In vivo acceleration of heart relaxation performance by parvalbumin gene delivery

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Defective cardiac muscle relaxation plays a causal role in heart failure. Shown here is the new in vivo application of parvalbumin, a calcium-binding protein that facilitates ultrafast relaxation of specialized skeletal muscles. Parvalbumin is not naturally expressed in the heart. We show that parvalbumin gene transfer to the heart in vivo produces levels of parvalbumin characteristic of fast skeletal muscles, causes a physiologically relevant acceleration of heart relaxation performance in normal hearts, and enhances relaxation performance in an animal model of slowed cardiac muscle relaxation. Parvalbumin may offer the unique potential to correct defective relaxation in energetically compromised failing hearts because the relaxation-enhancement effect of parvalbumin arises from an ATP-independent mechanism. Additionally, parvalbumin gene transfer may provide a new therapeutic approach to correct cellular disturbances in calcium signaling pathways that cause abnormal growth or damage in the heart or other organs.

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Introduction

In heart failure, which today represents the major cause of combined morbidity and mortality in the developed world (1, 2), cardiac mechanical performance deteriorates markedly, eventually becoming insufficient to meet the normal daily requirements of the body. In an estimated 40% of patients who suffer heart failure (3), disease progression results specifically from a slowing of myocardial relaxation (diastolic dysfunction). In these patients, the heart relaxes too slowly after each contraction, thus compromising the refilling of the cardiac chambers with blood for the next beat.

The normal cardiac contraction/relaxation cycle requires a precise, transient rise and fall of intracellular calcium ions, a process that is governed by the sarcoplasmic reticulum (SR) (4, 5). In relaxation, calcium ions are actively sequestered into the SR via ATP-dependent calcium pumps. Notably, the time required to remove activating calcium from the myoplasm is markedly prolonged in human cardiac tissue samples obtained from patients who have suffered heart failure or cardiomyopathy (4).

Recently, it has been shown that the decay phase of the calcium transient is accelerated by parvalbumin expression at the level of the isolated adult cardiac myocytes in vitro (6). Parvalbumin is a soluble, small–molecular weight intracellular calcium-binding protein that is highly expressed in ultrafast contracting/relaxing striated muscle fibers, but is not naturally expressed in the heart (7). Parvalbumin functions as a delayed calcium sink in fast muscle based on the relative affinities of its two calcium/magnesium binding sites (8, 9). Parvalbumin is therefore ideally designed to speed the rate of decline in intracellular calcium, with additional advantages of this occurring via a non–ATP-dependent process.

To our knowledge, there have been no previous reports documenting parvalbumin's potential to enhance heart relaxation performance in vivo. The translation of findings obtained in single heart cells in vitro to whole heart organ function in vivo faces a number of potential obstacles, including achieving a sufficient distribution and amount of parvalbumin expression in the left ventricle. Further, the pacing and mechanical loading conditions of the heart in vivo depart significantly from those of unloaded contractions in isolated myocytes in culture. Finally, heart organ function in vivo and under conditions of neurohumoral modulation is significantly more complex than what can be ascertained from single cells in vitro. Therefore, to test a new in vivo experimental strategy to speed directly heart relaxation performance, we delivered and expressed the human, full-length α-parvalbumin cDNA (ref. 10; Figure 1a) in the left ventricular (LV) free wall of adult, female Sprague-Dawley rodents. In this study, we specifically addressed the following

questions: (a) Could physiologically relevant levels of parvalbumin be achieved in the left ventricle at 6 days after gene transfer? (b) Under physiological conditions, can parvalbumin alter mechanical relaxation properties of the left ventricle? (c) Can parvalbumin improve heart performance in an experimental model of abnormally slow myocardial relaxation?

Methods

Cardiac gene transfer in vivo. Adult, female Sprague-Dawley rats (250 g) were anesthetized with sodium pentobarbital (30 mg/kg body weight intraperitoneally). Animals were intubated and placed on artificial ventilation (50–60 breaths per minute). A thoracotomy was performed over the third intercostal space of the left thorax to expose the heart. An insulin syringe was inserted into the LV free wall (apical to base), using the left anterior descending coronary artery as a guide, and then slowly injecting 50–100 μl solution (parvalbumin titer, 1.87×10^{10} PFU/ml; AdlacZ titer, 1.00×10^{10} PFU/ml, each in PBS/10% glycerol; vehicle was PBS/10% glycerol) while withdrawing the syringe. The chest was closed and negative pleural pressure reestablished before extubation. Animals were given antibiotics via the drinking water. At day 6 after gene (or vehicle) transfer, heart parvalbumin expression and/or heart mechanical function was evaluated. This procedure has been approved by the University Committee on the Use and Care of Animals at the University of Michigan.

Myocardial relaxation performance. At day 6 after gene transfer, hearts were isolated, retrogradely perfused with oxygenated Krebs-Henseleit buffer warmed to 37°C, and electrically paced at 4 Hz (supramaximal, 5-millisecond pulses; Model S5 Stimulator; Grass Instruments, Quincy, Massachusetts, USA). Cardiac twitch contractile parameters (see Figure 3) were obtained from Langendorff perfused hearts by placing monofilament sutures at the apical and the base regions of the LV wall (between the circumflex and lateral descending coronary arteries). The base suture was attached to a force transducer (Model BG-300; Kulite Semiconductor, Leonia New Jersey, USA) and the apical suture to a fixed post. Thus, the placement of the sutures demarcated the LV free wall target area for virus delivery and parvalbumin expression (see Figure 2i). Using three-way positioners, the myocardium between the sutures was gently stretched until optimal length was attained as judged by attaining peak twitch force output. This is a near-isometric condition that permits quantitative assessment of cardiac contractile function as described previously (11), and which also allows comparison to twitch contractile studies on skeletal muscle preparations.

In vivo hemodynamics by micromanometry. In vivo cardiovascular hemodynamics were analyzed by conductance micromanometry modified from methods described in small rodents previously (12). Rats were anesthetized (nonresponsive to toe pinch) by inhalation

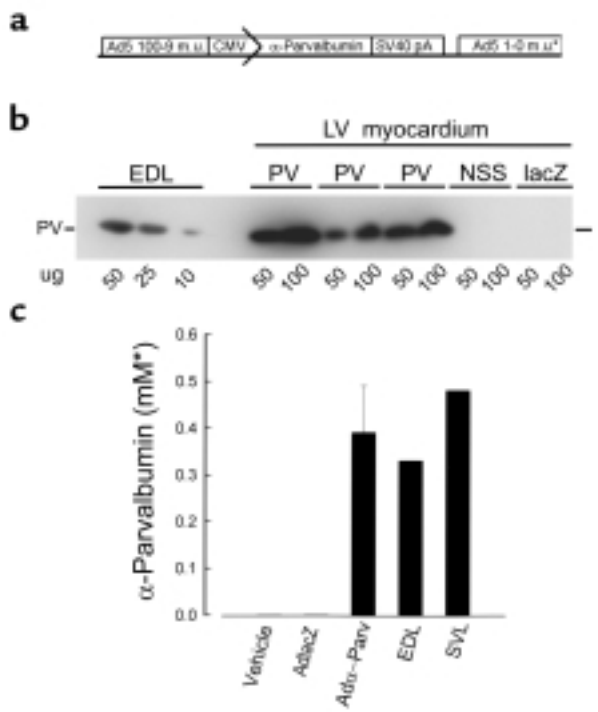
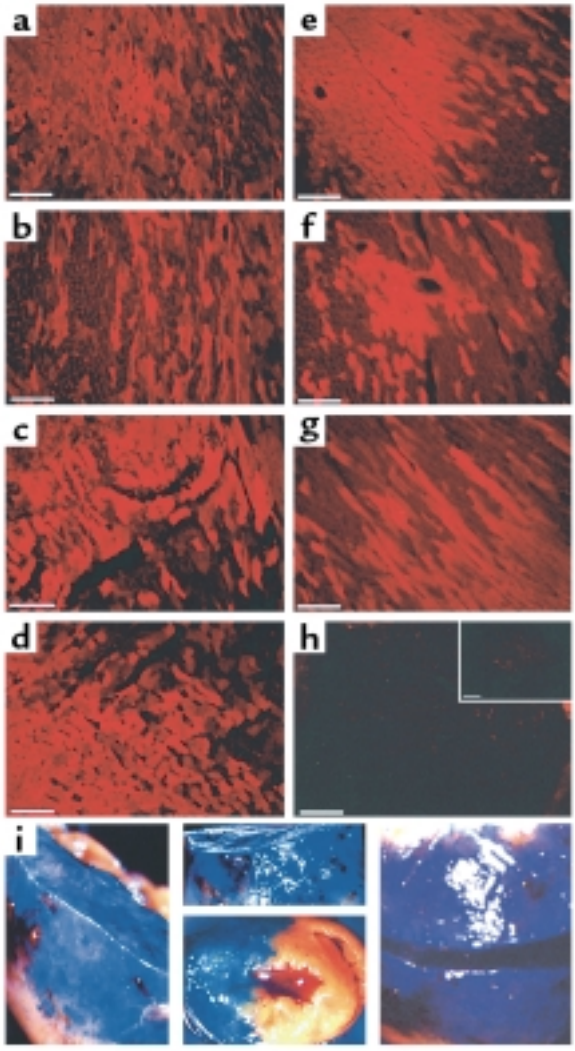


Figure 1

Parvalbumin gene transfer and expression in the LV free wall. (**a**) Recombinant adenovirus structure. The full-length human α-parvalbumin cDNA (10) was subcloned into *XbaI/HindIII* sites in the adenovirus shuttle plasmid pCA4 containing a human CMV promoter and SV40 polyadenylation site, and flanked by Ad5 serotype genetic sequences 0–1 map units (m.u.) and 9–16 m.u., and cotransfected with pJM17 into 293 cells to produce the recombinant adenovirus vector AdCMVαParvalbumin. AdCMVαParvalbumin plaques were identified by Southern blots, expanded, and virus-purified by CsCl banding; they were then dialyzed in PBS-10% glycerol overnight and stored in 100 μl volumes at –80°C as described elsewhere (27). (**b**) Western blot analysis of parvalbumin expression in the left ventricular (LV) free wall at day 6 after gene transfer. In each heart, the entire LV free wall was isolated, weighed, pulverized in liquid N2, and boiled in loading buffer and total protein content was determined (Bio-Rad Laboratories Inc., Hercules, California, USA). For each LV specimen, 50 and 100 μg of total protein was loaded in each lane, and densitometric analysis was conducted by comparing intensities to fast-twitch muscle samples (10, 25, and 50 μg samples; a range that is shown to be linear on these blots) from the rat extensor digitorum longus (EDL) and superficial vastus lateralis (SVL) as a guide on each blot to estimate the relative expression in the heart samples. PV, parvalbumin; NSS, normal, sterile saline-injected; *lacZ*, AdCMV*lacZ*-treated. Anti-parvalbumin antibody (PARV19; Sigma Chemical Co.) titer was 1:4,000, with detection by enhanced chemiluminescence as described previously (27). (**c**) Summary of parvalbumin expression in LV free wall samples. The mM* indicates that LV content is an estimate based on relative expression to EDL and SVL samples. Parvalbumin concentration in the LV free wall was estimated using Western blots (**b**) by scaling the relative immunostaining intensities of heart samples to that of a standard curve generated from rat fast-twitch EDL and SVL muscles, and converting relative intensities to estimated concentration by taking the published values (28) for parvalbumin expression in rat EDL (0.33 mM) and SVL (0.48 mM) muscles. Value for the parvalbumin group is mean ± SE (n = 8).

Figure 2
Immunofluorescence detection of parvalbumin in the heart. (**a**–**h**) The photographic montage assembled from representative serial cryosections extending from the apex to the base of a single heart at day 6 after gene transfer (representative of four experiments). (**a** and **b**) LV base sections; (**c** and **d**) LV midsection; (**e**–**g**) LV apical sections; (**h**) RV section. (**h**, inset) LV apical section from normal saline-injected control heart. Photographic exposure time was the same in **a**–**h**. There was extensive, parvalbumin-immunopositive reactivity in myocytes spanning the width, depth, and length of the left heart (apex to base). The primary antibody was anti-parvalbumin (PARV19; titer 1:500–1:1,000; Sigma Chemical Co.) and the secondary was goat anti-mouse IgG conjugated to Texas Red (titer 1:100; Molecular Probes Inc., Eugene, Oregon, USA), using methods described elsewhere (27). Dual labeling of single cells isolated from these hearts showed the colocalization of parvalbumin and sarcomeric actin, indicating that the cardiac myocytes were being transduced. Scale bar = 100 μm, **a**–**h**. (**i**) Low-power photomicrographs documenting the extent of *lacZ* gene expression (using X-gal, which results in blue staining of positive cells) after direct gene transfer to the heart in vivo. All panels are taken from the same gross dissection (sectioned in thirds) of a heart. Clockwise from left panel: side-view of apical one-third of heart with LV in foreground, demonstrating extensive transmural staining; side view of middle one-third of heart; side view of reconstruction of the apex/mid/base sections together; top view of midsection with base-facing section up (in this view, the spread of intense B-gal staining is evident across LV wall, with little or no detection in IVS or RV).



of 1.5–2% isoflurane, and body temperature was maintained at 37°C with a heating pad. Rats were intubated and placed on a ventilator. The jugular was cannulated and infused (~200 μl/kg/min) with 0.9% NaCl, 10% human serum albumin. A midline thoracotomy was performed, and a 1.4 French miniaturized pressure catheter (SPR-719; Millar Instruments Inc., Houston, Texas, USA) was inserted into the left ventricle via an apical stab made with a 25-gauge needle. Pressure was collected online at 1,000 Hz. Two-second data sweeps were analyzed with PVAN 2.7 software (Millar Instruments Inc.).

Echocardiography. Transthoracic echocardiograms were performed using a commercially available Acuson Systems (Model 125/XP) and 7.5 MHz linear transducer (Acuson, Mountain View, California, USA). Animals were sedated and placed in the supine/slightly left lateral decubitus position and the chest shaved. Acoustic gel was applied to the transducer and chest.

Using two-dimensional imaging, a short axis view of the left ventricle at the level of the papillary muscles was obtained. Two dimensionally guided M-mode recordings through the anterior and posterior walls of the left ventricle were obtained. LV end-systolic (LVESD) and end-diastolic (LVEDD) dimensions as well as systolic and diastolic wall thicknesses were measured from the M-mode tracings using the leading-edge convention of the American Society of Echocardiography. LV shortening fraction (LVSF) was calculated as a measure of LV function using the following formula: LVSF = (LVEDD–LVESD) / LVEDD.

Using two-dimensional imaging, the apical four-chamber view of the heart was obtained. Using color-flow Doppler as a guide, pulsed-wave Doppler spectral recording of mitral inflow and aortic outflow was obtained on the same recording strip. This was obtained by angling the transducer slightly anterior from the inflow tracing and widening the sample volume as needed. From the spectral Doppler tracing, the isovolumic relaxation time (IVRT) was measured using the appropriate valve closure Doppler signal whenever possible.

Data analysis. Values are mean ± SE. Statistical analysis was performed by a one-way ANOVA and Student-Newman-Keuls multiple comparison test, with $P < 0.05$ indicating significance.

Results

Parvalbumin gene transfer and expression. In the first experiments, the expression and localization of parvalbumin were evaluated at 6 days after adenoviral gene transfer of a single direct injection into the LV free wall. Western blot analysis of average parvalbumin expression throughout the entire LV free wall showed a calculated parvalbumin expression of 0.39 ± 0.10 mM (n = 8) (Figure 1, b and c), with little or no detectable expression in the right ventricle (RV) or interventricu-

lar septum (IVS). Thus this approach targets high expression to the LV but not to other regions of the heart. No parvalbumin was detected in vehicle-treated or Ad*lacZ*-treated LV free wall samples. Importantly, the ectopic parvalbumin expression level obtained in the LV of these hearts is in the physiological range found in rodent fast-twitch skeletal muscles (Figure 1c). In a test to address the possibility that during the 6 days after gene transfer there may be adaptive/compensatory alterations in SR function, we examined the expression levels of phospholamban, a known key SR regulatory protein, and calsequestrin, an important SR calcium-binding protein. Blots documenting parvalbumin expression were subsequently reprobed with antibodies to phospholamban and calsequestrin and showed no detected changes in their expression level (data not shown). This indicates that expression of these important proteins is not affected by parvalbumin gene transfer and expression. These findings, however, do not rule out other possible changes in expression levels or the phosphorylation status or activity of these or other important calcium handling proteins.

In separate experiments, hearts (n = 4) were fixed, cryosectioned from apex to base, and immunolabeled to document the localization and extent of parvalbumin expression throughout the heart at day 6 after gene transfer. Results demonstrated extensive parvalbumin immunostaining in cardiac myocytes across the entire LV free wall extending from sections obtained from the apex, midsection, and base (Figure 2). Parvalbumin expression was minimal and sporadic in the interventricular septum and RV. When some detection was seen in the RV or IVS, the extent of expression was always less than that in observed in the LV (Figure 2). Thus, this approach does not lead to global heart transduction, but instead results in marked expression throughout the LV free wall.

Cardiac muscle twitch kinetics. In a first test to determine whether cardiac muscle relaxation can be affected by parvalbumin expression, whole hearts were isolated at day 6 after gene transfer, retrograde perfused and electrically paced at 4 Hz, and cardiac muscle twitch kinetics were monitored at an optimal and near-isometric length to control for length-dependent alterations in contractile function. This method is similar to procedures used to analyze twitch relaxation parameters in isolated skeletal muscles. In addition, this approach enables a quantitative assessment of cardiac muscle relaxation parameters in the LV free wall (11). Results showed that the cardiac twitch one-half relaxation time was significantly shortened (Figure 3, a and b), and the rate constants describing the initial and late phases of relaxation (Figure 3, c and d) were significantly faster in parvalbumin-treated than in control groups. Other aspects of LV mechanical function, including time to peak twitch force, were not altered by parvalbumin gene transfer. These findings demonstrate for the first time to our knowledge that parvalbumin can specifically accelerate the mechanical relaxation properties of the intact myocardium under physiological pacing conditions.

In vivo hemodynamics. In a second series of functional experiments, in vivo LV hemodynamic function was determined directly by catheter-based micromanometry (12). After gene transfer, animals were anesthetized with isoflurane, intubated, and ventilated; following midline thoracotomy, a Millar pressure catheter was inserted into the LV chamber. In this in vivo setting, results demonstrated a significant effect of parvalbumin to increase the minimum –dP/dt (dP/dtmin), as well as to significantly shorten the time to 50% or 90% decrease in peak pressure (Figure 4). These hemodynamic measurements are well-accepted indices of myocardial relaxation function (13, 14) and thus directly show that parvalbumin can enhance cardiac relaxation function in the adult rat in vivo. Cardiac output, as judged by a flow probe placed on the aorta, was not different among groups. Interestingly, results also showed an effect of parvalbumin to increase peak LV pressure development (Figure 4).

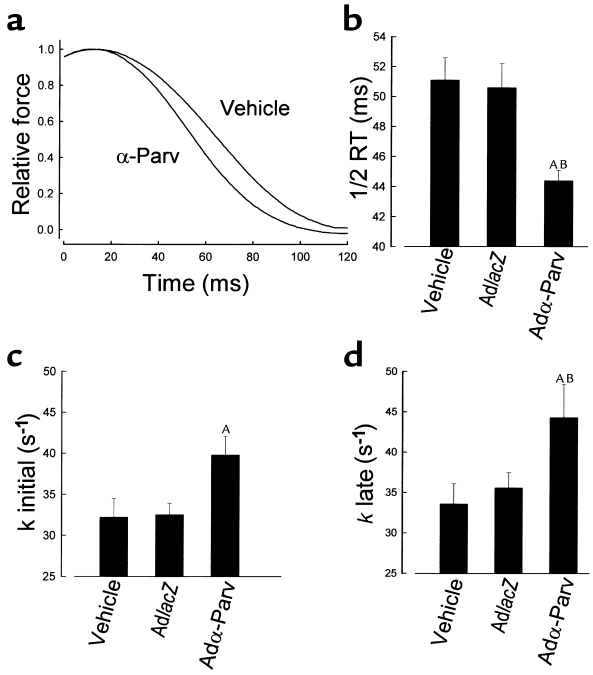


Figure 3

Acceleration of myocardial twitch relaxation rates by parvalbumin. (**a**) Representative traces of force relaxation in a parvalbumin-treated and a vehicle-treated heart. Force records are normalized to enable direct comparison of relaxation rates between groups. Maximum twitch forces were not different between groups. (**b**–**d**) Summaries of one-half relaxation times (1/2 RT) (**b**), and rate constants for the initial (**c**) and late (**d**) phases of relaxation of the cardiac twitch. 1/2 RT is calculated as the time interval from peak twitch force to one-half decay in peak force. Relaxation rate constants were obtained using single exponential fits of the initial phase (peak force to inflection point) and late phase (from inflection point to full relaxation) of relaxation. [A]Parvalbumin significantly different from Ad*lacZ* (P < 0.05; one-way ANOVA and Student-Newman-Keuls multiple comparison test). [B]Parvalbumin significantly different from vehicle (P < 0.05). Values are mean ± SE (n = 6–12). Time to peak twitch was not different between groups.

Echocardiography. In a separate series of experiments, transthoracic echocardiography was used for a noninvasive assessment of heart performance in vivo. Specifically, transmitral Doppler waveform analysis showed that LV isovolumic relaxation time (IVRT; time interval from aortic valve closure to mitral valve opening) and heart-rate corrected IVRT (IVRT/square root RR interval) were significantly shortened in parvalbumin-treated compared with control groups (Figure 5, a and b). Assuming that loading conditions are similar among groups (15), this result is evidence that parvalbumin directly accelerates cardiac relaxation performance under physiological conditions in vivo. In a subgroup of experiments (n = 15), both LV IVRT and parvalbumin content were determined in the same hearts. Results showed a significant, inverse correlation between IVRT and the relative parvalbumin expression in the LV free wall ($r^2 = 0.74$; $P < 0.0001$; Figure 5c), providing evidence that parvalbumin was causal for the shortening of IVRT, and strengthening the hypothesis that parvalbumin directly speeds the relaxation rate of the myocardium in vivo. Parvalbumin expression had no significant effect on echo-derived parameters, including LV ejection time, peak E, peak A, E/A ratio, shortening fraction or heart rate (Table 1). In lacZ animals, shortening fraction was reduced compared with vehicle (Table 1). In both parvalbumin and *lacZ*-treated groups the velocity of circumferential shortening was reduced compared with vehicle. The basis for this effect is not known; however, as parvalbumin gene transfer in vitro has no direct effect on the rate of myocyte shortening (6), these differences may be a nonspecific consequence of the immune response elicited by gene transfer in vivo.

In a final series of experiments, parvalbumin was tested in an animal model of slowed cardiac relaxation. Whereas it is acknowledged that there is no one perfect experimental model of slowed myocardial relaxation, we used here the hypothyroid rat model because it closely mimics the human disease characteristics of reduced calcium sequestration rate and slowed myocardial relaxation function that are associated with failing heart muscle (6, 16). Although numerous other muscle and nonmuscle alterations in function are inherent to this experimental model, it does nonetheless provide an experimental test bed to begin assessment of parvalbumin's potential to correct abnormal myocardial relaxation function in vivo. In the hypothyroid state, IVRT was markedly shortened in parvalbumin-treated compared with *lacZ*-treated animals, and, interestingly, IVRT in the parvalbumin group was not significantly different from IVRT in vehicle-treated euthyroid animals (Figure 5d). This suggests, but does not prove, that parvalbumin restored the in vivo relaxation performance of hypothyroid rats to the normal euthyroid condition. It was noted in the hypothyroid condition that the relaxation function of *lacZ*-treated animals (Figure 5d) was significantly slower than the vehicle-treated group (90.3 ± 4.3; n = 7), a difference in experimental groups (i.e., vehicle versus *lacZ*) not evident in the euthyroid state (Figure 5, a and b). This result could be attributed to the heightened immune response of hypothyroid rats (17). Despite this apparent effect of gene transfer to slow relaxation further in hypothyroid rats, parvalbumin expression restored normal diastolic function in these animals (Figure 5d).

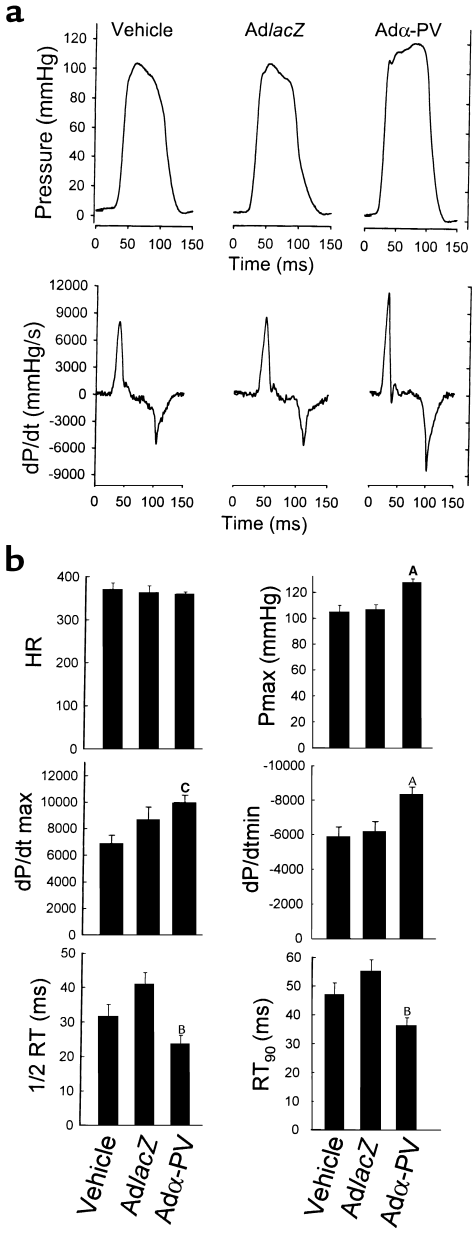
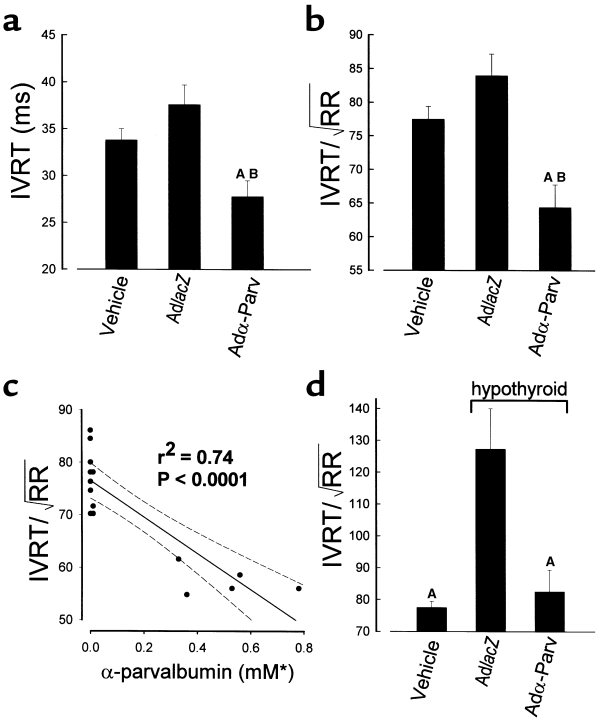


Figure 4
In vivo hemodynamics by catheter-based micromanometry. (**a**) Pressure (top traces) and first derivative of pressure (dP/dt; bottom traces) recordings at day 6 after vehicle or Ad*lacZ* or Adα-PV gene transfer. (**b**) Summary of hemodynamic data. HR, heart rate; Pmax, peak pressure; dP/dtmax, maximum positive dP/dt; dP/dtmin, maximum negative dP/dt; 1/2 RT, time from peak pressure to one-half decay in pressure; RT_{90}, time from peak pressure to 90% decay in pressure. Values are mean SEM (n = 9–10). [A]Adα-PV significantly different from vehicle and Ad*lacZ* ($P < 0.01$ for Pmax data and $P < 0.05$ for dP/dtmin data). [B]Adα-PV significantly different from Ad*lacZ*, $P < 0.01$. [C]Adα-PV significantly different from vehicle, $P < 0.05$.

Figure 5
Summary of parvalbumin-mediated enhancement of LV isovolumic relaxation time in vivo. (**a**) Absolute isovolumic relaxation times (IVRT) at day 6 after gene transfer in euthyroid animals. [A]Parvalbumin (n = 10) significantly (P < 0.05) different from vehicle (n = 9). [B]Parvalbumin significantly (P < 0.01) different from Ad*lacZ* (n = 10). (**b**) Normalized IVRT, correcting for variations in heart rate among euthyroid animals {IVRT / [square root of time interval between beats (RR interval in seconds)]}. [A]Parvalbumin significantly different from vehicle (P < 0.01). [B]Parvalbumin significantly different from Ad*lacZ* (P < 0.001). (**c**) Significant inverse correlation between IVRT and parvalbumin concentration. IVRT was normalized to heart rate. Solid line is best fit linear regression [y = –33.3 (x) + 76.1], and dashed lines are the 95% confidence limits. (**d**) Restoration of IVRT in hypothyroid animals by parvalbumin expression. IVRT was normalized to heart rate. [A]Parvalbumin-treated hypothyroid (n = 5) and vehicle-treated euthyroid (n = 9) significantly (P < 0.001) different from *lacZ*-treated hypothyroid group (n = 2). In the hypothyroid condition, the vehicle-treated group (90.3 ± 4.3, n = 7) was significantly different from the *lacZ*-treated group (**d**), owing possibly to the heightened immune response characteristic of the hypothyroid state in rats (17).



Discussion

In human patients, there is presently no direct clinical treatment for slowed cardiac relaxation performance in vivo. Whereas β-blockers appear to reduce mortality associated with heart failure, and indirectly improve diastolic function in failing hearts by prolonging the time interval between beats, these benefits come at the expense of reductions in exercise tolerance and quality of life (18). Recently, the rate of relaxation has been improved by overexpressing SR calcium ATPase pumps in isolated human heart cells (19), and in a mouse model of heart failure the release of inhibition of the SERCA-2a activity was shown to rescue function (20). One possible limitation of these approaches is that they require an obligate increase in the rate of ATP utilization to fuel the increased number/activity of the ATP-dependent pumps; accordingly, their potential therapeutic usefulness may be limited owing to the energetically compromised state of the failing heart (21, 22). Alternatively, parvalbumin appears uniquely designed to provide this gain-in-function to the energetically compromised and failing heart because the desired enhancement in the muscle's relaxation rate occurs via an ATP-independent mechanism (6, 9).

In three separate studies using a range of functional assays, including catheter-based micromanometry, our results consistently demonstrated that parvalbumin can produce an acceleration of myocardial relaxation under physiological conditions in vivo (Figures 3, 4, and 5) and can restore normal relaxation performance in an experimental animal model of abnormal relaxation in vivo (Figure 5). When considering all our findings, it is less clear whether parvalbumin may also influence systolic function of the intact heart. Catheter-based results showed an increase in peak LV pressure generation after parvalbumin gene transfer; conversely, in a separate set of experiments, echo-derived LV shortening fraction was not significantly altered in parvalbumin expressing hearts (Table 1). Considering that the catheter data provide a more reliable assessment of overall LV function, our results suggest that parvalbumin may additionally enhance systolic function. The possible mechanism underlying such an effect is not known. Parvalbumin did not affect the heart weight or heart/body weight ratio, so enhanced LV pressure could not be attributed to a hypertrophic response. It is interesting to note that the increase in LV pressure parallels, at least qualitatively, the enhanced pressure generation in mouse hearts after inactivation of the phospholamban gene (14). In that study, the authors speculated that the enhanced peak pressure may result from increased calcium loading of the SR. Whether a similar mechanism is shared in parvalbumin-expressing myocardium awaits further experimentation.

The present study focused on a short-term demonstration of proof-in-concept, as necessitated by the

Table 1
Summary of echocardiography-derived measurements of LV function

	SF	LVEDD	LVESD	Peak E/A	RR	ET	Vcfc
Vehicle	0.59 ± 0.02	0.59 ± 0.03	0.27 ± 0.03	1.14 ± 0.05	0.190 ± 0.008	0.072 ± 0.004	3.64 ± 0.22
AdlacZ	0.47 ± 0.02[A]	0.63 ± 0.04	0.35 ± 0.04	1.40 ± 0.11	0.201 ± 0.010	0.075 ± 0.002	2.78 ± 0.13[B]
Adα-PV	0.52 ± 0.03	0.57 ± 0.02	0.26 ± 0.02	1.35 ± 0.06	0.193 ± 0.010	0.079 ± 0.003	2.92 ± 0.16[B]

Values are mean ± SEM (n = 9–11 per group). [A]LacZ < vehicle, P < 0.01. [B]LacZ and PV < vehicle, P < 0.01. RR, r to r interval (s); ET, ejection time (msec); Peak E/A, ratio of early/late transmitral flow rates; Vcfc, velocity of circumferential shortening.

time-dependent immune response elicited by first-generation adenoviral vectors (23). Of note, there is some recent evidence that adeno-associated and/or gutted adenoviral vectors may provide long-term foreign gene expression in striated muscles in vivo (24, 25). It will be interesting and important in future studies using these new genetic vectors to determine, in other small and large animal experimental models of heart disease, the full therapeutic potential of parvalbumin to correct defective heart relaxation performance in vivo. Finally, it is tempting to speculate that parvalbumin's ability to directly buffer intracellular calcium in cardiac myocytes (6) may also protect the heart and other organs from maladaptive growth or damage arising from cellular disturbances in calcium signaling pathways (26).

Acknowledgments

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1. Chien, K.R. 1999. Stress pathways and heart failure. *Cell.* **98**:555–558.
2. O'Connell, J.B. 2000. The economic burden of heart failure. *Clin. Cardiol.* **23**:III6–III10.
3. Lorell, B.H. 1991. Significance of diastolic dysfunction of the heart. *Annu. Rev. Med.* **42**:411–436.
4. Morgan, J.P. 1991. Abnormal intracellular modulation of calcium as a major cause of cardiac contractile dysfunction. *N. Engl. J. Med.* **325**:625–632.
5. Ebashi, S. 1991. Excitation-contraction coupling and the mechanism of muscle contraction. *Annu. Rev. Physiol* **53**:1–16.
6. Wahr, P.A., Michele, D.E., and Metzger, J.M. 1999. Parvalbumin gene transfer corrects diastolic dysfunction in diseased cardiac myocytes. *Proc. Natl. Acad. Sci. USA.* **96**:11982–11985.
7. Celio, M.R., and Heizmann, C.W. 1982. Calcium-binding protein parvalbumin is associated with fast contracting muscle fibres. *Nature.* **297**:504–506.
8. Hou, T.T., Johnson, J.D., and Rall, J.A. 1991. Parvalbumin content and Ca2+ and Mg2+ dissociation rates correlated with changes in relaxation rate of frog muscle fibres. *J. Physiol.* **441**:285–304.
9. Rall, J.A. 1996. Role of parvalbumin in skeletal muscle contraction. *News in Physiological Science.* **11**:249–255.
10. Fohr, U.G., et al. 1993. Human alpha and beta parvalbumins. Structure and tissue-specific expression. *Eur. J. Biochem.* **215**:719–727.
11. Grupp, I.L., and Grupp, G. 1984. Isolated heart preparations perfused or superfused with balanced salt solutions. In *Methods in pharmacology*. A. Schwartz, editor. Plenum Press. New York, New York, USA. 111–128.
12. Georgakopoulos, D., et al. 1998. In vivo murine left ventricular pressure-volume relations by miniaturized conductance micromanometry. *Am. J. Physiol.* **274**:H1416–H1422.
13. Kass, D.A. 2000. Assessment of diastolic dysfunction. Invasive modalities. *Cardiol. Clin.* **18**:571–586.
14. Luo, W., et al. 1994. Targeted ablation of the phospholamban gene is associated with markedly enhanced myocardial contractility and loss of beta-agonist stimulation. *Circ. Res.* **75**:401–409.
15. Hoit, B.D., and Walsh, R.A. 1997. In vivo echocardiographic assessment of left ventricular function in transgenic and gene targeted mice. *Trends Cardiovasc. Med.* **7**:129–134.
16. Topol, E.J. 1998. *Textbook of cardiovascular medicine.* Lippencott-Raven. New York, New York, USA. 1–2732.
17. Ohashi, H., and Itoh, M. 1994. Effects of thyroid hormones on the lymphocyte phenotypes in rats: changes in lymphocyte subsets related to thyroid function. *Endocr. Regul.* **28**:117–123.
18. Braunwald, E., Colucci, W.S., and Grossman, W. 1997. Clinical aspects of heart failure: high output heart failure; pulmonary edema. In *Heart disease: a textbook of cardiovascular medicine.* E. Braunwald, editor. W.B. Saunders Co. Philadelphia, Pennsylvania, USA. 445–470.
19. delMonte, F., et al. 1999. Restoration of contractile function in isolated cardiomyocytes from failing human hearts by gene transfer of SERCA2a. *Circulation.* **100**:2308–2311.
20. Minamisawa, S., et al. 1999. Chronic phospholamban-sarcoplasmic reticulum calcium ATPase interaction is the critical calcium cycling defect in dilated cardiomyopathy. *Cell.* **99**:313–322.
21. Tian, R., Nascimben, L., Kaddurah-Daouk, R., and Ingwall, J.S. 1996. Depletion of energy reserve via the creatine kinase reaction during the evolution of heart failure in cardiomyopathic hamsters. *J. Mol. Cell. Cardiol.* **28**:755–765.
22. Ingwall, J.S. 1993. Is cardiac failure a consequence of decreased energy reserve? *Circulation.* **87**:VII-58–VII-62.
23. Yang, Y., Haecker, S.E., Su, Q., and Wilson, J.M. 1996. Immunology of gene therapy with adenoviral vectors in mouse skeletal muscle. *Hum. Mol. Genet.* **5**:1703–1712.
24. Svensson, E.C., et al. 1999. Efficient and stable transduction of cardiomyocytes after intramyocardial injection or intracoronary perfusion with recombinant adeno-associated virus vectors. *Circulation.* **99**:201–205.
25. Chen, H.H., et al. 1997. Persistence in muscle of an adenoviral vector that lacks all viral genes. *Proc. Natl. Acad. Sci. USA.* **94**:1645–1650.
26. Molkentin, J.D., et al. 1998. A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. *Cell.* **93**:215–228.
27. Westfall, M.V., Rust, E.M., Albayya, F., and Metzger, J.M. 1997. Adenovirus-mediated myofilament gene transfer into adult cardiac myocytes. *Methods Cell Biol.* **52**:307–322.
28. Green, H.J., et al. 1984. Exercise-induced fibre type transitions with regard to myosin, parvalbumin, and sarcoplasmic reticulum in muscles of the rat. *Pflugers Arch.* **400**:432–438.