generated a transgenic model harboring the V49G mutation in PLB, which we identified as a superinhibitor of SERCA2a Ca<sup>2+</sup> affinity in vitro, and studied the cardiac phenotype over the life span of the mouse. Overexpression of this mutant PLB was associated with significant depression in SR Ca<sup>2+</sup> uptake rates. The inhibited SR function resulted in impaired cardiac contractile parameters in isolated cardiomyocytes and intact mice at 3 months of age. In cardiomyocytes, the attenuated mechanical parameters reflected a significant prolongation in the rate of the Ca<sup>2+</sup> signal decay (T<sub>1/2</sub>Ca<sup>2+</sup>) without alteration in the peak amplitude of the Ca<sup>2+</sup> transient. The lack of peak amplitude alteration may reflect increased Ca<sup>2+</sup> influx through the L-type Ca<sup>2+</sup> channels, whose density was increased in transgenic myocytes, indicating an important compensatory response in an attempt to normalize systolic Ca<sup>2+</sup> levels in these cells. The V49G mutant myocytes also exhibited significantly slower I<sub>Ca</sub> inactivation, despite increased Ca<sup>2+</sup> influx through the L-type Ca<sup>2+</sup> channels, suggesting that the SR Ca<sup>2+</sup> release was diminished (26, 31). Importantly, similar phenotypic alterations were exhibited in a second line (line 4), which expressed the same levels of mutant PLB, indicating that the inhibitory effect
was due to transgene expression and not any possible inser-
tional mutation.

The mechanisms underlying the superinhibitory effects of V49G mutant PLB in vivo are not clear. However, this study together with a previous one, utilizing a V49A mutant (23), emphasize that the Val^{49} residue in PLB, which is highly conserved among species, is important for the PLB/SERCA2a interaction and changes in this region have profound effects on SR function and cardiac contractility. Thus, the V49G mutation, employed in this study, may influence the three-dimen-
sional structure of PLB and enhance its interaction with SERCA2a. Importantly, the normal ratio (10:1) of pentamers to monomers was not altered by this mutation in PLB, further supporting that the superinhibitory effect of the mutant may be due to its conformational change. An enhanced association between PLB and SERCA2a would be consistent with the in-
ability of isoproterenol to fully relieve the V49G mutant PLB superinhibitory effects, similar to previous findings with the N27A PLB mutant (24). In contrast, the reduced contractility in L37A and I40A PLB mutant mice could be completely over

It is of interest that cardiac-specific overexpression of the PLB V49G mutant induced cardiac hypertrophy in an attempt to normalize cardiac function (32, 33), which was severely depressed in mutant hearts. However, the molecular mecha-
nisms that link depressed SR Ca^{2+} cycling to reprogramming of gene expression in the PLB V49G hearts are not clear. Hyper-
trophy was associated with induction or re-expression of a fetal gene program, including ANF, b-myosin heavy chain, and a-skeletal actin in these hearts (34). The increases in the pro-
tein expression levels of the slow myosin heavy chain (b-MHC) isoform (35) may also contribute to the observed attenuation of contraction and relaxation rates in the mutant PLB hearts. Interestingly, the hypertrophic remodeling, which provided an initial compensatory phase, progressed to cardiac failure and premature death in this model expressing the V49G PLB superinhibitor. Previous studies on the N27A (24) and L37A and I40A (22) PLB superinhibitors also showed severely depressed SR and cardiac function in vivo, which were associated with hypertrophy by 3 months of age. However, the hypertrophic phenotype of the N27A, L37A, and I40A mutant mice did not progress to overt heart failure, as observed with the V49G mutant. The apparent differences between these transgenic models may be due to the expression levels of the various PLB mutants in vivo, the age of the mice studied, and/or the time course of transition between the compensatory hypertrophic response and heart failure. In addition, there may be differ-
ences in activated intrinsic hypertrophic signaling pathways, triggered by SR dysfunction, which lead to different courses of myocyte maladaptation among these transgenic mice. How-
ever, the present findings and those in other models (22–24, 37) suggest that multiple pathways, including disturbed SR Ca^{2+} homeostasis, may operate in concert to induce a hypertrophic response leading to heart failure.

Several studies have indicated that cardiovascular mortality is higher in males than in females, which frequently exhibit preserved cardiac performance, and suggested a gender-depen-
tent influence on the onset and progression of heart failure (36). In the present study, we also observed an early mortality in males (6 months) compared with females (15 months) in our model with genetically induced SR Ca^{2+}-handling defects. The striking survival benefit in females was not due to differences in their levels of PLB, compared with age-matched males. Therefore, we investigated gender-specific differences in cardiac function and in the progression to heart failure. Males and females were examined during compensatory hypertrophy and after the appearance of symptoms of heart failure, using echocardiography. LV-to-body mass was about 50% increased by 3 months of age, and cardiac function was significantly depressed in male PLB mutant mice. The remodeling process accelerated the progression to heart failure, characterized by significant chamber dilatation and a decrease in the ratio of wall thickness to chamber diameter (h/t) by 6 months of age. However, the progression to failure was delayed in females (37), indicating that, despite a similar degree of LV hypertrophy between males and females, there were significant gender differences in the LV-adaptive response to pathological hypertrophy and de-
pressed function, similar to recent studies in the TNF-a over-
expression model and in a rat model of pressure overload hypo-
 hypertrophy (38). The etiology of these differences is unclear but may be related to: (a) a reduced adaptive hypertrophic reserve in males (39); (b) the lower mitochondria respiratory and lysosomal enzyme activity in females (40); (c) a higher percentage of the V1 myosin isoform, which is up-regulated by estrogen in females (41); and (d) the intrinsic gender-specific differences in cardiac muscle physiology and biochemistry (42). Furthermore, estrogen signaling through the adult myocyte estrogen receptor may contribute to gender differences in gene expression in pathological hypertrophy (43). Interestingly, in humans with congenital aortic stenosis and cardiac hypertrophy, there ap-
ppears to be an overcompensation of myocardial contraction early on (44), which is similar to that described in mutant PLB females in this study.

In summary, our findings point to a primary defect in SR function as an inducer of a phenotype that resembles human dilated cardiomyopathy and ventricular remodeling. The en-
hanced inhibition of SERCA2a by the V49G mutant PLB, asso-
ciated with depressed myocyte calcium homeostasis, resulted in a remodeling process, which involved interaction between all components of the myocardium leading to overt heart failure. Furthermore, gender significantly influenced the evolution of the early responses to LV remodeling, including the transition to heart failure in the mutant PLB model. Future studies on the cellular and molecular mechanisms underlying these responses, using gene expression profiling and proteomics, may unveil specific molecules or pathways by which depressed SR function influences the onset of hypertrophy and the transition to dilated cardiomyopathy in this model.

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