The Emergence of Plasma Membrane Calcium Pump as a Novel Therapeutic Target for Heart Disease

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Abstract: The plasma membrane calcium/calmodulin dependent ATPase (PMCA) is a calcium-extruding enzymatic pump important in the control of intracellular calcium concentration. PMCA is the only system for calcium extrusion in the majority of cells. In excitable cells such as cardiomyocytes however, PMCA has been shown to play only a minor role in calcium homeostasis. In these cells the main mechanism of calcium extrusion is the sodium calcium exchanger. However, increasing evidence points to an important role for PMCA in signal transduction; in particular in the nitric oxide signalling pathway. In this review we will discuss recent advances that support a key role for PMCA in signal transduction and the potential for therapeutic targeting of this molecule in the treatment of cardiac diseases.

Keywords: Plasma membrane calcium pump, signal transduction, neuronal nitric oxide synthase, cardiovascular disease.

INTRODUCTION

Calcium has long been recognised as an important player in cell regulation. The involvement of calcium has been recorded in diverse fundamental processes including apoptosis [1], fertilization [2], transcription regulation [3, 4], synaptic plasticity [5] and exocytosis [5]. A unique feature of the calcium ion is its ability to act as a first, second or third messenger in cellular signalling [3]. This versatility enables calcium to control a number of important cell processes.

In heart muscle cells calcium is vital not only in maintaining cyclic contraction through the excitation-contraction coupling process [6], but also in the regulation of cell growth and hypertrophy [7, 8]. In the excitation-contraction cycle, a 100 fold increase in cytosolic calcium during systole is required for the activation of contractile molecules [9], while in the regulation of cardiac hypertrophy, calcium together with calmodulin, is required in the activation of several signalling molecules involved in hypertrophy such as protein kinase C, calcineurin and Cam Kinase II [8].

Despite the 100 fold increase during contraction, the intracellular calcium concentration ($[Ca^{2+}]_i$) is much lower compared to the extracellular free calcium. In excitable cells such as cardiomyocytes and neuronal cells, $[Ca^{2+}]_i$ fluctuates between 10nM and 1μM, [6, 10] whereas the extracellular free calcium concentration is approximately 1 mM [1]. The need to maintain low $[Ca^{2+}]_i$ is an absolute not only in myocytes but in most cell types since higher intracellular calcium levels can damage cells and furthermore, have been implicated in several diseases [11-13]. An effective calcium transport system is therefore essential to maintain the calcium gradient across the plasma membrane and ensure normal physiological function.

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Two outward transport mechanisms in the plasma membrane are known to regulate $[Ca^{2+}]_i$ levels: i)The plasma membrane calcium/calmodulin dependent ATPase (PMCA), which pumps calcium out of the cell using energy derived from the hydrolysis of ATP and is dependent on calmodulin [14] and ii) The sodium/calcium exchanger (NCX), which counter-transports one molecule of Ca^{2+} in exchange for three molecules of Na^+ utilising the sodium gradient across the plasma membrane as an energy source [15, 16].

In cardiomyocytes, NCX plays the dominant role in trans-sarcolemmal calcium ejection during the excitation-contraction coupling process. It has been shown that the NCX transports approximately 10-15 times more calcium (depending on species) than PMCA [6, 9]. Conventional views have therefore limited PMCA to its minor role in calcium transport, and the little or no contribution to the excitation-contraction coupling process. This marginal role however is challenged by interesting recent evidence suggesting a modulatory function for PMCA in signal transduction pathways. The wide-ranging potential of this signalling role is reflected in the ability of PMCA to interact with partner proteins *via* its PDZ binding domain, which is an amino acid sequence motif that is able to interact with PDZ motif (stands for PSD 95, Drosophila Discs large protein and Zona occludens-1) [17-19] and *via* non-PDZ binding regions [20, 21]. These novel insights could lead to the targeting of PMCA for new therapeutic interventions in important diseases including heart disease.

THE STRUCTURE OF PMCA

PMCA comprises 10 putative trans-membrane domains with four main units protruding into the cytoplasm (Fig. **1A**) [22]. The first intra-cytoplasmic unit is the N-terminal region encompassing the first 80-90 amino acids; the second one is the loop between trans-membrane domains 2 and 3 containing the phospholipid sensitive region. The third and largest unit is the loop between trans-membrane domains 4 and 5, which contains the catalytic sites including the ATP binding site and the site of aspartate residue which form aspartyl-phosphate during Ca^{2+} transport (PI). Finally, the

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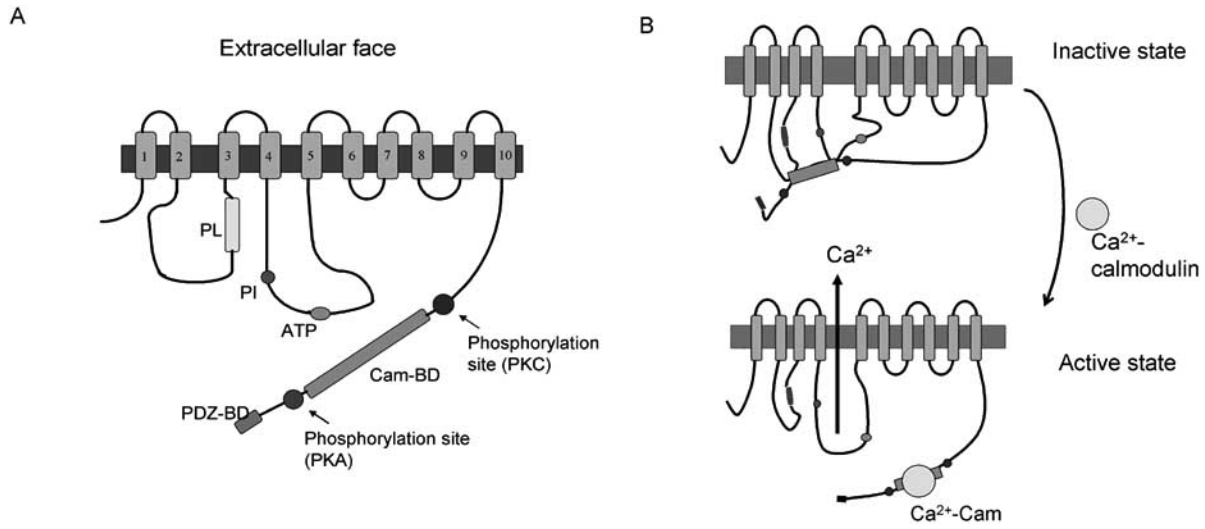

Fig. (1). Structure of Plasma membrane Ca^{2+} ATPase.

A) A schematic model of PMCA, the putative transmembrane domains (TM)are numbered from 1-10. The phospholipid-sensitive region (PL) is located between transmembrane domains 2 and 3. P is the site of aspartyl-phosphate formed during pump opening, and ATP is the ATP binding site. The calmodulin binding site (CamBD), the phosphorylation sites for PKA and PKC, and the PDZ binding domain are located at the extended C-terminal tail of the molecule. B) A schematic representation of PMCA at inactive state (top panel) and upon stimulation with Ca^{2+}-calmodulin (bottom panel). Binding of Ca^{2+}-calmodulin to the CamBD will release the autoinhibition of the pump.

fourth intra-cytoplasmic unit is the COOH terminal containing the regulatory domains including the calmodulin binding site [23] and the Protein Kinase A and Protein Kinase C phosphorylation sites [24]. Four PMCA isoforms encoded by four different genes (PMCA1-4) have been identified. The isoform diversity, splice variants and tissue distribution have been reviewed in great detail previously [22].

As with other Ca-calmodulin activated enzymes, PMCA has an autoinhibitory domain which is located in the COOH terminal tail. In the absence of Ca-calmodulin this 28 amino acid containing domain [25] interacts with two different regions of the molecule to keep the pump in an inactive state. The first binding site (residue 206-271) is located within the second cytoplasmic loop [26] and the second binding site (residue 537-544) within the third cytoplasmic loop [27]. In the presence of elevated calcium levels, the binding of Ca-calmodulin to the pump releases the autoinhibition and increases the calcium transport activity of the pump (Fig. **1B**). Phosphorylation by Protein Kinases has also been shown to activate this enzyme. Fig. **1A** depicts the potential sites for phosphorylation by Protein Kinase A or C. The specificity of phosphorylation appears to be isoform dependent, for example PMCA isoform 2,3 and 4 are phosphorylated by Protein Kinase C with variable effects depending on isoform type, whereas Protein Kinase A appears to activate PMCA1 only. [28]

SUBCELLULLAR LOCALISATION

A number of studies indicate that PMCA localisation varies depending on cell type. For example, studies using heart muscle from dog and hamster showed that PMCA is not abundant in the T-tubule but is abundant in the surface plasma membrane [29], whereas in rabbit skeletal muscle PMCA is found in both the T-tubule and surface plasma membrane [30]. A rather different location of PMCA is observed in salivary glands and kidney epithelial cells, where PMCA is detected in the basolateral membrane [31, 32]. The variation in PMCA localisation in different cell types reflects the varying cell functions and in particular cell calcium metabolism.

Recent evidence has shown that in many cell types, PMCA is concentrated within caveolae, which are invaginations of the plasma membrane [33-35]. Caveolae are believed to be the location of signal integration and modulation, containing a large number of receptors, signal transducers and effectors, and also linked to the cytoskeletal networks [36, 37]. As a consequence, PMCA may be integrated in the cellular signalling network, as well as to be anchored to the cytoskeletal network.

THE PDZ INTERACTION OF PMCA

Recent work has revealed that the 'b' splice variants of PMCA have a PDZ binding sequence at the COOH terminal region. The last six amino acid sequence of Ser-Leu/Val-Glu-Thr-Ser-Leu/Val-* (where * is stop codon) has been recognised as a ligand for PDZ domain [32, 38]. PDZ domain was named after the three proteins in which it was first described: synaptic protein PSD 95, Drosophila Discs large protein and the epithelial tight junction protein Zona occludens-1 [39, 40]. This domain is highly conserved and found largely in proteins responsible for anchoring and clustering structural and functional molecules, thus permitting the formation of large multiprotein complexes [41, 42]. An example of this is the PDZ domain in syntrophin, a cytoskeletal protein linked to the dystrophin network. This complex connects the extracellular matrix as well as membrane proteins to the cytoskeleton involving a number of proteins such as dystrobrevin, dytroglycan and dystrophin [43-45]. This network is of crucial importance in

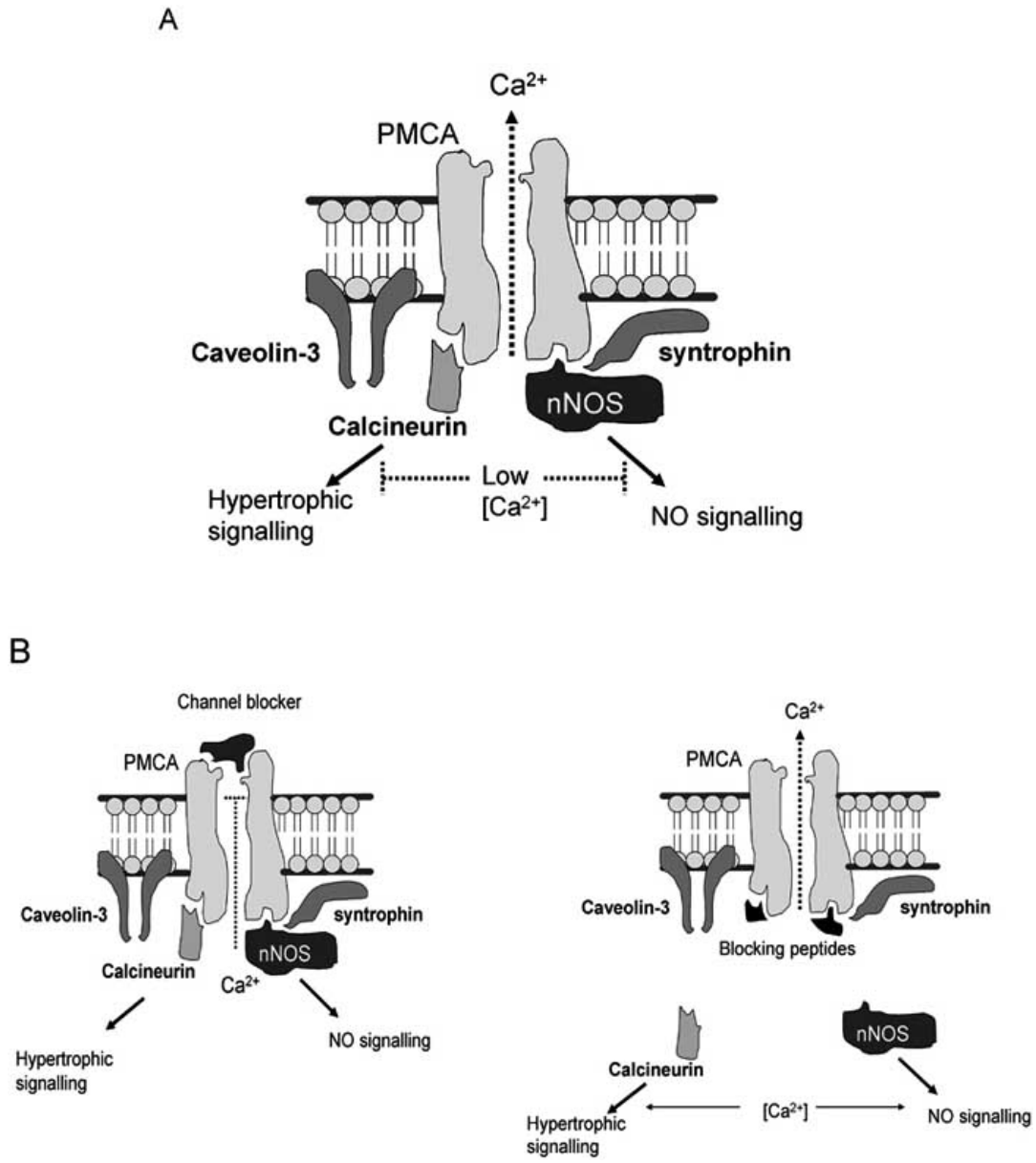

Fig. (2). Modulation of cellular signaling.

A) A schematic illustration of interactions between PMCA-nNOS and PMCA-calcineurin. PMCA modulates the functions of its interacting partners possibly by maintaining low local Ca^{2+} concentration. B) Strategies for targeting PMCA for therapeutical purpose. A channel blocker (left panel) will inhibit the Ca^{2+} pump activity and hence increase local Ca^{2+} concentration which subsequently upregulates the activities of interacting partners. Specific blocking peptides will prevent interactions (right panel) and hence will bring the interacting molecules to higher local Ca^{2+} concentration.

the myocardium as it is responsible for stretch signal transduction, and mutations within this complex lead to cardiomyopathy.

There is a significant body of evidence demonstrating the interaction of PMCA, particularly the 'b' splice variant, with proteins containing a PDZ domain. The interactions of PMCA2b and 4b with members of the membrane-associated guanylate kinase (MAGUK) family have been reported [17, 38]. In addition, PMCA4b also has functional interactions with calcium/calmodulin dependent protein kinase (CASK) [19], platelet cytoskeletal protein [46], a novel PMCA-interacting single PDZ protein (PISP) [47] and of major importance in cardiomyocyte biology, interaction with neuronal nitric oxide synthase (nNOS) [18].

In addition to PDZ-mediated binding, our group has also demonstrated interactions of non-PDZ regions of PMCA with the tumour suppressor RASSF1 [21] and calcineurin [20]. The cytoplamic loop between transmembrane domains 4 and 5 is involved in mediating these interactions. These interactions provide potentially significant functional implications in the critical processes of cardiomyocyte apoptosis and hypertrophy.

The subcellular localisation of PMCA in caveolae and its ability to bind to signalling molecules provides a new

dimension to our understanding of the role of PMCA in cardiomyocytes. The evidence of PMCA involvement in cellular signalling may answer the question of why, as a calcium pump, it plays such a minor role in excitation-contraction coupling; but it also leads to the question: exactly what is its role in signalling?

ROLE OF PMCA IN SIGNALLING: REGULATION OF CELL GROWTH

Involvement of PMCA in the regulation of cell growth and differentiation was first demonstrated in studies using PMCA over-expressing cells. The L6 myogenic cell line over-expressing human PMCA4b have an accelerated differentiation process reflected in multinucleated myotube formation [48], whereas over-expression of PMCA1a in vascular smooth muscle cells reduces the rate of cell proliferation [49]. The role of PMCA in cardiomyocyte growth has been studied further in transgenic rats over-expressing PMCA4b in the heart. Transgenic cardiomyocytes over-expressing PMCA4b displayed enhanced cellular growth in response to phenylephrine and isoproterenol stimulation [50], whereas in response to endothelin-1 infusion, the expressions of BNP, adrenomedulin and c-fos were reduced [51]. Other than the finding that over-expressed PMCA4b localises to the caveolae [50], where signalling processes are known to take place, there was no direct explanation of how PMCA4b regulates cardiomyocyte cell growth. However, our group has recently discovered a functional interaction between PMCA4b and calcineurin in mammalian cells [20]. Calcineurin is a calcium/calmodulin dependent protein phosphatase and an important transducer of hypertrophic signalling in cardiac myocytes [52]. During the pathological cardiac hypertrophy process calcineurin activates NFAT3, which subsequently induces the expression of hypertrophic genes [53]. The catalytic region of calcineurin A subunit was shown to physically interact with the catalytic region of PMCA4b, between trans-membrane domains 4 and 5; this interaction resulted in the down regulation of the transcriptional activity of one of its best characterised substrates, NFAT [20]. This finding provides an insight that modulation of calcineurin mediated hypertrophy by PMCA may be used as a novel target for controlling cardiac hypertrophy.

ROLE OF PMCA IN SIGNALLING: REGULATION OF NNOS

The interaction of PMCA4b and nNOS has been demonstrated in neuronal cells [18], cardiomyocytes and smooth muscle cells [54]. The interaction occurs between the C-terminal PDZ binding domain of PMCA4b with the PDZ domain of nNOS. In HEK 293 cells, over-expression of wild type PMCA4b dramatically reduces nNOS activity, whereas expression of a mutant PMCA4, which is unable to bind nNOS due to the deletion of the PDZ binding domain, fails to down regulate nNOS activity [18]. The physiological relevance of this interaction has been shown in studies using transgenic mice over-expressing PMCA4b in arterial smooth muscle cells. Using inducible [55] or constitutive [54] over-expression systems, the increased expression of PMCA4b in arterial smooth muscle resulted in increased blood pressure. Both authors showed that this phenomenon was attributable to the modulation of nNOS activity as opposed to changes in cellular calcium. It may be however that the regulation of local calcium concentration by PMCA is the mechanism responsible for modulation of nNOS activity since nNOS is a calcium calmodulin dependent enzyme. Indeed, over-expression of the mutant PMCA4b Asp672Glu, which reduces the calcium transport activity, failed to downregulate nNOS activity [18].

Recently, nNOS has moved to centre stage in cardiac physiology. It has been shown to regulate excitation contraction cycle both in isolated cardiomyocytes and in the heart [56, 57]. In cardiomyocytes nNOS is likely to localise in several cellular compartments including the sarcoplasmic reticulum, mitochondria and caveolae [58-60]. The co-localization of nNOS and PMCA4 in caveolae further strengthens the notion that this interaction is of physiological relevance in cardiomyocytes. Furthermore, the involvement of nNOS in calcium cycling [56], regulation of the beta adrenergic inotropic response [57, 61] and its up-regulation in heart failure [62, 63] strongly suggests that modulation of this enzyme may become an important therapeutic approach.

PMCA INHIBITORS: THE NEW THERAPEUTICAL APPROACH

The emerging evidence implicating PMCA in cardiac hypertrophic signalling and nNOS signalling (which subsequently regulates contractility) raises the possibility that targeting PMCA may be beneficial in patients with heart disease. For example, as PMCA downregulates nNOS activity by a tight molecular interaction, targeting PMCA will increase nNOS activity. This will be beneficial to increase the cardiac contractility because it has been shown that mice lacking nNOS in the heart have reduced β-adrenergic and force frequency response contractility [57, 64]. But the question is how to make PMCA drugable? As mentioned earlier, PMCA may modulate the function of its interacting signalling molecules by altering the local calcium concentration, therefore the approach to targeting PMCA signalling would be to inhibit the pump activity or to disrupt the interaction with signalling molecules.

Most inhibitors of calcium pumps traditionally used in the laboratory, such as carboxyeosin for PMCA [65] and nickel for NCX, are not suitable for therapeutic purposes due to their non-specificity and toxicity. Recently, however, a new compound that is able to inhibit the calcium pump transport activity of PMCA has been discovered. Caloxin2A1, a small peptide that binds to the second extracellular domain of PMCA, inhibits the pump activity in human erythrocyte leaky ghost preparations which express mainly PMCA4 [66]. The inhibition was not competitive with respect to ATP or calmodulin binding, leading to the suggestion that caloxin2A1 blocks PMCA activity by inhibiting the conformational changes required for the pump to eject calcium [67]. Caloxin2A1 was selected by a phage display screening of peptides which bind the second extracellular domain of PMCA1; thus the ability of this peptide to inhibit PMCA4 suggests a non-isoform specific activity. However, the fact that Caloxin2A1 inhibits PMCA at a high concentration (1 mM Caloxin2A1 for 80% inhibition of PMCA) [66] makes it difficult to be developed for therapeutic purpose since a good inhibitor should be

effective in a relatively low concentration (~1μM). Additionally, there are no studies examining potential toxicity of this compound for *in vivo* use. Nevertheless, the finding of Caloxin has opened new insights of the role of the extracellular domain, which may generate new ideas in the search for modulators of PMCA.

The more challenging aspect for drug discovery is targeting the protein-protein interaction. The difficulties in the identification of small compounds that inhibit protein-protein interactions are caused by a number of factors such as the lack of a lead compound, difficulties in distinguishing between real or artefactual binding and the small size of the molecule [68]. Discovery of a lead compound can be achieved using fragment based discovery methods [69], high throughput screening and computational screening. Understanding of the precise binding site is crucial and therefore X-ray crystallography and NMR studies are very useful. X-ray crystallography provides a high resolution static binding view of the interaction, whilst NMR allows construction of a three dimensional model of the interaction in solution [68].

There have been several recent reports on the use of blocking peptides to interrupt PDZ domain dependent protein interactions [70]. A number of peptides have been shown to inhibit the PDZ interaction of PICK1 (Protein interacting with C α kinase) with its interacting partners. Moreover, a few studies on the *in vivo* use of blocking peptides suggest potential therapeutic application of this approach. One example is the blocking of the PDZ interaction between the N-methyl-p-aspartate receptor (NMDAR) and PSD95 in whole animal models providing protection against ischemic brain damage [71].

In the case of PMCA, however, substantial more work is required before we can find candidate blocking peptides. In particular, domain mapping using site directed mutagenesis is needed to determine the precise interaction site; X-ray crystallography, as well as NMR studies, are also important to provide a basis for the design of inhibitory compounds. Alternatively, high throughput screening of small compounds library using NMR technology [72] or time-resolved fluorescence resonance energy transfer (TR-FRET) methods [68] can also be used to identify molecules that block interaction of PMCA with its interacting partners.

CONCLUDING REMARKS

After about two decades since the first cloning of PMCA and demonstrating its expression in the heart, a large number of studies have been conducted to characterise the role of PMCA in cardiac cells. Recent evidence, particularly in the case of isoform 4b, has re-directed our attention from its limited role as a calcium transporter in the excitation-contraction coupling process, to a more dominant role in cellular signalling and hence the regulation of cardiac contractility and hypertrophy. This has provided a potentially exciting opportunity for a new therapeutic target. Further studies of PDZ interaction of PMCA however are essential in order to fully understand the functional role of PMCA in health and disease and whether it can be manipulated for therapeutic purpose.

REFERENCES

[1] Orrenius, S.; Zhivotovsky, B.; Nicotera, P. *Nat. Rev. Mol. Cell. Biol.*, **2003**, *4*, 552-65.
[2] Stricker, S.A. *Dev. Biol.*, **1999**, *211*, 157-76.
[3] Carafoli, E. *Proc. Natl. Acad. Sci. USA,* **2002**, *99*, 1115-22.
[4] Carafoli, E.; Santella, L.; Branca, D.; Brini, M. *Crit. Rev. Biochem. Mol. Biol.*, **2001**, *36*, 107-260.
[5] Zucker, R.S. *Curr. Opin. Neurobiol.*, **1999**, *9*, 305-13.
[6] Bers, D.M. *Circ. Res.*, **2000**, *87*, 275-81.
[7] Frey, N.; Katus, H.A.; Olson, E.N.; Hill, J.A. *Circulation*, **2004**, *109*, 1580-9.
[8] Molkentin, J.D.; Dorn, I.G., 2nd. *Annu. Rev. Physiol.*, **2001**, *63*, 391-426.
[9] Bers, D.M. *Nature*, **2002**, *415*, 198-205.
[10] Tsien, R.Y. *Nature*, **1981**, *290*, 527-8.
[11] Mattson, M.P.; Chan, S.L. *Cell Calcium*, **2003**, *34*, 385-97.
[12] Halliday, N.J. *J. Craniofac. Surg.*, **2003**, *14*, 800-2.
[13] Legrand, G.; Humez, S.; Slomianny, C.; Dewailly, E.; Vanden Abeele, F.; Mariot, P.; Wuytack, F.; Prevarskaya, N. *J. Biol. Chem.*, **2001**, *276*, 47608-14.
[14] Carafoli, E. *Physiol. Rev.*, **1991**, *71*, 129-53.
[15] Philipson, K.D.; Nicoll, D.A. *Annu. Rev. Physiol.*, **2000**, *62*, 111-33.
[16] Blaustein, M.P.; Lederer, W.J. *Physiol. Rev.*, **1999**, *79*, 763-854.
[17] Kim, E.; DeMarco, S.J.; Marfatia, S.M.; Chishti, A.H.; Sheng, M.; Strehler, E.E. *J. Biol. Chem.*, **1998**, *273*, 1591-5.
[18] Schuh, K.; Uldrijan, S.; Telkamp, M.; Rothlein, N.; Neyses, L. *J. Cell Biol.*, **2001**, *155*, 201-5.
[19] Schuh, K.; Uldrijan, S.; Gambaryan, S.; Roethlein, N.; Neyses, L. *J. Biol. Chem.*, **2003**, *278*, 9778-83.
[20] Buch, M.H.; Pickard, A.; Rodriguez, A.; Gillies, S.; Maass, A.H.; Emerson, M.; Cartwright, E.J.; Williams, J.C.; Oceandy, D.; Redondo, J.M.; Neyses, L.; Armesilla, A.L. *J. Biol. Chem.*, **2005**, *280*, 29479-87.
[21] Armesilla, A.L.; Williams, J.C.; Buch, M.H.; Pickard, A.; Emerson, M.; Cartwright, E.J.; Oceandy, D.; Vos, M.D.; Gillies, S.; Clark, G.J.; Neyses, L. *J. Biol. Chem.*, **2004**, *279*, 31318-28.
[22] Strehler, E.E.; Zacharias, D.A. *Physiol. Rev.*, **2001**, *81*, 21-50.
[23] James, P.; Maeda, M.; Fischer, R.; Verma, A.K.; Krebs, J.; Penniston, J.T.; Carafoli, E. *J. Biol. Chem.*, **1988**, *263*, 2905-10.
[24] Enyedi, A.; Verma, A.K.; Filoteo, A.G.; Penniston, J.T. *J. Biol. Chem.*, **1996**, *271*, 32461-7.
[25] Vorherr, T.; Chiesi, M.; Schwaller, R.; Carafoli, E. *Biochemistry*, **1992**, *31*, 371-6.
[26] Falchetto, R.; Vorherr, T.; Carafoli, E. *Protein Sci.*, **1992**, *1*, 1613-21.
[27] Falchetto, R.; Vorherr, T.; Brunner, J.; Carafoli, E. *J. Biol. Chem.*, **1991**, *266*, 2930-6.
[28] Penniston, J.T.; Enyedi, A. *J. Membr. Biol.*, **1998**, *165*, 101-9.
[29] Iwata, Y.; Hanada, H.; Takahashi, M.; Shigekawa, M. *FEBS Lett.*, **1994**, *355*, 65-8.
[30] Sacchetto, R.; Margreth, A.; Pelosi, M.; Carafoli, E. *Eur. J. Biochem.*, **1996**, *237*, 483-8.
[31] Magyar, C.E.; White, K.E.; Rojas, R.; Apodaca, G.; Friedman, P.A. *Am. J. Physiol. Renal. Physiol.*, **2002**, *283*, F29-40.
[32] Lee, M.G.; Xu, X.; Zeng, W.; Diaz, J.; Kuo, T.H.; Wuytack, F.; Racymaekers, L.; Muallem, S. *J. Biol. Chem.*, **1997**, *272*, 15771-6.
[33] Schnitzer, J.E.; Oh, P.; Jacobson, B.S.; Dvorak, A.M. *Proc. Natl. Acad. Sci. USA,* **1995**, *92*, 1759-63.
[34] Amino, K.; Honda, Y.; Ide, C.; Fujimoto, T. *Curr. Eye Res.*, **1997**, *16*, 1088-95.
[35] Fujimoto, T. *J. Cell Biol.*, **1993**, *120*, 1147-57.
[36] Shaul, P.W.; Anderson, R.G. *Am. J. Physiol.*, **1998**, *275*, L843-51.
[37] Razani, B.; Woodman, S.E.; Lisanti, M.P. *Pharmacol. Rev.*, **2002**, *54*, 431-67.
[38] DeMarco, S.J.; Strehler, E.E. *J. Biol. Chem.*, **2001**, *276*, 21594-600.
[39] Hung, A.Y.; Sheng, M. *J. Biol. Chem.*, **2002**, *277*, 5699-702.
[40] Sheng, M.; Sala, C. *Annu. Rev. Neurosci.*, **2001**, *24*, 1-29.
[41] Fanning, A.S.; Anderson, J.M. *J. Clin. Invest.*, **1999**, *103*, 767-72.
[42] Fanning, A.S.; Anderson, J.M. *Curr. Opin. Cell Biol.*, **1999**, *11*, 432-9.
[43] Miyagoe-Suzuki, Y.; Takeda, S.I. *Microsc. Res. Tech.*, **2001**, *55*, 164-70.

[44] Michele, D.E.; Campbell, K.P. *J. Biol. Chem.*, **2003**, *278*, 15457-60.
[45] Lapidos, K.A.; Kakkar, R.; McNally, E.M. *Circ. Res.*, **2004**, *94*, 1023-31.
[46] Zabe, M.; Dean, W.L. *J. Biol. Chem.*, **2001**, *276*, 14704-9.
[47] Goellner, G.M.; DeMarco, S.J.; Strehler, E.E. *Ann. N. Y. Acad. Sci.*, **2003**, *986*, 461-71.
[48] Hammes, A.; Oberdorf-Maass, S.; Jenatschke, S.; Pelzer, T.; Maass, A.; Gollnick, F.; Meyer, R.; Afflerbach, J.; Neyses, L. *J. Biol. Chem.*, **1996**, *271*, 30816-22.
[49] Husain, M.; Jiang, L.; See, V.; Bein, K.; Simons, M.; Alper, S.L.; Rosenberg, R.D. *Am. J. Physiol.*, **1997**, *272*, C1947-59.
[50] Hammes, A.; Oberdorf-Maass, S.; Rother, T.; Nething, K.; Gollnick, F.; Linz, K.W.; Meyer, R.; Hu, K.; Han, H.; Gaudron, P.; Ertl, G.; Hoffmann, S.; Ganten, U.; Vetter, R.; Schuh, K.; Benkwitz, C.; Zimmer, H.G.; Neyses, L. *Circ. Res.*, **1998**, *83*, 877-88.
[51] Piuhola, J.; Hammes, A.; Schuh, K.; Neyses, L.; Vuolteenaho, O.; Ruskoaho, H. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **2001**, *281*, R699-705.
[52] Vega, R.B.; Bassel-Duby, R.; Olson, E.N. *J. Biol. Chem.*, **2003**, *278*, 36981-4.
[53] Molkentin, J.D.; Lu, J.R.; Antos, C.L.; Markham, B.; Richardson, J.; Robbins, J.; Grant, S.R.; Olson, E.N. *Cell*, **1998**, *93*, 215-28.
[54] Schuh, K.; Quaschning, T.; Knauer, S.; Hu, K.; Kocak, S.; Roethlein, N.; Neyses, L. *J. Biol. Chem.*, **2003**, *278*, 41246-52.
[55] Gros, R.; Afroze, T.; You, X.M.; Kabir, G.; Van Wert, R.; Kalair, W.; Hoque, A.E.; Mungrue, I.N.; Husain, M. *Circ. Res.*, **2003**, *93*, 614-21.
[56] Sears, C.E.; Bryant, S.M.; Ashley, E.A.; Lygate, C.A.; Rakovic, S.; Wallis, H.L.; Neubauer, S.; Terrar, D.A.; Casadei, B. *Circ. Res.*, **2003**, *92*, e52-9.
[57] Barouch, L.A.; Harrison, R.W.; Skaf, M.W.; Rosas, G.O.; Cappola, T.P.; Kobeissi, Z.A.; Hobai, I.A.; Lemmon, C.A.; Burnett, A.L.; O'Rourke, B.; Rodriguez, E.R.; Huang, P.L.; Lima, J.A.; Berkowitz, D.E.; Hare, J.M. *Nature*, **2002**, *416*, 337-9.
[58] Kanai, A.J.; Pearce, L.L.; Clemens, P.R.; Birder, L.A.; VanBibber, M.M.; Choi, S.Y.; de Groat, W.C.; Peterson, J. *Proc. Natl. Acad. Sci. USA,* **2001**, *98*, 14126-31.
[59] Xu, K.Y.; Huso, D.L.; Dawson, T.M.; Bredt, D.S.; Becker, L.C. *Proc. Natl. Acad. Sci. USA,* **1999**, *96*, 657-62.
[60] Venema, V.J.; Ju, H.; Zou, R.; Venema, R.C. *J. Biol. Chem.*, **1997**, *272*, 28187-90.
[61] Ashley, E.A.; Sears, C.E.; Bryant, S.M.; Watkins, H.C.; Casadei, B. *Circulation*, **2002**, *105*, 3011-6.
[62] Bendall, J.K.; Damy, T.; Ratajczak, P.; Loyer, X.; Monceau, V.; Marty, I.; Milliez, P.; Robidel, E.; Marotte, F.; Samuel, J.L.; Heymes, C. *Circulation*, **2004**, *110*, 2368-75.
[63] Damy, T.; Ratajczak, P.; Shah, A.M.; Camors, E.; Marty, I.; Hasenfuss, G.; Marotte, F.; Samuel, J.L.; Heymes, C. *Lancet*, **2004**, *363*, 1365-7.
[64] Khan, S.A.; Skaf, M.W.; Harrison, R.W.; Lee, K.; Minhas, K.M.; Kumar, A.; Fradley, M.; Shoukas, A.A.; Berkowitz, D.E.; Hare, J.M. *Circ. Res.*, **2003**, *92*, 1322-9.
[65] Choi, H.S.; Eisner, D.A. *Pflugers Arch.*, **1999**, *437*, 966-71.
[66] Chaudhary, J.; Walia, M.; Matharu, J.; Escher, E.; Grover, A.K. *Am. J. Physiol. Cell Physiol.*, **2001**, *280*, C1027-30.
[67] Holmes, M.E.; Chaudhary, J.; Grover, A.K. *Cell Calcium*, **2003**, *33*, 241-5.
[68] Arkin, M.R.; Wells, J.A. *Nat. Rev. Drug Discov.*, **2004**, *3*, 301-17.
[69] Rees, D.C.; Congreve, M.; Murray, C.W.; Carr, R. *Nat. Rev. Drug Discov.*, **2004**, *3*, 660-72.
[70] Dev, K.K. *Nat. Rev. Drug Discov.*, **2004**, *3*, 1047-56.
[71] Aarts, M.; Liu, Y.; Liu, L.; Besshoh, S.; Arundine, M.; Gurd, J.W.; Wang, Y.T.; Salter, M.W.; Tymianski, M. *Science*, **2002**, *298*, 846-50.
[72] Moore, J.M. *Biopolymers*, **1999**, *51*, 221-43.

Received: October 5, 2005 **Revised: December 15, 2005** **Accepted: December 16, 2005**