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Editorial

Calcium cycling in heart failure: how the fast became too furious

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See article by Yanczewski et al. [11] (pages 468–480) in this issue.

> . . .After them, the (scientific) philosophers appear. . .who proclaim that only they are wise and all other mortals are but shadows flapping about. . . . [The philosophers] build uncountable worlds as . . .they hypothesize without hesitation causes for . . .unexplainable things, as if they were secretaries of Nature, architects of all things, as if they appeared among us from a meeting of gods. Meanwhile, Nature laughs at them and their speculations, because that they have uncovered nothing is demonstrated by the fact that they immediately cross their swords on each single issue without results. . .

From Moriae Encomium (the Praise of Folly) by Desiderius Erasmus (1509 AD).

For the maintenance of cardiac function, there may be no more important molecule than calcium. During each heartbeat, a small leak of calcium through the voltage-dependent L-type calcium channels (VDLC) triggers massive calcium release from an internal storage pool in the sarcoplasmatic reticulum (SR) through SR-bound ryanodine receptors (RyR) into the cytoplasm of heart muscle cells, which triggers muscle contraction. Cardiac relaxation is initiated by the concerted action of the sodium/calcium exchanger (NCX) and muscle-specific SR calcium ATPase-2 (SERCA2), which resequesters calcium into the internal calcium storage pool, allowing for the next quantal release of calcium. SERCA2 thereby promotes both cardiac relaxation and contractility. Phospholamban, an endogenous muscle-specific SERCA2 inhibitor, interacts with SERCA2, and determines the rate of calcium reuptake into the SR. On top of this elaborate system of calcium pumps, the cardiac β-adrenergic receptor system controls the magnitude of the calcium transient by means of activating protein kinase A (PKA), a kinase that by virtue of its direct phosphorylation of the VDLC, RyR, SERCA2 and phospholamban allows for intracellular calcium transients with higher amplitude and faster reuptake into the SR calcium storage. Specifically, PKA-mediated phosphorylation of phospholamban dissociates the latter from SERCA2, allowing for faster calcium reuptake into the SR (Fig. 1).

For over 20 years, our principal therapeutic approach to heart failure has been largely palliative, aimed at relieving symptoms rather than interrupting defined molecular pathways that drive disease progression. Diminished calcium-transient amplitudes and slowed rates of SR calcium reuptake have been observed in cardiac muscle cells from failing human hearts as well as altered expression and phosphorylation status of key SR components directly regulating the calcium transient, fueling speculations that correcting the calcium transient may form a rationale to recover cardiac contractility.

One landmark observation in line with this hypothesis was made by Luo et al. [1] when they reported that *phospholamban* gene-targeted mice (i.e., complete absence of the SERCA2 inhibitor) displayed maximal cardiac contractility at baseline and were unresponsive to further augmentation with β-adrenergic stimulation, suggesting that the most significant contractile effect of β-adrenergic stimulation was mediated through phospholamban and its ability to amend SERCA2 function. Next, Minamisawa et al. [2] exposed yet another function for phospholamban when cross-breeding *phospholamban* null mice with the muscle-LIM protein (MLP) knockout mouse model of dilated cardiomyopathy. Not only was cardiac contractility in double knockout mice was significantly improved, but ventricular hypertrophy, fibrosis, and survival were virtually eradicated, implying that correcting the calcium transient could also alleviate maladaptive structural demise in heart failure. Phospholamban gene ablation further prevented cardiac hypertrophy and ventricular dysfunction in calsequestrin-overexpressing mice [3], while viral delivery of antisense phospholamban to the myocardium improved calcium han-

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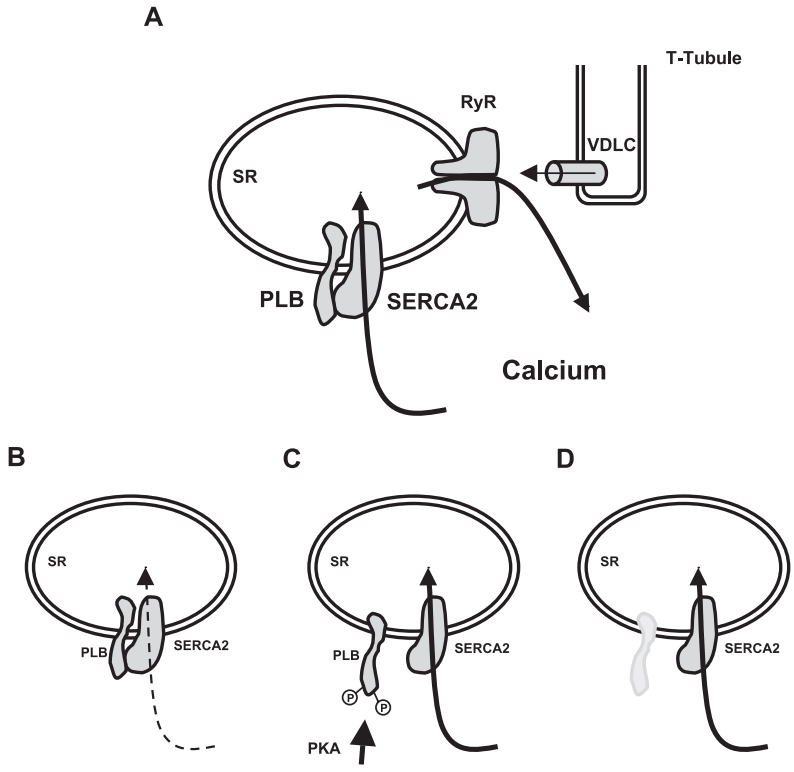

Fig. 1. (A) Calcium cycling involves the concerted action of major calcium pumps on the T-tubule, the SR, and sarcolemma (see text). (B) Reuptake of calcium into the SR, the main site of calcium storage, is determined by the activity of phospholamban (PLB), which in a "normal" situation has a repressive action on SERCA2. (C) Following β-adrenergic stimulation, maximal SERCA2 activity is achieved by direct phosphorylation of PLB and its dissociation from SERCA2. (D) Complete absence of PLB results in a basal increase in cardiomyocyte contractility in the absence of catecholamines.

dling and contractile parameters of human myocytes from explanted failing hearts [4] and provided full rescue to a hamster model of dilated cardiomyopathy [5].

Unfortunately, a growing number of reports consign a more critical note to the contention that SERCA2–phospholamban dysfunction may form a universal defect in experimental and human heart failure. First, phospholamban erasure does not cure hypertrophy or overall ventricular function in the setting of experimental heart failure due to overexpression of tropomodulin [6], Gαq [7], or a mutant myosin binding protein C [7], although the characteristically prolonged cardiomyocyte calcium transients and enhanced unloaded fractional shortening were rescued. Likewise, phospholamban ablation only incompletely healed a mouse model of hypertrophic cardiomyopathy due to expression of a mutant myosin heavy chain [8], and, of more concern, did not improve the progressive demise of the pressure-overloaded mouse heart resulting from chronic aortic stenosis [9]. Finally, Haghighi et al. [10] recently identified two human families with null-like polymorphisms in the human *phospholamban* gene, with affected members displaying dilated cardiomyopathy, requiring heart transplantation as young adults.

In this issue of Cardiovascular Research, a report from the group of Janczewski et al. [11] fuels the controversy on the "phospholamban hypothesis" in heart failure. Janczewski et al. analyzed mice engineered to overexpress the cytokine tumor necrosis factor-α (TNF-α), which develop a well-characterized path to heart failure resembling the human disease encompassing diminished amplitudes and slowed kinetics of intracellular calcium transients and structural and functional demise. The mice finally succumbed to overt congestive heart failure by 6 months of age. The authors went great lengths to demonstrate that phospholamban ablation normalized the calcium transients in TNF-α-overexpressing mice, but overall ventricular geometry and dysfunction remained unaltered, if not worsened. In other words, calcium-handling defects were cured without favorable effects on heart failure initiation and progression [11].

How should we interpret these conflicting results and what may be their ramifications for the future therapeutic potential to correct SR defects in human heart failure? Supporters of the hypothesis would point to the concept that diverse triggers ignite relatively consistent changes in heart cells, including defective SR calcium loading, the successes obtained so far by phospholamban antagonism in a variety of model systems, and the attractive prospect that one simple genetic rescue could overcome a highly complex disease such as heart failure. Additionally, one may argue that all negative reports were obtained in mice. Indeed, phospholamban knockouts are

healthy and their hearts display supercontractility in the absence of structural defects, while humans with the same genotype *develop* heart failure. This paradox signals a cautionary note for molecular medicine in general, which embraces the mouse as the ultimate genetic system to study human disease, although in this particular case the human truncation mutation does not produce a true null but a truncated protein that may have toxic myocardial consequences.

Notwithstanding these valid arguments, the mere contention that heart failure remains unchanged in the face of normalized calcium transients [11] remains a sobering biological observation, demanding a fundamental shift in our perception of the (patho)physiology of cardiac calcium cycling and its players involved. Indeed, posttranslational modifications of the RyR alone seem sufficient to provoke characteristic changes in SR calcium content, calcium transient amplitudes and kinetics previously attributed to defects in SERCA2 function [12]. In addition, novel players enter the field. Hearts from protein kinase C-α-deficient mice were recently characterized to be hypercontractile due to favorable changes in phoshoplamban phosphorylation status and calcium handling, indicating an as of yet unexpected regulatory role for this signaling molecule in excitation–contraction coupling [13].

As heart failure is more likely a clinical entity characterized largely by its overwhelming complexity rather than by the instigating cause(s), it starts to seem unlikely that one single approach (e.g., phosholamban antagonism) will ever benefit all cases of human heart failure. A uniform conclusion that does emerge from these efforts is that we still have very limited understanding about the full complexity of one single aspect of heart muscle physiology (calcium handling), let alone the exponential complexity of human heart disease in general. Living in the time of the Renaissance, characterized by revision of most prevailing concepts, the Dutch scholar Erasmus embraced the shortcomings of his contemporaries when writing his famous satire on mankind's vanities in Praise of Folly five centuries ago. Instead of immersing in self-satisfaction, modern day scholars may want to remember the message of this ancient philosopher and continue their pursuit to uncover the particulars of the heartbeat, the beneficiaries ultimately being the victims of heart failure.

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