Adenoviral Gene Transfer of Mutant Phospholamban Rescues Contractile Dysfunction in Failing Rabbit Myocytes With Relatively Preserved SERCA Function

Mark T. Ziolo, Jody L. Martin, Julie Bossuyt, Donald M. Bers, Steven M. Pogwizd

In heart failure (HF) a main factor in reduced contractility is reduced SR Ca\(^{2+}\) content and reversed force-frequency response (FFR), ie, from positive to negative. Our arrhythmogenic rabbit HF model exhibits decreased contractility mainly due to an increase in Na/Ca exchange (NCX) activity (with only modest decrease in SR Ca\(^{2+}\) contractility mainly due to an increase in Na/Ca exchange) Our arrhythmogenic rabbit HF model exhibits decreased frequency response (FFR), ie, from positive to negative.1 This is analogous to our nonischemic rabbit HF model, where contractile function and SR Ca\(^{2+}\) load are reduced mainly because of increased NCX function rather than depressed SERCA function or SR Ca\(^{2+}\) leak.10–12

The purpose of this study was to determine whether (and how) increasing SERCA function, via ectopic expression of a PLB dominant-negative mutant, can reverse contractile dysfunction.

Materials and Methods

Rabbit Heart Failure Model and Cardiac Myocyte Isolation

In New Zealand White rabbits, HF was induced by aortic insufficiency and 2 to 4 weeks later by aortic constriction.10,11 HF progression was assessed by echocardiography and rabbits were studied 7.1 ± 1.9 months after aortic constriction when LV end-systolic dimension exceeded 1.4 cm.11 Left ventricular (LV) myocytes were isolated as previously described.10,11

Adenoviral Gene Transfer and Cell Culture

HF myocytes were cultured for adenoviral infection in modified M199 medium for 24 to 36 hours after infection. Mycocytes were infected with adenovirus (MOI=100 to 200, 1 to 2 hour infection) encoding either β-galactosidase (Adβgal, served as control) or a dominant-negative PLB mutant [AdPLB-dn, with amino acid substitutions at positions 3 and 14 (K3E/R14E), which inhibits PLB function].13

Western Blots

Antibodies for PLB (Badrilla), SERCA (Affinity Bioreagents), and NCX (Affinity Bioregants) were used and data normalized to sarcomeric α-actin (Sigma).10

Measurement of Ca\(^{2+}\) Transients

Myocytes were field-stimulated and superfused with (in mmol/L): 140 NaCl, 4 KCl, 1 MgCl\(_2\), 2 CaCl\(_2\), 10 glucose, and 5 HEPES (pH=7.4). [Ca\(^{2+}\)]\(_i\) was measured by Fluo-3 epifluorescence with excitation at 480 nm and emission at 530 nm, with recorded fluorescence (F) normalized to baseline fluorescence (F\(_0\)).14

Results

HF Rabbits

HF rabbits exhibited significant depression of LV systolic function; with increases in both LV end-diastolic (by 50%) and end-systolic dimension (by 73%; P<0.001) and a 27%
decrease in LV fractional shortening \( (P<0.01 \text{ versus baseline}).\)

**AdPLB-dn Enhances \( \text{Ca}^{2+} \) Transients, Force-Frequency Response, and SR \( \text{Ca}^{2+} \) Load**

Cells exposed to AdPLB-dn (versus Ad\( \beta \)gal) showed increased PLB expression and the characteristic\(^{13} \) decrease in electrophoretic mobility of pentamers in Western blots (Figure 1A). In addition, infection with AdPLB-dn or Ad\( \beta \)gal did not alter expression of either NCX or SERCA2a versus acutely isolated HF myocytes (Figure 1B).

Figure 1C and 1D shows that steady-state \( \text{Ca}^{2+} \) transient amplitude in HF myocytes was increased by 58% after AdPLB-dn versus Ad\( \beta \)gal infection. Notably, Ad\( \beta \)gal had similar \( \text{Ca}^{2+} \) transient amplitude compared with acutely isolated noncultured, noninfected HF myocytes (from the same HF hearts used for adenoviral infection). Figure 1E shows that HF myocytes acutely isolated or infected with Ad\( \beta \)gal displayed the negative FFR typical of HF, whereas HF myocytes infected with AdPLB-dn had a positive FFR.

We also assessed whether the enhanced function in AdPLB-dn is due to increased SERCA function and SR \( \text{Ca}^{2+} \) load (despite unaltered SERCA or NCX expression). The rate constant of [\( \text{Ca}^{2+} \)] decline in myocytes infected with AdPLB-dn was 42% faster than that in Ad\( \beta \)gal (Figure 2A), consistent with enhanced SERCA function. Likewise, SR \( \text{Ca}^{2+} \) content was significantly increased in AdPLB-dn versus Ad\( \beta \)gal (Figure 2B).

**Discussion**

HF myocytes typically demonstrate decreased SR \( \text{Ca}^{2+} \) load, \( \text{Ca}^{2+} \) transient amplitude, and a negative FFR.\(^{1,10,12} \) Although this is frequently attributed to decreased SR \( \text{Ca}^{2+} \) uptake and SERCA function,\(^{2,3} \) reduced SR \( \text{Ca}^{2+} \) load in HF models and human HF can arise from upregulation of the NCX.\(^{9,10} \) Moreover, adenoviral overexpression of NCX in normal rabbit ventricular myocytes decreased SR \( \text{Ca}^{2+} \) content and \( \text{Ca}^{2+} \) transients.\(^{15} \)

In this study, we found that, even after HF development, inhibition of PLB function enhanced \( \text{Ca}^{2+} \) transient amplitude, rate of [\( \text{Ca}^{2+} \)] decline, and SR \( \text{Ca}^{2+} \) content and restored a positive FFR in LV HF myocytes (without altered NCX or SERCA expression). This HF model exhibits severely depressed LV and myocyte function and reduced SR \( \text{Ca}^{2+} \) content, 2-fold upregulation of NCX expression and function, enhanced SR \( \text{Ca}^{2+} \) leak, and unchanged SERCA expression (but detailed quantitative analysis resolved a 24% decrease in SERCA function).\(^{10-12} \) The main cause of depressed SR \( \text{Ca}^{2+} \) content was found to be the increased NCX function (not altered SERCA or SR \( \text{Ca}^{2+} \) leak).\(^{12} \)

![Figure 1](image1.png)

**Figure 1.** AdPLB-dn and \( \text{Ca}^{2+} \) handling in HF myocytes. A, Western blot of HF rabbit myocytes infected with AdPLB-dn or Ad\( \beta \)gal (MOI 100 or 200), showing heteropentamer shift due to mutant PLB, as in ref 13. B, Pooled data of NCX and SERCA expression after infection with AdPLBdn or Ad\( \beta \)gal (vs acutely isolated HF myocytes). C, \( \text{Ca}^{2+} \) transients in HF myocytes infected with Ad\( \beta \)gal or AdPLBdn (0.5 Hz). D, Pooled data for \( \text{Ca}^{2+} \) transient amplitude and (E) force-frequency relationship (FFR). Data are mean±SEM. *\( P<0.05 \) vs acute and Ad\( \beta \)gal (n=6 to 10 cells from 4 HF hearts).

![Figure 2](image2.png)

**Figure 2.** AdPLBdn: \( \text{Ca}^{2+} \) transient kinetics and SR \( \text{Ca} \) content in HF myocytes. A, Half-time of [\( \text{Ca} \)] decline during twitches and (B) SR \( \text{Ca}^{2+} \) load assessed by rapid application of 10 mmol/L caffeine. Pooled data (mean±SEM; *\( P<0.05 \) vs Ad\( \beta \)gal; n=8 to 10 cells from 4 HF hearts).
We show, for the first time in HF myocytes, that PLB inhibition increased SR Ca\(^{2+}\) load and restored a positive FFR, which can explain most of the beneficial effects observed by us and others.\(^{4-8}\) This indicates that enhanced SERCA function can overcome the NCX upregulation and functional normalization of Ca\(^{2+}\) transients.

As in HF myocytes, nonfailing rabbit ventricular myocytes also showed significantly enhanced Ca\(^{2+}\) transient amplitude (by 43\%) and rate of \([\text{Ca}]+\) decline (by 20\%), as previously reported.\(^{13}\) Precisely how this dominant-negative PLB effect is mediated is unclear, but it may either sequester endogenous PLB in pentameric form (Figure 1A) relieving SERCA of inhibition, or displace endogenous PLB from SERCA2 (without inhibiting the pump itself). A quantitative limitation is that we cannot assess the effect of AdPLB-dn on endogenous PLB expression because the antibody used recognizes both endogenous and mutant PLB.

SERCA function in HF can be stimulated by either SERCA overexpression or interfering with the inhibition of SERCA by PLB (PLB knockout or by viral gene transfer of antisense PLB RNA or of a pseudophosphorylated form of PLB mutant). These can all improve myocyte and cardiac contractile function and prevent the development or progression of HF, including in some animal models of HF.\(^{4-8}\) Our data indicates that we are able to restore contractility even long after the onset of HF. In addition, it may be more advantageous to inhibit PLB function versus SERCA overexpression because too much SERCA may increase cytosolic Ca\(^{2+}\) buffering and accelerate \([\text{Ca}]+\) decline in a manner that limits myofilament activation.\(^{16}\) Inhibition of the NCX may also be beneficial,\(^{17}\) but excessive NCX inhibition may prevent necessary Ca\(^{2+}\) removal from the cell. Our results attest to the potential of PLB as a therapeutic target for the treatment of HF even when reduced SERCA activity is not the major abnormality.

In conclusion, enhanced SERCA function via gene transfer of a dominant-negative PLB mutant reversed the contractile dysfunction in HF myocytes by increasing SR Ca\(^{2+}\) load and restoring the positive FFR. This may justify future tests of this rescue approach in vivo.

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**References**


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