Review

Parvalbumin: Targeting calcium handling in cardiac diastolic dysfunction

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Abstract. Diastolic heart failure (DHF) is a clinical syndrome characterized by depressed myocardial relaxation performance and poor ventricular refilling. Defective intracellular calcium (Ca^{2+}) handling underlies one of the fundamental mechanisms of DHF. Manipulating the content and function of Ca^{2+} handling proteins in the heart has been the focus of intense study to develop effective therapies for DHF patients. Parvalbumin (Parv), a skeletal muscle Ca^{2+} binding protein, has been shown to facilitate myocardial relaxation both *in vitro* and *in vivo*. Parv acts as a unique "delayed" Ca^{2+} buffer and facilitates Ca^{2+} sequestration from cytosol. Here, we summarize studies employing gene transfer of Parv in cultured adult cardiac myocytes and in vivo to redress depressed diastolic function. By targeting defects in cardiac Ca^{2+} handling, Parv represents a promising therapeutic candidate for alleviating diastolic dysfunction in DHF.

Key words: Parvalbumin — Calcium handling — Diastolic heart failure

Cardiac diastolic dysfunction and calcium handling

Congestive heart failure (CHF) is a clinical syndrome of compromised pumping function of the heart. Diastolic dysfunction can dominate the early phase of CHF before significant decrease in cardiac output occurs. Moreover, about 40% of the CHF patients experience isolated diastolic heart failure (DHF) in the absence of significant systolic dysfunction (Chinnaiyan et al. 2007; Owan and Redfield 2005). Importantly, DHF is more prominent among elderly population (Kitzman et al. 2001). Mechanistically, DHF is a chronic condition that involves one or more pathological abnormalities of the myocardium, including impaired cardiac myocyte relaxation, increased ventricular wall stiffness / thickness and restricted pericardial structures. These pathological conditions are often caused by dysregulated intracellular Ca^{2+} handling, myocyte hypertrophy, extracellular collagen deposition, elevated afterload, and confined ventricular wall movements (Zile and Brutsaert 2002). Defective Ca^{2+} removal is a key factor underlying the pathogenesis of DHF. Studies in human and animals suggest that delayed Ca^{2+} sequestration is an important factor leading to slowed Ca^{2+} transient decay and myocyte relaxation as well as elevated diastolic Ca^{2+} levels (Houser et al. 2000; Zile and Brutsaert 2002).

Normal cardiac function relies on seamlessly coupled processes of myocyte contraction and relaxation driven by oscillations in cytosolic Ca^{2+}. Timely control of intracellular Ca^{2+} levels requires the released Ca^{2+} be removed promptly. The decay of the Ca^{2+} transient involves sequential processes including Ca^{2+} dissociation from troponin C, Ca^{2+} reuptake into sarcoplasmic reticulum (SR) by phospholamban (PLN) regulated sarcoplasmic (endoplasmic) reticulum Ca^{2+} ATPase (SERCA) and Ca^{2+} export through Na^{+}/Ca^{2+} exchanger (NCX). The function and abundance of these key Ca^{2+} cycling molecules are known to be altered during DHF. Such alterations include decreased SERCA2a expression and activity, decreased SERCA2a/PLN ratio, up-regulated NCX and increased ryanodine receptor (RyR) open probability and Ca^{2+} leak (Arai et al. 1994; Houser et al. 2000; Wehrens et al. 2005). These changes ultimately lead to delayed Ca^{2+} removal, diminished Ca^{2+} storage and release, elevated resting cytosolic Ca^{2+}, and increased vulnerability to arrhythmia (Minamisawa et al. 2004; Molkentin 2005; Pieske et al. 1999).

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A direct gene transfer approach that manipulates Ca^{2+} regulating proteins has been used to develop new therapies for diastolic dysfunction. Promising results in rodents have been obtained from studies such as overexpression of SERCA2a, sorcin and S100A1 or depletion of PLN, in which diastolic and systolic dysfunction are improved (del Monte and Hajjar 2003; Hoshijima 2005; Minamisawa et al. 1999; Most et al. 2004). However, several critical issues remain unsolved regarding the effectiveness of targeting endogenous Ca^{2+} cycling process in DHF. For instance, NCX has been shown to be elevated in HF, but experimental NCX overexpression exerts either beneficial or deleterious effects (Schillinger et al. 2000; Terracciano et al. 1998). The results of sorcin gene transfer in regulating contractility, relaxation and SR Ca^{2+} content are conflicting among studies (Seidler et al. 2003; Suarez et al. 2004). SERCA2a gene transfer may cause diminished responsiveness to β-adrenergic stimulation (Hirsch et al. 2004), due to imbalance between SERCA and PLN. Further, energy insufficiency, a common complication of HF (Neubauer 2007), may significantly hamper the effects of these approaches, since they rely on the energy consuming process of Ca^{2+} recycling.

Parvalbumin: Delayed Calcium Buffer Ameliorates Cardiac Diastolic Dysfunction

Parvalbumin (Parv), a 11-kDa EF-hand Ca^{2+} binding protein, has recently been identified as an energy-efficient approach to treat DHF. Parv contains three highly conserved helix-loop-helix EF-hand Ca^{2+} binding motifs with the two C-terminal motifs functioning in physiological metal binding. Both motifs show high affinity for Ca^{2+} ($K_{Ca}{}^{2+}$ = 10^7-10^9 M^{-1}) and moderate-low affinity for Mg^{2+} ($K_{Mg}{}^{2+}$ = 10^3-10^5 M^{-1}) (Pauls et al. 1996). Since Ca^{2+} competes with Mg^{2+} to bind Parv, Parv's actual Ca^{2+} binding is critically influenced by several factors, including the relative concentrations of Mg^{2+} versus Ca^{2+} and the association and dissociation rates of these metals. In mammals, abundant Parv exists in fast skeletal muscles, where Parv plays a critical role in fast twitch muscle relaxation (Muntener et al. 1995; Schwaller et al. 1999). Parv is modeled as a "delayed" Ca^{2+} buffer (Coutu P. et al. 2003) that binds Ca^{2+} in diastole rather than in the systolic phase of muscle contraction, thus facilitating removal of Ca^{2+} from the myoplasm after force generation (Schwaller et al. 1999).

We and others have employed ectopic gene transfer of Parv in cardiac muscle and evaluated Parv's role in regulating cardiac Ca^{2+} cycling, contraction and relaxation. By using adenovirus-mediated gene transfer techniques, Parv transduces adult cardiac myocytes with high efficiency (Coutu et al. 2004; Hirsch et al. 2004; Wahr et al. 1999). *In vitro* and *in vivo* functional assessments have been carried out to verify Parv's effects on cardiac performance. Parv dramatically increases the rate of Ca^{2+} transient decay and accelerates myocyte mechanical relaxation in a dose-dependent manner in normal adult cardiac myocytes from rodents and canines (Coutu et al. 2004; Hirsch et al. 2004; Rodenbaugh et al. 2007; Wahr et al. 1999). Parv expressing myocytes also retain their ability to fully respond to β-adrenergic stimulation (Hirsch et al. 2004). *In vivo* gene transfer of Parv via direct intra-myocardial injection achieves physiologically relevant concentrations of Parv in vivo (Coutu et al. 2004; Michele et al. 2004; Szatkowski et al. 2001). Parv expressing hearts exhibit increased relaxation speed measured by working heart strip preparation, *in vivo* micromanometry and echocardiography (Szatkowski et al. 2001). In aged rats, *in vivo* Parv gene transfer has a reduced efficiency, yet Parv still corrects aging-related slow cardiac relaxation (Michele et al. 2004; Schmidt et al. 2005).

Parv's beneficial effect on myocyte relaxation can be fine-tuned by altering several factors, such as the cytosolic expressing level of Parv and Parv's relative metal binding affinities. We have developed a mathematical model that incorporates Parv-based Ca^{2+} buffering into cardiac E-C coupling (Coutu et al. 2003). The model emphasizes the delayed Ca^{2+} binding feature of Parv and accurately simulates results of experimental data (Coutu and Metzger 2002). In addition to providing increased understanding of Parv's role in cardiac Ca^{2+} regulation, the model also helps to determine the optimal range of Parv's concentration in cardiac myocytes, which is estimated to be ~10-100 μM (Coutu et al. 2003; Coutu and Metzger 2002, 2005). Within this range, Parv's Ca^{2+} buffering is primarily confined within the relaxation phase with minimal impact on Ca^{2+} transient amplitude. As a result, contractile parameters remain unaffected while diastolic relaxation is accelerated (Szatkowski et al. 2001). Since the exact expression level of Parv is an important variable to control, manipulating Parv's metal binding affinities and kinetics may offer an alternative approach to optimize Parv's function. For example, two naturally existing Parv isoforms, α and β Parv, which have distinct Ca^{2+}/Mg^{2+} binding affinities, demonstrate different efficiencies in buffering Ca^{2+} and accelerating cell relaxation (Rodenbaugh et al. 2007). The higher Ca^{2+} binding affinity and lower Mg^{2+} binding affinity enables β Parv twice as potent as α Parv in accelerating Ca^{2+} transient decay and myocyte relaxation (Rodenbaugh et al. 2007). Thus, biochemical properties of Parv represent targets for determining Parv's physiological functions in cardiac myocytes. Through designing metal binding properties of Parv, gain-of-function may be achieved, in which a much lower expressing level of Parv can have similar or even better ability to improve cardiac performance.

The effectiveness of Parv in accelerating Ca^{2+} transient decay and myocyte relaxation has been tested in a number of studies using DHF animal models (Wang and Metzger

2008). First, acute Parv gene transfer accelerates the slow relaxation in senescent cardiac myocytes (Huq et al. 2004; Michele et al. 2004). A similar Parv gene transfer procedure also restored the impaired relaxation function of myocytes from hypothyroid or hypertensive DHF rats (Rodenbaugh et al. 2007; Wahr et al. 1999). In a cell model of HCM, generated by expressing mutant α-tropomyosin in cultured adult cardiac myocytes, Parv gene transfer increased Ca^{2+} removal rate and reversed the characteristic slow relaxation of HCM (Coutu et al. 2004). Further, in a canine HF model, Parv gene transfer had comparative efficacy in hastening myocyte relaxation as SERCA gene transfer (Hirsch et al. 2004). *In vivo* Parv gene transfer also demonstrated efficacy in studies using various DHF models (Michele et al. 2004; Sakata et al. 2007; Schmidt et al. 2005; Szatkowski et al. 2001). Importantly, Parv gene transfer has been shown to augment myocyte energy utilization into a more efficient state in the failing heart (Sakata et al. 2007). Taken together, Parv accelerates relaxation in a variety of cellular and animal models of diastolic dysfunction indicating its potential in translating into therapies for HF patient.

Conclusions

Gene transfer strategies can effectively introduce Ca^{2+} regulatory proteins in myocytes to remediate DHF. Ectopic expression of the skeletal muscle Ca^{2+} binding protein parvalbumin in cardiac muscle achieves improved cardiac diastolic function under both normal and disease conditions. The delayed Ca^{2+} buffering feature of wild-type Parv enables it to specifically target diastolic rather than systolic Ca^{2+} under controlled expression conditions. Alternatively, distinct Parv isoforms in cardiac myocytes demonstrates the rationale of tuning Parv's biological effects through manipulating its metal binding affinity and expression level. The low energy requirement of Parv in Ca^{2+} buffering is an additional advantage for Parv's usage in energy-deprived failing cardiac myocytes. It should be noted that buffering intracellular Ca^{2+} may exert effects beyond cell contraction and relaxation. It is possible that Parv, through regulating cytosolic free Ca^{2+} concentration, may influence overall intracellular ion regulation and/or sustained Ca^{2+} signaling, affecting diverse cell processes ranging from action potential generation to cell growth and cell death. Further studies are needed to determine whether the results obtained in adult cardiac myocytes and in small mammals can be effectively translated into the complexities of diseased heart of larger animals and humans.

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