Basic Res Cardiol 92: Suppl. 1, 17 – 24

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The relative phospholamban and SERCA2 ratio: a critical determinant of myocardial contractility

Abstract Phospholamban is a regulatory phosphoprotein which modulates the active transport of Ca^{2+} by the cardiac sarcoplasmic reticular Ca^{2+}-ATPase enzyme (SERCA2) into the lumen of the sarcoplasmic reticulum. Phospholamban, which is a reversible inhibitor of SERCA2, represses the enzyme's activity, and this inhibition is relieved upon phosphorylation of phospholamban in response to β-adrenergic stimulation. In this way, phospholamban is an important regulator of SERCA2-mediated myocardial relaxation during diastole. This report centers on the hypothesis that the relative levels of phospholamban: SERCA2 in cardiac muscle plays an important role in the muscle's overall contractility status. This hypothesis was tested by comparing the contractile parameters of: a) murine atrial and ventricular muscles, which differentially express phospholamban, and b) murine wild-type and phospholamban knock-out hearts. These comparisons revealed that atrial muscles, which have a 4.2-fold lower phospholamban: SERCA2 ratio than ventricular muscles, exhibited rates of force development and relaxation of tension, which were three-fold faster that these parameters for ventricular muscles. Similar comparisons were made via analyses of left-ventricular pressure development recorded for isolated, work-performing hearts from wild-type and phospholamban knock-out mice. In these studies, hearts from phospholamban knock-out mice, which were devoid of phospholamban, exhibited enhanced parameters of left-ventricular contractility in comparison to wild-type hearts. These results suggest that the relative phospholamban: SERCA2 ratio is critical in the regulation of myocardial contractility and alterations in this ratio may contribute to the functional deterioration observed during heart failure.

Key words Phospholamban – SERCA2 – atrium – ventricle – gene-targeting

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Introduction

Phospholamban is a regulatory phosphoprotein of cardiac sarcoplasmic reticulum membrane, which is phosphorylated *in vivo* in response to β-adrenergic agonist stimulation (1). This relatively small phosphoprotein, which is 6080 D in size, modulates the active transport of Ca^{2+} into the cardiac sarcoplasmic reticulum via reversible regulation of the sarcoplasmic reticular Ca^{2+}-ATPase enzyme (SERCA2) (2, 3, 4). In the dephosphorylated sate, phospholamban inhibits Ca^{2+}-ATPase activity by decreasing the affinity of the enzyme for Ca^{2+} (5). Such inhibition of the Ca^{2+}-ATPase enzyme by phospholamban increases the EC_{50} of the enzyme for Ca^{2+} resulting in decreased Ca^{2+}-uptake into the cardiac sarcoplasmic reticulum, ultimately prolonging myofibrillar relaxation time (6). Inversely, phosphorylation of phospholamban, in response to β-adrenergic stimulation, relieves this inhibition, producing an increase in the affinity of the enzyme for Ca^{2+} and increasing the rate of myofibrillar relaxation

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(7). Therefore, the phosphorylation status of phospholamban is a primary determinant of calcium sequestration and of myocardial relaxation.

Recently, it has been demonstrated that phospholamban is not only a regulator of myocardial relaxation, but it is also an important regulator of the basal cardiac contraction cycle and that phosphorylation of phospholamban in intact hearts is a major regulatory pathway involved in the heart's responses to β-adrenergic stimulation. To determine the regulatory role of phospholamban in basal myocardial contractility, mice were generated in which the phospholamban gene was ablated using gene-targeting methodologies (8). These phospholamban knock-out mice, which have disrupted phospholamban alleles, exhibited hyperdynamic cardiac function including increased rates of basal myocardial contraction as well as increased rates of basal myocardial relaxation (8–10), demonstrating the functional role of phospholamban in both the contractile and relaxational phases of the cardiac cycle. In addition, hearts from the phospholamban knock-out mice, when studied as isolated, work-performing preparations, were refractory to administration of isoproterenol (8, 9). These experiments were instrumental in the delineation of the important functional role of phospholamban in the myocardial β-adrenergic signaling pathway.

This report centers on the hypothesis that phospholamban is an important regulator of the basal cardiac contraction cycle and is founded on studies of the phospholamban knock-out mouse, which demonstrate that phospholamban is a potent repressor of both myocardial contractility and myocardial relaxation (9, 10). Furthermore, this hypothesis is supported by recent studies on the differential contractility parameters of murine atrial and ventricular muscles, which are correlated with the respective differential expression of phospholamban in the muscles (9, 11). Our data indicate that the relative levels of phospholamban: SERCA2 in cardiac muscle have a determinant function as to the muscle's overall contractility status. The basal contractility parameters for muscles with relatively high phospholamban: SERCA2 ratios, are lower in comparison to those for muscles with relatively low phospholamban: SERCA2 ratios (8–11). These studies suggest that variations in phospholamban expression levels, which alter sarcoplasmic reticular function, are associated with concomitant variations in myocardial contractility. Thus, the relationship between phospholamban expression levels and myocardial contractility may be an important aspect of myocardial loss-of-function during cardiomyopathic disease, as well as an important consideration for restoration-of-function in cardiomyopathic therapy.

Methods

In situ hybridization of cardiopulmonary tissue sections

Cardiopulmonary tissues were excised from young adult female mice, rinsed with PBS, fixed in 4 % paraformaldehyde, and cryopreserved as previously described (11). All solutions were prepared under RNase-free conditions as outlined by Sambrook et al. (12). Tissues were sectioned, mounted, and permeabilized as previously described (11) and were then hybridized with [^{35}S]-labeled riboprobes, which were synthesized as phospholamban cRNA products using a template consisting of a DNA polymerase chain reaction product, subcloned into PBS SK$^-$ (11). The cRNA sequence and hybridization procedure have been previously reported (11). Hybridized tissue sections were processed according to previously described methods (13, 14) and were photographed using the dark-field optics of an Olympus BHTU microscope.

Analysis of cardiac RNA

In preparation for dot blot quantitation, total RNA was extracted and spectrophotometrically quantitated from right and left atrial flaps (auricles), ventricular apices comprised of left and right ventricular muscle, and whole hearts as previously described (11). Three RNA pools were collected from total RNA extracts from 180 atrial flaps, 30 ventricular apices, and eight whole-hearts. Serial dilutions of RNA extracts were blotted, three consecutive times each, onto triplicate nylon membranes, and quantitation of phospolamban and SERCA2 mRNA transcripts, relative to α-MyHC mRNA were performed as previously described (11). The phospholamban oligonucleotide probes, utilized in these studies, were analyzed for specificity on both Southern and Northern blots prior to being used in dot blot hybridization (11).

Isometric contractility measurements for isolated, superfused atrial and ventricular muscles

Cardiac muscles were dissected from anesthetized, heparinized female FVB/N mice according to the method of Grupp and Grupp (15). For atrial measurements, the left atrial appendages (auricles) were utilized, as the right auricles had to be excluded due to automatic pacemaker activity. For ventricular measurements, muscle strips containing the ventricular out-flow tract were dissected from right ventricles, while the left ventricles were excluded due to size (11). The muscles were mounted in baths and

superfused at 35 °C. This was accomplished by mounting two atrial or two ventricular muscles in the same bath using double-electrode clamp holders. One end of each muscle was clamped firmly on top of two electrodes, while the other end was connected via suture filament to the mechanical arm of a force transducer. The muscles were electrically stimulated as previously described (11). Great care was taken so that muscle orientation was identical between experiments as described by Koss et al. (11). Resting tension was set via micrometer, and following equilibration, length-tension curves were established. Final equilibration was achieved close to maximum length-tension (L_{max}), and all muscles were normalized for loading conditions at L_{max} as previously described (11). Muscles were stimulated with isoproterenol as previously described (11). For each muscle, developed force, resting tension, the rate of force development (+dF/dt), and the rate of relaxation of tension (−dF/dt) were recorded via polygraph. Measurements of time-to-peak tension (TPT) and time to half-relaxation of tension ($RT_{1/2}$) were made by digital micrometer. These measurements were normalized to the developed force and are expressed as mean ms/mg ± S.E.M. Measurements of +dF/dt and −dF/dt are reported as mean mg/s ± S.E.M. Significance between paired comparisons was determined using paired t-test analyses.

Generation of phospolamban-deficient (phospholamban knock-out) mice

Phospholamban knock-out mice were generated using gene targeting methodologies as described by Luo et al. (8). Heterozygous mice carrying a phospholamban-ablated allele were bred to homozygosity, generating phospholamban knock-out mice (8). Wild-type, littermate mice of identical mixed-strain background were bred simultaneously for use in control experiments (8).

Contractility measurements for isolated, work-performing hearts from phospholamban knock-out and wild-type mice

Work-performing heart preparations were carried-out according to the methodology described by Grupp et al. (10). Measurements of developed left-ventricular pressure (DP), rate of left-ventricular pressure development (+dP/dt), and rate of pressure release (−dP/dt) were recorded as previously described (8, 10). These are reported as mean developed mm Hg/s ± S.E.M. under control conditions and at maximal isoproterenol stimulation, as previously described (8). The times to left-ventricular peak pressure development (TPP) and to half-relaxation of left ventricular pressure ($RT_{1/2}$) were measured via digital micrometer and normalized to DP. These are reported as mean ms/mm Hg ± S.E.M. under control conditions and during maximal isoproterenol stimulation, as previously described (8). Significance of paired comparisons was determined using paired t-test analyses.

Results

Phospholamban and SERCA2 transcript levels in atrial and ventricular muscles

In order to study the distribution of phospholamban gene transcripts in the murine heart, we performed *in situ* hybridization studies of murine cardiopulmonary sections (Fig. 1). Hybridization of these tissue sections with a phospholamban riboprobe (antisense to the murine phospholamban gene) revealed a differential pattern of intensities of the phospolamban antisense hybridization signal between atrial and ventricular cardiac compartments (Fig. 1; left). The tissue-autoradiogram shown in Fig. 1, shows a higher intensity of phospholamban hybridization signal in the ventricle than in the atrium. This finding is similar to that which we have previously reported for other murine cardiac tissues, hybridized with radiolabeled phospholamban cRNA (11). Background hybridization for the phospolamban antisense cRNA probe is depicted in the lung tissue (Fig. 1; left). The degree of non-specific hybridization to the tissues was evaluated using a phospholamban sense riboprobe, which is demonstrated in the representative autoradiogram shown in Fig. 1 (right).

The differential pattern of phospholamban gene transcript expression observed between murine atrial and ventricular muscles in cardiac tissue sections, prompted us to extend our studies to include the relative *in vitro* quantitation of phospholamban mRNA in these muscles. Phospholamban gene transcripts were quantitated in RNA extracts from atrial and ventricular muscles, using previously described dot blot techniques (11). A 60-bp oligonucleotide, antisense to a portion of the murine phospolamban gene coding region, was used as a probe for this analysis. Prior to use on dot blots, the efficacy and specificity of this probe was demonstrated on Southern blots of murine genomic DNA and on Northern blots of murine cardiac RNA (11). A representative autoradiogram of a dot blot, probed for phospholamban mRNA is shown in Fig. 2. In addition to RNA extracts from atrial and ventricular muscles, RNA extracts from whole hearts

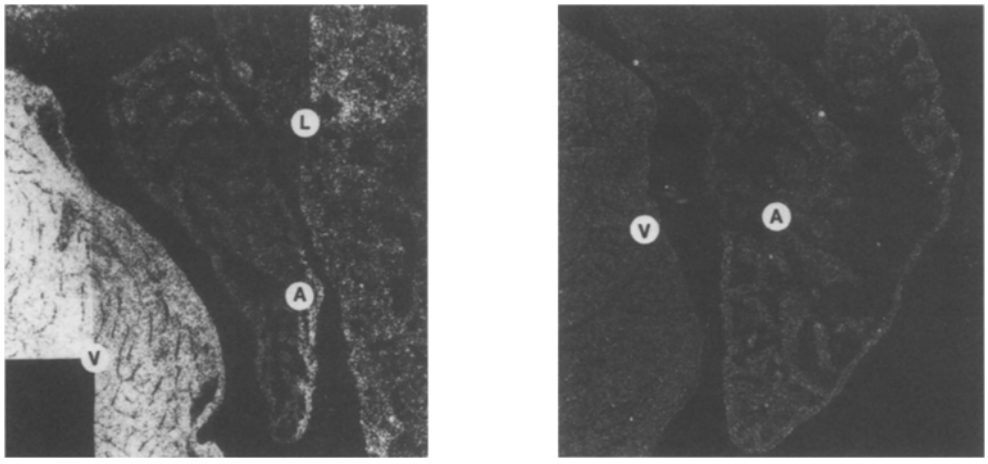

Fig. 1 Differential phospholamban gene transcript expression visualized by *in situ* hybridization of cardiopulmonary sections from the FVB/N mouse. Tissue sections from wild-type FVB/N mice were probed with either cRNA, antisense to the phospholamban gene (left) or phospholamban sense-strand cRNA (right) as a control. All photomicrographs were taken under dark-field illumination. The composite photomicrographs allow for visualization of phospholamban gene expression in murine atrium (A) and ventricle (V). Background hybridization of the antisense cRNA probe is depicted in the lung (L).

were blotted onto the same membrane and served as standards for normalization of RNA loading onto the membranes. In addition, this provided for quantitation of relative expression levels of atrial and ventricular gene transcripts to the whole heart. Each blot also contained: 1) tRNA blotted at the same concentration as the cardiac RNA samples, serving as a negative control; and 2) the diluent (SSC) blotted as a blank, demonstrating no carryover or contamination of the diluent in serial dilutions (Fig. 2). As previously described, the RNA samples were additionally probed for quantitation of alpha myosin heavy chain (α-MyHC) mRNA, and phospolamban gene transcripts were expressed relative to α-MyHC transcripts in order to normalize these measurements relative to cardiac muscle (11).

Quantitative results obtained from dot blot analyses indicate that phospholamban gene transcripts are significantly lower in atrial muscles than in ventricular muscles, when expressed relative to the whole heart (Fig. 2). Our data indicate that the relative ratio of phospholamban to α-MyHC was 3.2-fold higher in the murine ventricles as compared to murine atrial muscles (Fig. 3). This ratio is similar to that previously reported comparing murine ventricular phospholamban mRNA copy numbers to those of the atrium (11). That report showed ventricular mRNA to

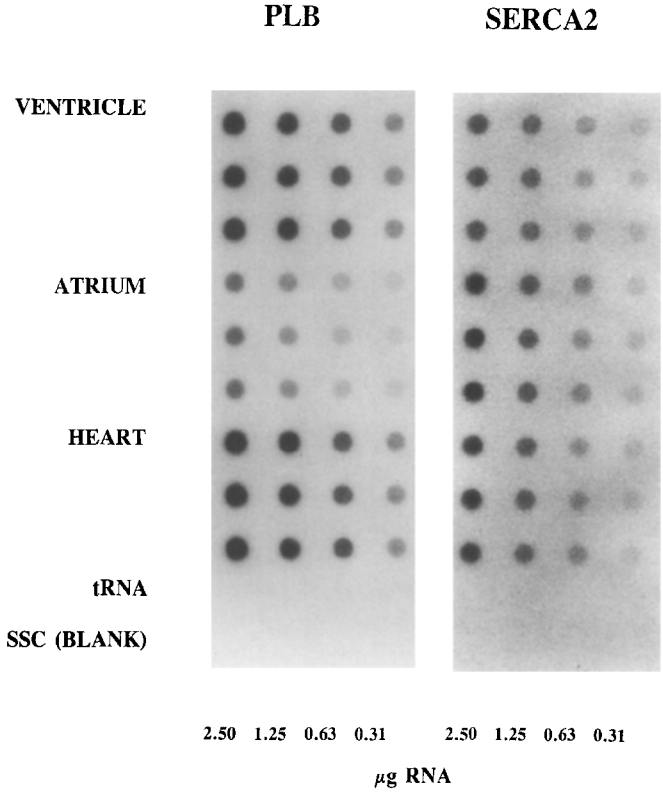

Fig. 2 Dot blot quantitation of phospholamban and SERCA2 gene transcripts in murine atrium and ventricle. Representative autoradiograms are shown in the photos depicting total cardiac RNA extracts, which were blotted onto nylon membranes and probed with [^{32}P]-end labeled DNA oligonucleotides. Blots were probed with either a 60-bp oligonucleotide, antisense to the murine phospholamban gene or a 60-bp oligonucleotide, antisense to the murine SERCA2 gene. Measurements of phospholamban and SERCA2 mRNAs were normalized to α-MyHC mRNA by blotting of the pooled RNA samples onto separate membranes, which were probed with an oligonucleotide, antisense to the murine α-MyHC gene. All dot blots were standardized for RNA membrane-loading via subsequent re-probing with a 60-bp oligonucleotide, antisense to the murine 18S gene.

be 2.5-fold above atrial mRNA in measurements which were not normalized to a cardiac muscle indicator such as α-MyHC.

To determine whether SERCA2 is also differentially expressed between murine atrial and ventricular compartments, we similarly analyzed the transcript ratios of SERCA2 relative to α-MyHC mRNA, using dot blots of the same cardiac RNA samples used in the quantitation of phospholamban gene transcripts (Fig. 3). Blotted RNA was probed with a 60–bp oligonucleotide, antisense to a portion of the murine SERCA2 gene. The specificity of this probe was previously determined via hybridization of murine cardiac mRNA on Northern blots (data not shown). These results demonstrate that ventricular SERCA2 mRNA is 20 % below that found in atrial muscle (Fig. 2). Therefore, the relative ratio of phospolamban: SERCA2 is 4.2-fold higher in the murine ventricle than in the murine atrium.

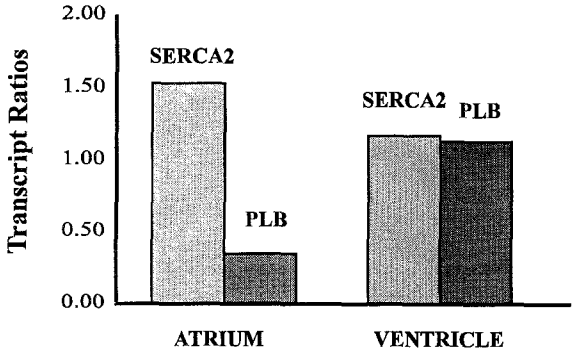

Fig. 3 Relative gene expression levels of phospholamban and SERCA2 gene transcripts to cardiac α-MyHC in murine atrium and ventricle. Atrial SERCA2 mRNA is 1.2-fold above that of ventricular muscle, while atrial phospholamban mRNA is three-fold below that of ventricular muscle. All mRNA levels were measured by phosphorimager analysis of hybridized RNA dot blots and reported relative to whole heart mRNA. Values represent the mean of six determinations.

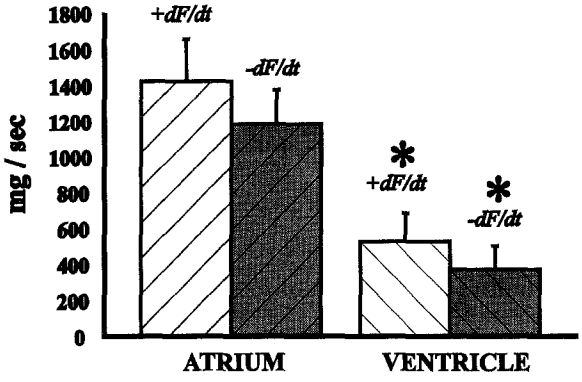

Fig. 4 Average rates of atrial and ventricular force development (+dF/dt) and relaxation of tension (−dF/dt). The rate of ventricular contraction (+dF/dt) is approx. three-fold slower than the rate of contraction for atrial muscle. Similarly, the rate of ventricular relaxation is nearly three times slower than the rate of atrial relaxation. Each average rate represents the mean ± S.E.M. for five to six individual muscles, recorded at maximal length-tension, which provided for normalization of the load on the muscles.

Contractility measurements for atrial and ventricular muscle tissues

Since phospholamban is an important regulator of basal myocardial contractility in the mouse (8–10) and since it appears that phospholamban is differentially expressed between murine cardiac compartments (11), parameters of atrial and ventricular contractility were assessed for isolated, superfused atrial and ventricular muscles in order to determine whether there exists a correlation between varying levels of phospholamban and contractility for these muscles. For each isolated muscle, resting tension (mg), developed force (mg), rate of force development (+dF/dt in mg/s), and rate of relaxation of tension (−dF/dt in mg/s) were recorded. Each muscle was normalized for loading conditions to maximal length-tension (L_{max}) and isoproterenol responses were then recorded. Our findings demonstrate that at L_{max} under basal isometric stimulation, the atrial muscles exhibited on average a three-fold faster rate of contraction than did the ventricular muscles ($p < 0.05$) (Fig. 4). This relationship also held true for the rates of atrial vs. ventricular relaxation of tension, which on average were nearly three-fold faster for atria as compared to ventricles ($p < 0.05$) (Fig. 4). It is interesting to note that the slower rates of ventricular contraction and relaxation, which are three-fold slower than those observed for atrial muscles, correlate with the higher level of ventricular phospholamban transcript expression, which is three-fold higher in the ventricle than in the atrium (Figs. 3 and 4).

Contractile parameters for the time-to-peak tension (TPT) and the time to half-relaxation of tension ($RT_{1/2}$) were measured from atrial and ventricular recordings of developed force via digital micrometer for each muscle. These parameters were calculated in ms and were normalized to the extent of developed force (mg). Time parameters, reported as mean ms/mg are shown in Table 1. These data demonstrate that at L_{max}, the ventricular muscles exhibited significantly greater TPT and $RT_{1/2}$ values than did atrial muscles, indicating that these muscles required more time to develop a specific level of force as well as more time to relax following force development. This relationship also held true during isoproterenol

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stimulation of the muscles (Table 1). While isoproterenol significantly decreased TPT and $RT_{1/2}$ parameters for both muscles groups (Table 1), at maximal isoproterenol response, the ventricular muscles still required more time to contract and to relax than did the atrial muscles (Table 1). Such greater time parameters for ventricular muscles as compared to atrial muscles correlate with the greater levels of phospholamban compared to SERCA2 gene expression in the ventricular muscles compared to atria (Figs. 1, 2 and 3).

Contractility measurements for isolated, work-performing hearts from phospholamban knock-out and wild-type mice

In order to determine the role of phospholamban in murine myocardial contractility, measurements of developed left-ventricular pressure (DP), rate of left-ventricular pressure development (+dP/dt), and rate of pressure release (−dP/dt) were recorded for isolated work-performing hearts from both wild-type and phospholamban knock-out mice. These studies demonstrate that in the absence of phospholamban, both the rate of left-ventricular pressure-development and the rate of left-ventricular pressure-release are significantly enhanced when compared to similar measurements for hearts from aged-matched, wild-type litter-mate mice (Fig. 5A). In addition, time parameters (in ms) of left-ventricular contractility were calculated from recordings of left-ventricular force development for each heart and were normalized to the extent of left-ventricular pressure development (in mm Hg) for that respective heart. These parameters, reported as mean ms/mm Hg are shown in Fig. 5B. In the

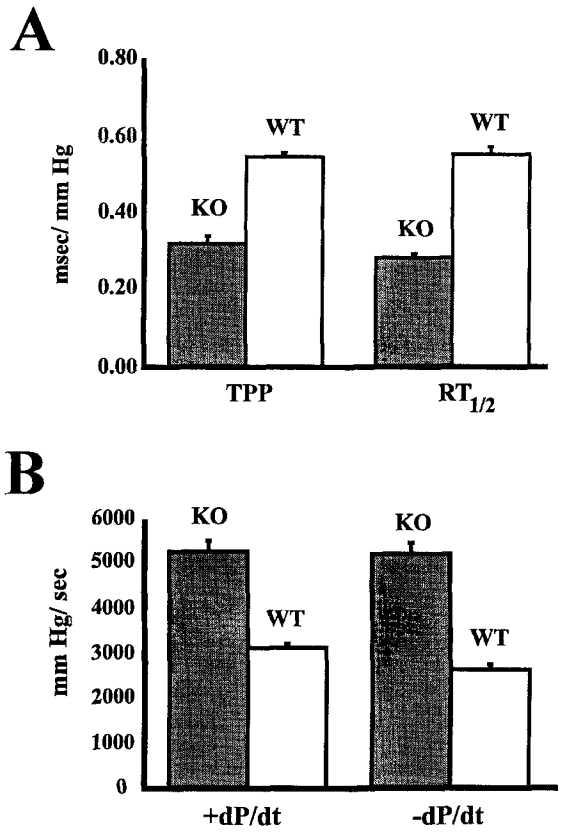

Fig. 5 Contractility parameters for isolated, perfused hearts from wild-type (WT) and phospholamban knock-out (KO) mice. The average time to left-ventricular peak pressure development (TPP) and average time to half-relaxation of developed pressure ($RT_{1/2}$) are represented in Fig. 5A as mean ms ± S.E.M., normalized to mm Hg of developed pressure (DP). The average rate of left-ventricular pressure development (+dP/dt) and rate of pressure release (−dP/dt) are depicted in Fig. 5B as mean mm Hg/ms ± S.E.M.

Table 1 Contractility parameters for isolated, superfused myocardial muscles. Reported as ms/mg developed force.

	Atria (n = 7)	Ventricles (n = 4)	p Values* (df = 9)
TPT(L_{max})	0.66 ± 0.88	2.75 ± 0.50	0.0004
TPT(ISO_{-max})	0.25 ± 0.03	0.85 ± 0.27	0.0136
p values**	0.0006	0.016	
$RT_{1/2}$(L_{max})	0.41 ± 0.05	2.14 ± 0.34	0.0001
$RT_{1/2}$(ISO_{-max})	0.14 ± 0.02	0.52 ± 0.12	0.003
p values**	0.0006	0.004	

n indicates number of muscles; $RT_{1/2}$ is the time to half-relaxation; TPT is the time to peak-tension; df indicates degrees of freedom.

* Assessed by *t*-test for paired comparisons between atrial and ventricular strips.

** Assessed by *t*-test for paired comparisons between basal and maximal for a given tissue.

phospolamban knock-out heart, both the TPP and $RT_{1/2}$ parameters are significantly shortened with respect to these parameters in wild-type hearts (Fig. 5B). These shortened parameters indicate that in the absence of phospholamban, the heart cannot only relax more quickly, but can also contract more quickly compared to hearts in which phospolamban is normally expressed.

Administration of isoproterenol via cumulative dosage failed to inotropically stimulate the phospholamban knock-out hearts, in contrast to wild-type hearts, which displayed enhanced contractility parameters for DP, +dP/dt, −dP/dt, TPP, and $RT_{1/2}$ (8–10). These parameters were not significantly affected, even at isoproterenol doses, which maximally stimulated the wild-type hearts (8–10). However, heart rate increased in both wild-type and phospholamban knock-out hearts during isoproterenol exposure (8). It is also interesting to note that the maximally

stimulated contractile parameters in wild-type hearts were similar to the basal levels of these parameters in the phospholamban knock-out hearts (8–10).

Discussion

In this report, we suggest that the phospholamban: SERCA2 ratio is a critical regulator of contractility in cardiac muscle. This suggestion is based on our findings in murine atrial and ventricular muscles and in phospholamban knock-out hearts. Murine atrial muscles, which have relatively low phospholamban: SERCA2 ratios, have faster rates of contraction and relaxation, as well as shorter times to peak tension development than do ventricular muscles, which have a higher phospholamban: SERCA2 ratio (11). Our data demonstrate that while SERCA2 expression is similar between atrial and ventricular muscles, phospholamban is differentially expressed between these two muscles with the ventricle having three-fold greater phospholamban than the atrium. This differential phospholamban gene expression gives rise to the different phospholamban: SERCA2 ratios observed for these muscles. While it is clear that the relatively low phospholamban: SERCA2 ratio is not the only factor affecting differences between atrial and ventricular contractility, the suggestion that this low ratio is an important determinant of enhanced atrial contractility is consistent with observations recently reported for hearts from hyperthyroid rats which displayed decreased levels of phospholamban and enhanced sarcoplasmic reticular Ca^{2+}-uptake (17). However, effects due to other atrial factors such as: 1) shortened action potential duration, 2) abbreviated calcium currents, or 3) differential myosin light-chain expression may also have a role in the enhanced contractility of atrial muscles as compared to ventricles (11).

Comparative association of the enhanced contractile properties of atrial muscles to ventricular muscles with the relatively lower phospholamban content of atrial muscle, is in keeping with our recent observations on enhanced myocardial contractility in a phospholamban knock-out mouse (8–10). Hearts from these mice, which are devoid of phospholamban, express a Ca^{2+}-ATPase enzyme, which is unregulated by phospholamban (8–10). Measurements of left-ventricular contractility made from isolated, work-performing heart preparations, demonstrated that the phospholamban knock-out hearts displayed greatly enhanced contractile parameters in comparison to hearts from wild-type mice. The increased inotropic characteristics of the phospholamban knock-out hearts includes shortened times to left-ventricular peak pressure development, shortened times to half-relaxation of tension, enhanced rates of pressure development, and enhanced rates of pressure release (8–10). In addition, these hearts exhibited no chronotropic differences in comparison to wild-type controls (8). Consistent with these observations on the critical role of the phospholamban: SERCA2 ratio in contractility, recent studies in our laboratory indicated that phospholamban over-expression in the murine heart results in an increased phospholamban: SERCA2 ratio and decreses in the contractile parameters (unpublished observations). Furthermore, it is interesting to note that hearts from phospholamban knock-out mice were refractory to the inotropic effects of isoproterenol, which was effective in stimulating wild-type hearts (8, 9). The lack of isoproterenol response in phospholamban knock-out hearts suggests that phosphorylation of phospholamban is a major signaling pathway through which the β-adrenergic stimulus is transduced in murine myocardium. A second explanation for the lack of isoproterenol stimulation of phospholamban knock-out hearts may simply be the fact that these hearts exist in a maximally-stimulated inotropic state, which is incapable of further enhancement. While measurements of both right and left wild-type ventricles displayed marked inotropic enhancement in response to isoproterenol (8–11), it is interesting to note that isoproterenol also had a marked inotropic effect on wild-type atrial muscle, despite the fact that phospholamban levels in this muscle were three-fold below those of the ventricles (11).

The mechanism through which murine phospholamban gene expression is differentially regulated in murine atrium and ventricle is at present, unknown. Our recent studies on phospholamban and SERCA2 gene transcript expression in these cardiac compartments indicates that the expression of these genes is not regulated in a coordinate manner (11). This assertion is supported by reports in rat heart (17) and in rabbit heart (20), where hyperthyroidism and hypothyroidism affected the expression of phospholamban and SERCA2 in a dis-coordinate manner. Future studies using genetically altered, diseased, or aged animals will lend great insight as to the regulation of expression of these two cardiac sarcoplasmic reticular genes, which are so important in the regulation of myocardial contractility. In addition, molecular studies designed to isolate and express the phospholamban gene promoter will eventually lead to a greater understanding as to how the differential expression of this gene is regulated in cardiac compartments. This understanding will be an important tool for deciphering the role of altered phospholamban gene expression in cardiac disease.

Acknowledgements This work was supported by National Institutes of Health Grants HL08901 (K.L.K.) and HL26057 and HL22619 (E.G.K.).

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