Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 369 (2008) 182–187
BBRC
www.elsevier.com/locate/ybbrc

The Ca^{2+} ATPase of cardiac sarcoplasmic reticulum: Physiological role and relevance to diseases

Giuseppe Inesi *, Anand Mohan Prasad, Rajendra Pilankatta

California Pacific Medical Center Research Institute, 475 Brannan Street, San Francisco, CA 94107, USA

Received 27 September 2007
Available online 20 December 2007

Abstract

The Ca^{2+} ATPase of sarcoplasmic reticulum has a prominent role in excitation/contraction coupling of cardiac muscle, as it induces relaxation by sequestering Ca^{2+} from the cytoplasm. The stored Ca^{2+} is in turn released to trigger contraction. We review here experiments demonstrating that in cardiac myocytes Ca^{2+} signaling and contractile activation are strongly altered by pharmacological inhibition or transcriptional down-regulation of SERCA. On the other hand, kinetics, and intensity of Ca^{2+} signaling are improved by SERCA overexpression following delivery of exogenous cDNA by adenovirus vectors. Experiments on adrenergic hypertrophy demonstrate SERCA down-regulation, consistent with its pathogenetic involvement in cardiac hypertrophy and failure, as also shown in other experimental models and clinical studies. Compensation by alternate Ca^{2+} signaling proteins, including functional activation and increased expression of Na^{+}/Ca^{2+} exchanger and TRPC proteins has been observed. These compensatory mechanisms, including calcineurin activation, remain to be clarified and are a most important subject of current studies.

Keywords: Ca^{2+} transport; Sarcoplasmic reticulum; Ca^{2+} signaling in cardiac muscle; Excitation–contraction coupling; Adrenergic hypertrophy; SERCA as a pathogenetic factor in cardiac diseases

On occasion of Professor Ebashi's visit to the Cardiovascular Research Institute of the University of California in San Francisco, the earliest preparations of vesicular fragments of cardiac sarcoplasmic reticulum (SR) were obtained under his guidance [1]. A warm memory remains with us of Professor Ebashi's influence and guidance through many hours in the laboratory, continuing at the end of day with stimulating and inspiring discussions at dinner in various San Francisco restaurants. At that time, SR membrane vesicles were referred to as "Relaxing Factor" since they prevented "superprecipitation" (i.e., contraction analog) of native actomyosin (containing myosin, actin, and the troponin complex) upon addition of ATP. It was soon established that this effect was produced by ATP dependent sequestration of Ca^{2+} by the vesicles from the reaction medium. In fact, the same relaxing effect could be produced simply by Ca^{2+} chelation with EGTA added to the medium. The functional behavior of isolated cardiac SR vesicle *in vitro* turned out to be similar to that exhibited by SR vesicles isolated from skeletal muscle [2,3], due to the Ca^{2+} activated Sarco-Endoplasmic Reticulum ATPase (SERCA) which is a prominent protein component of both preparations. The catalytic and transport cycle of SERCA includes ATP utilization by formation of a phosphorylated enzyme intermediate, translocation of bound Ca^{2+} across the SR membrane against a concentration gradient, and final hydrolytic cleavage of Pi from the phosphoenzyme intermediate [4]. We review here information presently available on the role of SERCA in physiology and diseases of heart muscle, discovered by several laboratories working in the field, and illustrated by experimental results obtained in our own laboratory.

* Corresponding author. Fax: +1 415 6001725.
E-mail address: ginesi@cpmcri.com (G. Inesi).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.11.161

SERCA and Ca^{2+} signaling in cardiac muscle

The cardiac SR ATPase (SERCA2a) is encoded by the (human nomenclature) ATP2A2 gene, which also yields the SERCA2b isoform found in the endoplasmic reticulum of most cells [5,6]. It is by now well-established that SERCA2 plays an important role in relaxation of heart muscle, as it sequesters cytosolic Ca^{2+} by active transport into intracellular stores delimited by the SR membranes. The stored Ca^{2+} is in turn released into the cytoplasm upon membrane excitation to induce contraction. This can be convincingly demonstrated on primary cultures of cardiac myocytes, as they provide a rather simple system to study complementary features of SERCA transport activity and cytosolic Ca^{2+} signaling.

The cytosolic Ca^{2+} signaling transients observed in myocytes subjected to electric stimuli include a rapid rise followed by a slower decay (Fig. 1). The rapid Ca^{2+} rise does not show significant dependence on temperature, as expected of passive flux through a channel. On the contrary, the rate of decay exhibits strong temperature dependence, similar to that exhibited by the transport activity of isolated SR vesicles (Fig. 1). This parallel behavior is consistent with dependence of the decay phase of Ca^{2+} transients on active transport by the SERCA enzyme [7].

The dependence of cytosolic Ca^{2+} signaling on transport by SERCA can be demonstrated directly by inhibiting its catalytic and transport activity [8] with thapsigargin (TG), a plant derived sesquiterpene lactone. Specific inhibition of SERCA is obtained *in situ* by exposing cardiac myocytes to nanomolar concentrations of TG in the culture medium (Fig. 2). Such low concentrations of TG inhibit specifically SERCA activity, without apparent effects on cell growth or other cell characteristics [9]. An alternative experimental approach is down-regulation of SERCA expression by the use of short interfering RNA [10], with consequent reduction of overall transport activity (Fig. 2). Additional evidence is obtained in myocytes subjected to adrenergic hypertrophy [9], as significant reduction of SERCA expression is produced relative to general protein expression. In all cases, reduction of Ca^{2+} transport activity, due to either SERCA inhibition or down-regulation, results in pronounced alterations of cytosolic transients and marked slowing of the decay phase (Fig. 3). It is of interest that SERCA inhibition with TG in myocytes, is followed by compensatory increase in Na^+/Ca^{2+} exchange activity (Fig. 4), as a compensatory mechanism to eliminate cytosolic Ca^{2+} through the plasma membrane. In fact, if Na^+/Ca^{2+} exchange is prevented by placing the myocytes in Na^+ free medium, the basal ("diastolic") level of cytosolic Ca^{2+}, between consecutive stimuli, is progressively increased (Fig. 4).

As a further demonstration of SERCA involvements in Ca^{2+} transient kinetics, faster decay of the transients can be obtained by SERCA overexpression, encoded by exogenous cDNA. For this purpose, we have used adenoviral vectors for efficient introduction of exogenous SERCA cDNA in cardiac myocytes, under control of constitutive or cardiac cell specific promoters [11]. Most importantly,

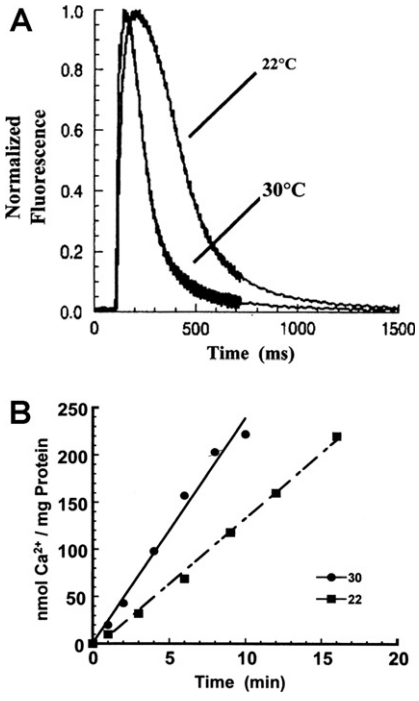

Fig. 1. Temperature dependence of cytosolic Ca^{2+} transients and active Ca^{2+} transport by cardiac SR vesicles. The Ca^{2+} transients were recorded using a fluorescence indicator in cultured neonatal rat myocytes (A). ATP dependent Ca^{2+} active transport (B) was measured with homogenized myocytes using radioactive tracer [7].

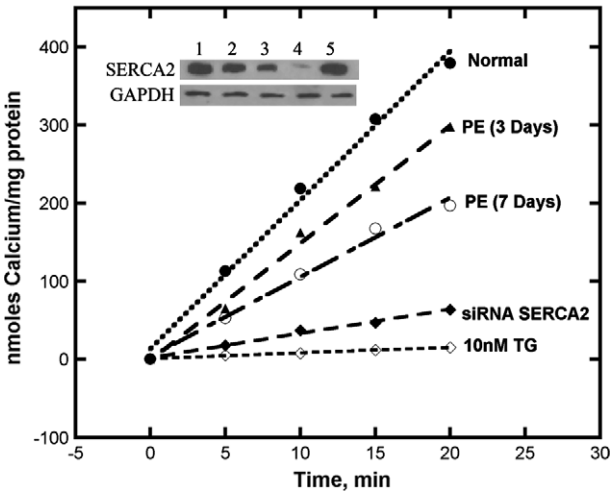

Fig. 2. Protein levels and ATP dependent Ca^{2+} transport in homogenates of myocytes exposed to 20 μM PE, siSERCA2 or 10 nM TG. Control myocytes [1] are compared to myocytes exposed to PE for three [2] or seven days [3], or siSERCA2 for three days [4], or 10 nM TG [5] for three days. Protein levels were measured by Western blotting and compared to the levels of GAPDH protein. ATP dependent calcium transport was measured using homogenates of cultured cardiac myocytes, harvested after three or seven days of treatment as specified above [9].

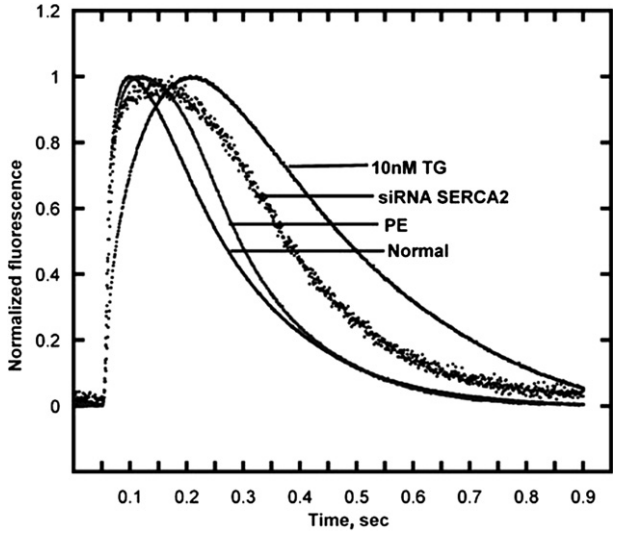

Fig. 3. Alteration of Ca^{2+} signaling kinetics in myocytes exposed to PE, siSERCA2 or TG. The myocytes were exposed to 20 μM PE for seven days, or to siSERCA2 for three days, or to 10 nM TG for three days. Cytosolic Ca^{2+} transients were measured in single cells using fluo-4. The cells were subjected to field stimulation (1 Hz pulsing). Each trace represents the average of transients obtained from 30 to 70 cells over five different preparations. The fluorescence signal was normalized to the maximal change per each transient [9].

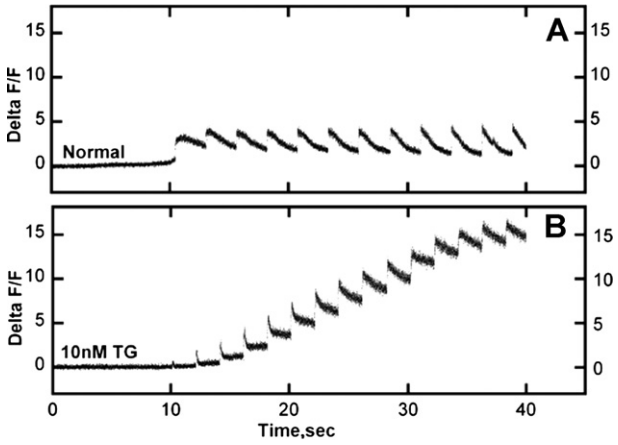

Fig. 4. Compensatory increase in Na^+/Ca^{2+} exchange activity following SERCA inhibition by TG in cardiac myocytes. Control myocytes (A) and myocytes exposed to 10 nM TG for three days (B) were subjected to a series of voltage clamp depolarizations to −10 mV at 2 s intervals. Ca^{2+} transients were then measured in Na^+ free bathing solution, thereby eliminating Na^+/Ca^{2+} exchange. It is clear that, in the absence of Na^+/Ca^{2+} exchange, the myocytes with inhibited SERCA show a progressive increase of the basal cytosolic Ca^{2+} level as a consequence of consecutive stimuli [9].

it can be shown by the use of diverse SERCA isoforms and specific antibodies [12], that the protein encoded by the exogenous cDNA is targeted exactly to the same membrane location as the protein encoded by the endogenous gene (Fig. 5). In all cases, the resulting effect on Ca^{2+} transients is a shorter decay phase, as expected from a faster Ca^{2+} removal from the cytoplasm (Fig. 6).

Physiologic and pathogenetic role of SERCA in excitation–contraction coupling

An intriguing question is whether, in addition to kinetic effects, the maximal level of Ca^{2+} stored within the lumen of the SR is affected by changes in overall SERCA activity. Assuming the same internal volume delimited by the SR membrane, the maximal Ca^{2+} concentration that can be possibly reached in the lumen of SR depends on Ca^{2+} dissociation constant from the ATPase inward oriented Ca^{2+} binding sites following utilization of ATP. In principle, this limit is independent of the overall Ca^{2+} transport velocity. On the other hand, if the frequency of consecutive Ca^{2+} release stimuli is increased, the time interval between stimuli becomes a limiting factor, and the overall transport velocity then determines the level of maximal filling. This is in fact observed when myocytes are subjected to trains of stimuli with progressively shorter intervals, whereby the amount of released Ca^{2+} becomes progressively smaller, as it is limited by the luminal Ca^{2+} load reached within the short interval. In this case, compensation is obtained by overexpression of exogenous SERCA, as a greater number of SERCA molecules allows a higher overall transport velocity and a more effective filling of the SR lumen within a limited time interval between stimuli (Fig. 7).

With regard to the relevance of Ca^{2+} transport to contractile activity, it has been clearly shown that specific inhibition of SERCA by TG *in situ*, interferes [13] with the contractile response of cardiac myocytes to membrane excitation. This effect is certainly relevant to cardiac pathology. In fact, many experimental and clinical studies indicate that inadequate function of the Ca^{2+} transport ATPase (SERCA2) and the consequent alterations of cytosolic Ca^{2+} signaling are pathogenetic features of cardiac hypertrophy and failure [14–24]. Even expression of SERCA isoforms with higher than normal Ca^{2+} affinity can lead to cardiac hypertrophy and failure [25].

An important feature of SERCA2 is its regulation by phospholamban in cardiac muscle, as originally described by Kirchberger et al. [26] and subsequently studied extensively in several laboratories, especially by E.G. Kranias [27]. Phospholamban is a small protein that interacts with the transmembrane and stalk regions of the SERCA protein. The functional effect resulting from this interaction is a displacement of the ATPase activation curve to a higher Ca^{2+} concentrations range, therefore yielding lower activity at relevant cytosolic Ca^{2+} concentrations [28]. This effect is overcome by phospholamban phosphorylation following adrenergic activation of protein kinase, thereby providing an elegant explanation for the increased cardiac contraction upon sympathetic discharge. Numerous and elegant studies with transgenic animals have shown convincingly that phospholamban is a key determinant of cardiac function and dysfunction [29]. In addition, specific mutations in the human phospholamban gene may result in severe cardiomyopathies [30].

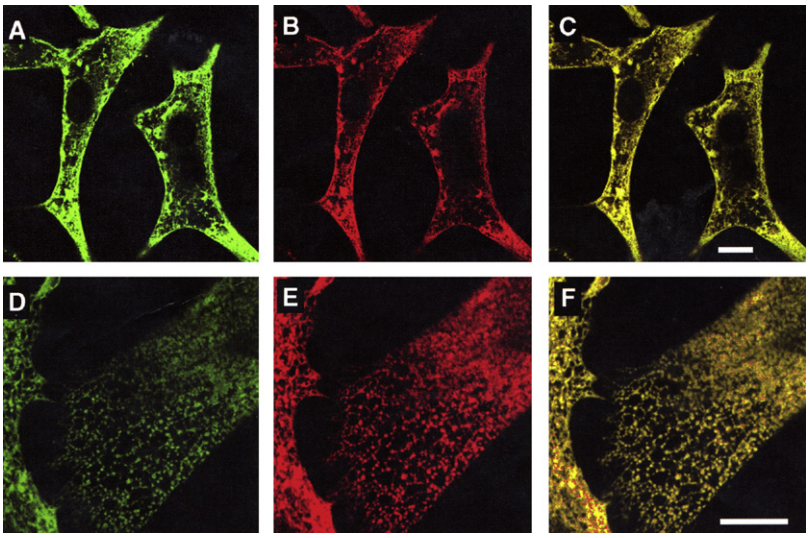

Fig. 5. Intracellular targeting of SERCA1 gene product following delivery of exogenous cDNA by means of adenovirus vector under control of cardiac cell specific promotor. Colocalization of exogenous SERCA1 with endogenous SERCA2 is demonstrated by specific immunostaining for endogenous SERCA2 (A and D), exogenous SERCA 1 (B and E), and combined staining for SERCA2 and SERCA1 (C and F). Scale bars correspond to 10 μm [12].

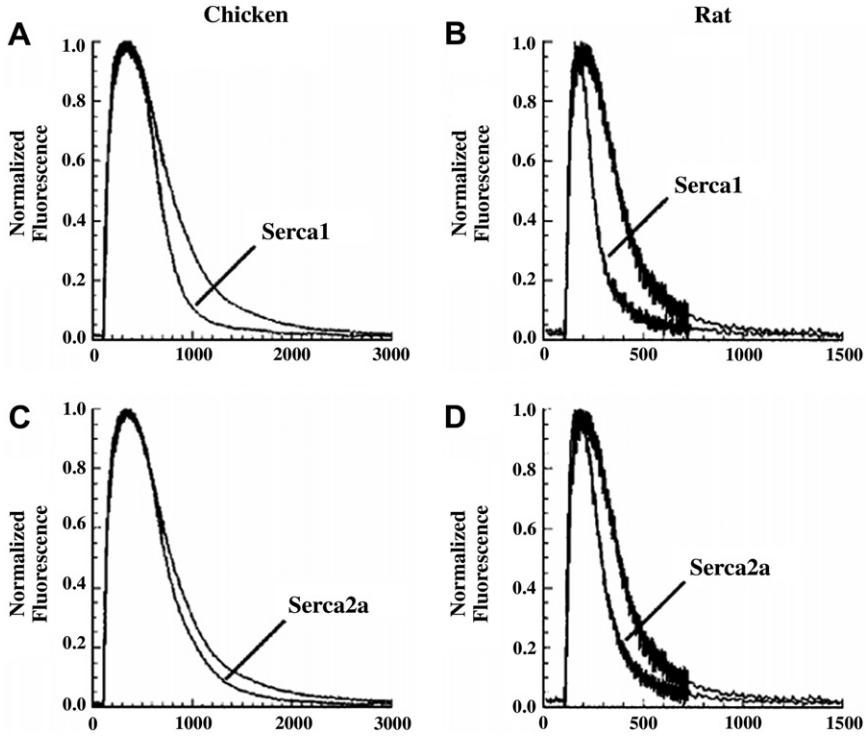

Fig. 6. Ca^{2+} signaling transients in control cardiac myocytes, and following overexpression of exogenous SERCA. Cytosolic Ca^{2+} transients were measured in chicken embryo or neonatal rat myocytes using fluo-4. (A–D) Myocytes overexpressing exogenous SERCA1 or SERCA2 (following infection with adenovirus vectors) are compared with control myocytes. The cells were subjected to field stimulation (1 Hz pulsing). Each trace represents the average of transients obtained from 15–30 cells over 3–5 different preparations. The fluorescence signal was normalized to the maximal change per each transient [12].

The evidence indicating that deficiencies of SERCA2 expression, function and regulation may be a significant factor in the pathogenesis of cardiac hypertrophy and failure [14–24], has led to attempts to relieve related shortcomings by over-expression through introduction of *exogenous* SERCA cDNA [31–37]. On the other hand, it is now becoming apparent that regulation of *endogenous* expression is an important factor to be considered. In the experimental setting, it is clear that myocytes undergoing adrenergic hypertrophy [38] under-express SERCA, as the enzyme does not seem to be included in the hypertrophy transcriptional program [9]. In addition, alterations

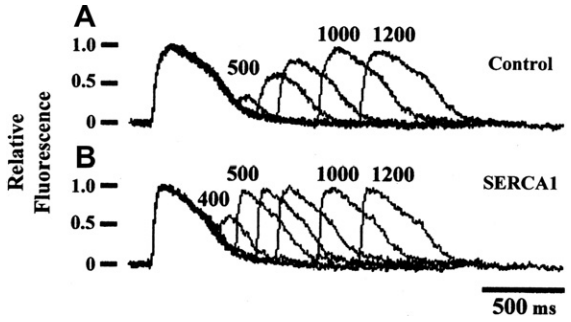

Fig. 7. Ca^{2+} signaling transients (A) following stimuli at progressively shorter intervals in control myocytes and following overexpression of exogenous SERCA. It is shown that the amount of released Ca^{2+} is reduced (B) as the interval between consecutive stimuli is shorter, indicating a time limit for Ca^{2+} refilling of SR stores between stimuli. This limit is significantly overcome by overexpression of exogenous SERCA1 [7].

of cytosolic Ca^{2+} homeostasis trigger over-expression of other Ca^{2+} handling proteins such as Na^{+}/Ca^{2+} exchanger and the TRPC proteins [10]. Calcineurin, a Ca^{2+}/calmodulin activated phosphatase and transcriptional activator [39,40] that plays an important role in heart development and remodeling [41,42], is likely to be involved in adaptive responses to alterations of cytosolic Ca^{2+} homeostasis [43–45]. However, the consequences of its activation are likely to be interwoven with additional mechanisms of transcriptional regulation [46] that may enhance or counteract the overall effect, directing it to expression of specific proteins, and excluding others. Presently, clarification of adaptive mechanisms for up- and down-regulation of endogenous SERCA and other Ca^{2+} handling proteins is a most promising endeavor in order to gain understanding and hopefully improving treatment of cardiac hypertrophy and failure.

Acknowledgments

Continuous support by the National Institutes of Health (Heart, Blood, and Lung Institute) is gratefully acknowledged.

References

[1] G. Inesi, S. Ebashi, S. Watanabe, Preparation of vesicular relaxing factor from bovine heart muscle, Am. J. Physiol. 207 (1964) 1339–1344.
[2] S. Ebashi, F. Lipmann, Adenosine-triphosphate linked concentration of calcium ions in a particulate fraction of rabbit muscle, J. Cell. Biol. 14 (1962) 389–400.
[3] W. Hasselbach, M. Makinose, ATP and active transport, Biochem. Biophys. Res. Commun. 7 (1962) 132–136.
[4] G. Inesi, C. Sumbilla, M.E. Kirtley, Relationships of molecular structure and function in Ca^{2+}-transport ATPase, Physiol. Rev. 70 (1990) 749–760.
[5] J. Lytton, A. Zarain-Herzberg, M. Periasamy, D.H. MacLennan, Molecular cloning of the mammalian smooth muscle sarco(endo)plasmic reticulum Ca^{2+}-ATPase, J. Biol. Chem. 264 (1989) 7059–7065.
[6] A. Zarain-Herzberg, D.H. MacLennan, M. Periasamy, Characterization of rabbit cardiac sarco(endo)plasmic reticulum Ca^{2+}-ATPase gene, J. Biol. Chem. 265 (1990) 4670–4677.
[7] M. Cavagna, J.M. O'Donnell, C. Sumbilla, G. Inesi, M.G. Klein, Exogenous Ca^{2+}-ATPase isoform effects on Ca^{2+} transients of embryonic chicken and neonatal rat cardiac myocytes, J. Physiol. 528 (2000) 53–63.
[8] Y. Sagara, G. Inesi, Inhibition of the sarcoplasmic reticulum Ca^{2+} transport ATPase by thapsigargin at subnanomolar concentrations, J. Biol. Chem. 266 (1991) 13503–13506.
[9] A.M. Prasad, H. Ma, C. Sumbilla, D.I. Lee, M.G. Klein, G. Inesi, Phenylephrine hypertrophy Ca^{2+}-ATPase (SERCA2), and Ca^{2+} signaling in neonatal rat cardiac myocytes, Am. J. Physiol. Cell Physiol. 292 (2007) C2269–C2275.
[10] M. Seth, C. Sumbilla, S.P. Mullen, D. Lewis, M.G. Klein, A. Hussain, J. Soboloff, D.L. Gill, G. Inesi, Sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA) gene silencing and remodeling of the Ca^{2+} signaling mechanism in cardiac myocytes, Proc. Natl. Acad. Sci. USA 101 (2004) 16683–16688.
[11] G. Inesi, D. Lewis, C. Sumbilla, A. Nandi, C. Strock, K.W. Huff, T.B. Rogers, D. C Johns, P.D. Kessler, C.P. Ordahl, Cell-specific promoter in adenovirus vector for transgenic expression of SERCA1 ATPase in cardiac myocytes, Am. J. Physiol. 274 (1998) C645–C653.
[12] H. Ma, C. Sumbilla, I.K. Farrance, M.G. Klein, G. Inesi, Cell-specific expression of SERCA the exogenous Ca^{2+} transport ATPase in cardiac myocytes, Am. J. Physiol. 286 (2004) C556–C564.
[13] M.S. Kirby, Y. Sagara, S. Gaa, G. Inesi, W.J. Lederer, T.B. Rogers, Thapsigargin inhibits contraction and Ca^{2+} transient in cardiac cells by specific inhibition of the sarcoplasmic reticulum Ca^{2+} pump, J. Biol. Chem. 267 (1992) 12545–12551.
[14] K.R. Chien, Meeting Koch's postulates for calcium signaling in cardiac hypertrophy, J. Clin. Invest. 105 (2000) 1339–1342.
[15] D.M. Bers, D.A. Eisner, H.H. Valdivia, Sarcoplasmic reticulum Ca^{2+} and heart failure: roles of diastolic leak and Ca^{2+} transport, Circ. Res. 93 (2003) 487–490.
[16] M. Zheng, K. Dilly, C.J. Dos Santos, M. Li, Y. Gu, J.A. Ursitti, J. Chen, J. Ross Jr., K.R. Chien, J.W. Lederer, Y. Wang, Sarcoplasmic reticulum calcium defect in Ras-induced hypertrophic cardiomyopathy heart, Am. J. Physiol. 286 (2004) H424–H433.
[17] J.J. Schultz, B.J. Glascock, S.A.S.A. Witt, M.L. Nieman, K.J. Nattamai, L.H. Liu, J.N. Lorenz, G.E. Shull, T.R. Kimball, M. Periasamy, Accelerated onset of heart failure in mice during pressure overload with chronically decreased SERCA2 calcium pump activity, Am. J. Physiol. 286 (2004) H1146–H1153.
[18] H. Okayama, M. Hamada, H. Kawakami, S. Ikeda, H. Hashida, Y. Shigematsu, K. Hiwada, Alterations in expression of sarcoplasmic reticulum gene in Dahl rats during the transition from compensatory myocardial hypertrophy to heart failure, J. Hypertens. 15 (1997) 1767–1774.
[19] M. Arai, N.R. Alpert, D.H. MacLennan, P. Barton, M. Periasami, Alterations in sarcoplasmic reticulum gene expression in human heart failure A possible mechanism for alterations in systolic and diastolic properties of the failing myocardium, Circ. Res. 72 (1993) 463–469.
[20] G. Hasenfuss, M. Meyer, W. Schillinger, M. Preuss, B. Pieske, H. Just, Calcium handling proteins in the failing human heart, Basic Res. Cardiol. 92 (Suppl. 1) (1997) 87–93.
[21] G. Hasenfuss, H. Reinecke, R. Studer, M. Meyer, B. Pieske, J. Holtz, C. Holubarsch, H. Posival, H. Just, H. Drexler, Relation between myocardial function and expression of sarcoplasmic reticulum Ca^{2+} ATPase in failing and nonfailing human myocardium, Circ. Res. 75 (1994) 434–442.
[22] M. Meyer, W. Schillinger, B. Pieske, C. Holubarsch, C. Heilmann, H. Posival, G. Kuwajima, K. Mikoshiba, H. Just, G. Hesenfuss, Alterations of sarcoplasmic reticulum proteins in failing human dilated cardiomyopathy, Circulation 92 (1995) 778–784.
[23] S.H. Lehnart, W. Schillinger, B. Pieske, J. Prestle, H. Just, G. Hasenfuss, Sarcoplasmic reticulum proteins in heart failure, Ann. N.Y. Acad. Sci. 853 (1998) 220–230.

[24] J.J. Mercadier, A.M. Lompre, P. Duc, K.R. Boheler, J.B. Fraysse, C. Wisnewsky, P.D. Allen, M. Komajda, K. Schwartz, Altered sarcoplasmic reticulum Ca^{2+} ATPase gene expression in the human ventricle during end-stage heart failure, J. Clin. Invest. 85 (1990) 305–309.

[25] P. Vangheluwe, M. Tjwa, A. Van Den Bergh, W.E. Louch, M. Beullens, L. Dode, P. Carmeliet, E. Kranias, P. Herijgers, K.R. Sipido, L. Raeymaekers, F. Wuytack, A SERCA2 pump with an increased Ca^{2+} affinity can lead to severe cardiac hypertrophy stress intolerance and reduced life span, J. Mol. Cell. Cardiol. 41 (2006) 308–317.

[26] M.A. Kirchberger, M. Tada, A.M. Katz, Phospholamban: a regulatory protein of the cardiac sarcoplasmic reticulum, Recent Adv. Stud. Cardiac Struct. Metab. 5 (1975) 103–115.

[27] D.H. MacLennan, E.G. Kranias, Phospholamban: a crucial regulator of cardiac contractility, Nat. Rev. Mol. Cell Biol. 4 (2003) 566–577.

[28] T. Cantilina, Y. Sagara, G. Inesi, L.R. Jones, Comparative studies of cardiac and skeletal sarcoplasmic reticulum ATPases Effect of a phospholamban antibody on enzyme activation by Ca^{2+}, J. Biol. Chem. 268 (1993) 17018–17025.

[29] P. Rodriguez, E.G. Kranias, Phospholamban: a key determinant of cardiac function and dysfunction, Arch. Mal. Coeur Vaiss. 98 (2005) 1239–1243.

[30] K. Haghighi, K. Kolokathis, A.O. Gramolini, J.R. Waggoner, L. Pater, R.A. Lynch, G.C. Fan, D. Tsiapras, R.R. Parekh, G.W. Dorn 2nd, D.H. MacLennan, D.T. Kremastinos, E.G. Kranias, A mutation in the human phospholamban gene, deleting arginine 14, results in lethal, hereditary cardiomyopathy, Proc. Natl. Acad. Sci. USA 103 (2006) 1388–1393.

[31] F. Del Monte, R.J. Hajjar, Targeting calcium cycling proteins in heart failure through gene transfer, J. Physiol. 546 (2003) 49–61.

[32] F. Del Monte, E. Williams, D. Lebeche, U. Schmidt, A. Rosenzweig, J.K. Gwathmey, E.D. Lewandowski, R.J. Hajjar, Improvement in survival and cardiac metabolism after gene transfer of sarcoplasmic reticulum Ca^{2+}-ATPase in a rat model of heart failure, Circulation 104 (2001) 1424–1429.

[33] M. Flesch, R.H. Schwinger, P. Schnabel, F. Schiffer, I. van Gelder, U. Bavendiek, M. Sudkamp, F. Kuhn-Regnier, M. Bohm, Sarcoplasmic reticulum Ca^{2+} ATPase and phospholamban mRNA and protein levels in end stage heart failure due to ischemic or dilated cardiomyopathy, J. Mol. Med. 74 (1996) 321–332.

[34] F.J. Giordano, H. He, P. McDonough, M. Meyer, M.R. Sayen, W.H. Dillmann, Adenovirus-mediated gene transfer reconstitutes depressed sarcoplasmic reticulum Ca^{2+}-ATPase levels and shortens prolonged cardiac myocyte Ca^{2+} transients, Circulation 96 (1997) 400–403.

[35] H. He, F.J. Giordano, R. Hilal-Dandan, D.J. Choi, H.A. Rockman, P.M. McDonough, W.F. Bluhm, M. Meyer, M.R. Sayen, E. Swanson, W.H. Dillmann, Overexpression of the rat sarcoplasmic reticulum Ca^{2+} ATPase gene in the heart of transgenic mice accelerates calcium transients and cardiac relaxation, J. Clin. Invest. 100 (1997) 380–389.

[36] E. Loukianov, Y. Ji, I.L. Grupp, D.L. Kirkpatrick, D.L. Baker, T. Loukianova, G. Grupp, J. Lytton, R.A. Walsh, M. Periasamy, Enhanced myocardial contractility and increased Ca^{2+} transport function in transgenic hearts expressing the fast-twitch skeletal muscle sarcoplasmic reticulum Ca^{2+}-ATPase, Circ. Res. 83 (1998) 889–897.

[37] M.I. Miyamoto, F. del Monte, U. Schmidt, T.S. DiSalvo, Z.B. Kang, T. Matsui, J.L. Guerrero, J.K. Gwathmey, A. Rosenzweig, R.J. Hajjar, Adenoviral gene transfer of SERCA2a improves left-ventricular function in aortic-banded rats in transition to heart failure, Proc. Natl. Acad. Sci. USA 97 (2000) 793–798.

[38] P. Simpson, A. McGrath, S. Savion, Myocyte hypertrophy in neonatal rat heart cultures and its regulation by serum and by catecholamines, Circ. Res. 51 (1982) 787–801.

[39] C.B. Klee, H. Ren, X. Wang, Regulation of the calmodulin-stimulated protein phosphatase calcineurin, J. Biol. Chem. 273 (1998) 13367–13370.

[40] G.R. Crabtree, Calcium calcineurin and the control of transcription, J. Biol. Chem. 276 (2001) 2313–2316.

[41] R.S. Williams, P. Rosenberg, Calcium-dependent Gene Regulation in Myocytes hypertrophy and Remodeling, Cold Spring Harbor Symposia on Quantitative Biology 67 (2002) 339–345.

[42] B.J. Wilkins, J.D. Molkentin, Calcineurin and cardiac hypertrophy: where have we been? Where are we going? J. Physiol. 541 (2002) 1–8.

[43] G. Munch, B. Bolck, P. Karczewski, R.H. Schwinger, Evidence for calcineurin-mediated regulation of SERCA 2a activity in human myocardium, J. Mol. Cell. Cardiol. 34 (2002) 321–334.

[44] A.G. Brittsan, K.S. Ginsburg, G. Chu, A. Yatani, B.M. Wolska, A.G. Schmidt, M. Asahi, D.H. MacLennan, D.M. Bers, E.G. Kranias, Chronic SR Ca^{2+}-ATPase inhibition causes adaptive changes in cellular Ca^{2+} transport (Increased L-type Ca^{2+} current (ICa) density), Circ. Res. 92 (2003) 769–776.

[45] E.W. Bush, D.B. Hood, P.J. Papst, J.A. Chapo, W. Minobe, M.R. Bristow, E.N. Olson, T.A. McKinsey, Canonical transient receptor potential channels promote cardiomyocyte hypertrophy through activation of calcineurin signaling, J. Biol. Chem. 281 (2006) 33487–33496.

[46] S. Abbasi, J.D. Lee, B. Su, X. Chen, J.L. Alcon, J. Yang, R.E. Kellems, Y. Xia, Protein kinase-mediated regulation of calcineurin through the phosphorylation of modulatory calcineurin-interacting protein 1, J. Biol. Chem. 281 (2006) 7717–7726.