The regulatory function of plasma-membrane Ca\(^{2+}\)-ATPase (PMCA) in the heart

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Abstract
The PMCA (plasma-membrane Ca\(^{2+}\)-ATPase) is a ubiquitously expressed calcium-extruding enzymatic pump important in the control of intracellular calcium concentration. Unlike in non-excitable cells, where PMCA is the only system for calcium extrusion, in excitable cells, such as cardiomyocytes, PMCA has been shown to play only a minor role in calcium homeostasis compared with the NCX (sodium/calcium exchanger), another system of calcium extrusion. However, increasing evidence points to an important role for PMCA in signal transduction; of particular interest in cardiac physiology is the modulation of nNOS (neuronal nitric oxide synthase) by isoform 4b of PMCA. In the present paper, we will discuss recent advances that support a key role for PMCA4 in modulating the nitric oxide signalling pathway in the heart.

Introduction
Calcium is one of the most important ionic regulators in the heart, where it has a pivotal role in mediating the contraction/relaxation cycle. In cardiomyocytes, calcium traffic across the plasma membrane and within the cell is co-ordinated by several transporters located at the sarcolemma (plasma membrane), sarcoplasmic reticulum and mitochondrial membrane. Calcium influx is mediated by voltage-gated calcium channels, receptor-operated channels or non-selective cation channels [1]. It will subsequently trigger calcium release from the intracellular calcium stores (sarcoplasmic reticulum), which initiates contraction. During relaxation, most of the intracellular calcium will be transported back into the sarcoplasmic reticulum by the SERCA (sarcoplasmic/endo-plasmic-reticulum Ca\(^{2+}\)-ATPase) and a smaller proportion of calcium will be ejected out of the cell [2].

Two outward transport mechanisms in the sarcolemma are responsible for calcium extrusion: (i) the PMCA (plasma-membrane Ca\(^{2+}\)-ATPase), which ejects calcium to the extracellular compartment using energy derived from ATP hydrolysis and is dependent on calmodulin [3], and (ii) the NCX (sodium/calcium exchanger), which counter-transport one molecule of calcium in exchange for three molecules of sodium utilizing the sodium gradient across the plasma membrane as an energy source [4,5].

In cardiomyocytes, the trans-sarcolemmal calcium ejection is performed mostly by the NCX. Accumulating evidence suggests that the NCX transports approx. 10–15 times more calcium (depending on species) than PMCA [2,6]. It is believed therefore that PMCA in cardiomyocytes has only a limited role in calcium transport, and makes a minor contribution to the excitation–contraction coupling process. This marginal role, however, is challenged by interesting recent findings suggesting a modulatory function for PMCA in signal transduction pathways. The present review will discuss recent evidence suggesting that PMCA has an essential role in signal transduction in the myocardium.

PMCA is a signalling molecule
In cardiomyocytes, calcium has a crucial role not only in maintaining cyclic contraction through the excitation–contraction coupling process but also as a key messenger in signal transduction pathways in the heart. For example, it has been accepted that calcium/calmodulin-dependent enzymes, such as calcineurin, nNOS (neuronal nitric oxide synthase), eNOS (endothelial nitric oxide synthase) and CaMKs (Ca\(^{2+}\)/calmodulin-dependent protein kinases), are capable of carrying localized intracellular signals regulating hypertrophy and redox equilibrium in the heart [7,8]. However, the mechanisms by which the myocardial cell distinguishes the contractile and signalling roles of calcium remain unclear. One hypothesis is that a calcium transporter that is not involved in the contractile cycle (such as PMCA) may carry non-contractile signals.

Evidence is accumulating to support the idea that PMCA has a more dominant role in signalling. PMCA displays two main characteristics of a signalling molecule, which will be discussed in more detail below: (i) the ability to interact with signalling proteins [9], and (ii) its subcellular localization in caveolae [10].

PMCA is encoded by a family of four genes (PMCA1–PMCA4) with more than 20 isoforms existing due to alternative splicing [11]. The predominant PMCA genes expressed in the heart are PMCA1 and PMCA4 [11,12]. Splice variants ‘b’ of PMCA, for example PMCA1b or PMCA4b, contain a PDZ-binding sequence at the C-terminal region.

Key words: calcium/calmodulin-dependent enzyme, calcium transport, cardiomyocyte, heart, neuronal nitric oxide synthase (nNOS), plasma-membrane Ca\(^{2+}\)-ATPase (PMCA).

Abbreviations used: NCX, sodium/calcium exchanger; nNOS, neuronal nitric oxide synthase; PMCA, plasma-membrane Ca\(^{2+}\)-ATPase; Rassf1, Ras-associated factor 1.

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PDZ domain was first identified in three proteins: synaptic protein PSD-95 (postsynaptic density 95), Drosophila discs large protein and the epithelial tight junction protein ZO-1 (zonula occludens 1 protein) [13,14], and named after these proteins. This domain is highly conserved and found mostly in proteins responsible for anchoring and clustering structural and functional molecules and so allowing the formation of large multiprotein complexes.

A number of PDZ domain-containing proteins that interact with PMCA have been identified. These proteins consist of members of the MAGUK (membrane-associated guanylate kinase) family [15,16], cytoskeletal proteins [17], NHERF2 (Na+/H+ -exchanger regulatory factor 2) [18], nNOS [19], CASK (calcium/calmodulin-dependent serine protein kinase) [20] and the PISP (PMCA-interacting single PDZ protein) [21]. In addition, a number of novel protein–protein interactions involving other domains of PMCA have also been demonstrated including the interaction between the N-terminal region of PMCA and the phosphorylation modulator molecule 14-3-3 [22], interaction between the second intracellular loop of PMCA with tumour suppressor molecule Rassf1 (Ras-associated factor 1) [23], calcineurin [24] and a cytoskeletal protein α1-syntrophin [25]. An important characteristic of protein–protein interactions involving PMCA is their functionality. Most of the interactions will result in the modification of PMCA or its interacting partner activity. For example, nNOS and calcineurin activities were down-regulated by PMCA [19,24], whereas 14-3-3 proteins inhibited PMCA activity [22].

The subcellular localization of PMCA in caveolae has been discovered in various cell types [10,26,27]. Caveolae, which are invaginations of the plasma membrane, are thought to be the location of signal integration and modulation. They contain a large number of receptors, signal transducers and effectors and are also linked to the cytoskeletal networks [28,29]. Consequently, PMCA may be integrated into the cellular signalling network, as well as being anchored to the cytoskeletal network.

The subcellular localization 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 (Figure 1). It suggests that PMCA may be integrated into important cellular signalling networks and function as a signalling molecule rather than as a regulator of global intracellular calcium.

Isoform 4b of PMCA (PMCA4b) is a regulator of nNOS

nNOS plays a very important role in cardiomyocyte biology. Two independent groups have shown that nNOS regulates excitation–contraction coupling both in isolated cardiomyocytes and in vivo in the heart [30,31]. Furthermore, nNOS has been described to be involved in the regulation of the β-adrenergic inotropic response [30], redox equilibrium [7] and development of heart failure [32,33].

We have demonstrated that isoform 4b of PMCA (PMCA4b), one of the isoforms expressed in the heart, interacts with nNOS [19]. The interaction occurs between the PDZ-binding domain located at the C-terminus of PMCA4b and the PDZ domain of nNOS. The functionality of the PMCA4b–nNOS interaction was first identified using a cellular system: overexpression of PMCA4b in HEK-293 cells (human embryonic kidney cells) dramatically down-regulated 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 reduce nNOS activity [19]. Since nNOS is a calcium/calmodulin-dependent enzyme, it is possible that a lower calcium concentration in the local vicinity due to PMCA activity is the mechanism responsible for nNOS down-regulation (Figure 2). In keeping with that idea, overexpression of the mutant PMCA4 D672E, which reduces the calcium transport activity, failed to down-regulate nNOS activity [19].

Recently, our group has demonstrated that the PMCA4–nNOS interaction also occurs in the heart [25]. Given the importance of nNOS in the heart, this finding raises the question of whether the modulation of nNOS function by PMCA affects various important aspects of cardiovascular physiology.

Lessons from the transgenic animals

Several recent studies using genetically modified animals have provided experimental evidence to address the question whether the signalling role of PMCA4 has in vivo consequences. The first published data come from studies using transgenic rats overexpressing human PMCA4b using the
cardiac-specific promoter MLC2v [34]. This rat displayed a normal cardiac function, normal blood pressure and normal intracellular calcium transient. However, cardiomyocytes isolated from this strain of rat displayed a higher growth rate and further analysis showed that endothelin-1-mediated hypertrophy was differently regulated in transgenic cardiomyocytes [35]. This indicates the role of PMCA4b in the regulation of cardiac growth and hypertrophy and less involvement of this protein in the regulation of global intracellular calcium.

Interesting findings from mice overexpressing PMCA4b in vascular smooth-muscle cells have been reported by our group [36] and others [37]. Both groups have found higher peripheral blood pressure in the transgenic mice overexpressing PMCA4b. If PMCA4b were involved directly in intracellular calcium regulation, one would expect a reduction in vascular tone due to a reduction in intracellular calcium. Surprisingly, no significant changes in intracellular calcium were detected but a significant reduction of nNOS function was revealed [37]. These results suggest that PMCA4b regulates peripheral vascular tone not by modulation of calcium transient within the smooth-muscle cells but by modulation of nNOS function.

Recently, the in vivo role of PMCA4b in cardiac myocytes has been discovered through a comprehensive study using transgenic mice overexpressing PMCA4b specifically in the heart [38]. In contrast with experiments performed using the transgenic rat model, in this study more robust cardiac contractility measurements using pressure–volume loops and transthoracic echocardiographic analyses have been used. As a result, mice overexpressing PMCA4b displayed a reduced inotropic response to β-adrenergic stimulation. Further analyses using transgenic mice overexpressing mutant PMCA ct120, which is unable to bind and regulate nNOS, revealed that modulation of nNOS function was the probable mechanism for this phenotype [38]. A further phenotype of this strain of transgenic mouse was the increase of a hypertrophic response under chronic stimulation with a β-adrenergic agonist. This study provides evidence that PMCA4b plays important roles in cardiomyocytes, including regulation of contractility and hypertrophic response that are likely through modulation of cellular signalling pathways.

Although PMCA4-knockout mice have been established by two independent groups [39,40], there were only limited cardiovascular phenotypes that have been reported. It is possible that the general embryonic knockout of this molecule will induce an overexpression of other PMCA isoforms to compensate for the loss of PMCA4. Therefore further studies using a tissue-specific and an inducible knockout model of PMCA4 would be very interesting. Nevertheless, a comprehensive study by Shull and co-workers [41] in the bladder smooth-muscle cells suggested that PMCA4-knockout mice displayed a reduction of the bladder smooth-muscle contractility probably caused by modulation of signalling pathways.

**Conclusion**

Since the first discovery of PMCA expression in the heart, a large number of studies have been conducted to characterize the role of PMCA in cardiac cells. A growing body of evidence, particularly in the case of isoform 4b, has redirected our attention from its limited role as a calcium transporter 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 for heart failure in the future.

**References**


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