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EDITORIAL COMMENT

Negative Adrenergic Feedback Specific to Phospholamban*

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In the heart, the sarcoplasmic reticulum (SR) acts as an important calcium (Ca^{2+}) store, which is of paramount relevance for the control of cardiac function. Ca^{2+} is released from the SR during systole through cardiac ryanodine receptor (RyR2) Ca^{2+} release channels. Ca^{2+} binding to troponin C on the thin filaments activates acto-myosin cross-bridge cycling (**Figure 1**). Hence, the amount and rate of SR Ca^{2+} release and Ca^{2+} binding to troponin C correlates with cross-bridge activation and myocardial force generation. Vice versa, for diastolic relaxation, Ca^{2+} needs to dissociate from troponin C to terminate cross-bridge cycling. Hence, the cytosolic Ca^{2+} concentration has to be decreased to ~100 nmol/l mainly via Ca^{2+} reuptake into the SR through the cardiac sarcoplasmic reticulum Ca^{2+} pump 2a (SERCA2a) at the cost of ATP consumption (1). Although SERCA2a activity is crucial for normal diastolic function, dysregulation of SERCA2a represents a central molecular defect in heart failure.

In a simplified model (**Figure 1**), systolic transsarcolemmal Ca^{2+} influx through L-type Ca^{2+} channels, matched by net diastolic efflux through Na^{+}/Ca^{2+} exchangers, activates RyR2 channels via Ca^{2+}-induced Ca^{2+} release. Because relatively large SR Ca^{2+} fluxes are essential for Ca^{2+}-induced Ca^{2+} release, SR Ca^{2+} uptake, storage, and release are critical for proper function of cardiac myocytes. This contrasts with end-stage heart failure, disturbed SR Ca^{2+} handling, and contractile dysfunction documented in patients (2). Of note, Ca^{2+} handling can be disrupted by increased diastolic Ca^{2+} leak through defective RyR2 channels and decreased Ca^{2+} uptake either from reduced SERCA2a protein levels (i.e., fewer SERCA2a pumps) or decreased SERCA2a function. Within this challenging multifactorial context, Akaike et al. (3), in this issue of *JACC: Basic to Translational Science*, identify a new regulatory mechanism of SERCA2a with potentially important therapeutic implications.

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Phospholamban (PLN), a small peptide in the SR, inhibits SERCA2a function in its dephosphorylated state (4). SERCA2a inhibition is relieved during catecholaminergic stimulation through increased PLN phosphorylation by protein kinase A (PKA) at Ser-16 and Ca^{2+} calmodulin-dependent kinase (CaMK) at Thr-17, augmenting SR Ca^{2+} load and contractility. Whereas RyR2 channels are hyperphosphorylated in heart failure, PLN was *hypo*-phosphorylated in the same hearts with regard to PKA, indicating differential SERCA2a regulatory mechanisms (5). Depressed SR function in human heart failure results in reduced systolic contractility, disturbed diastolic relaxation, and inversion of the myocardial force-frequency relation (6). Moreover, cytosolic Ca^{2+} overload can alter gene expression and enzyme activity, energy metabolism, and apoptosis, and can contribute to heart failure progression.

Vice versa, restoration of disturbed Ca^{2+} uptake represents a major therapeutic strategy in heart

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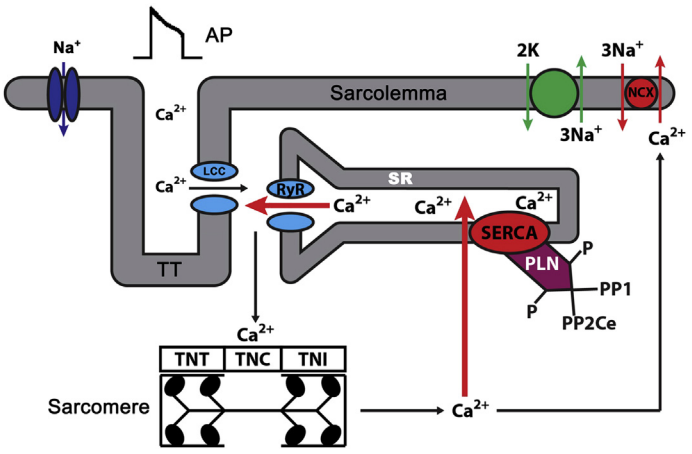
FIGURE 1 PLN Dephosphorylation Regulates Cardiac Excitation-Contraction Coupling



An incoming action potential (AP) activates voltage-dependent Na^+ and L-type Ca^{2+} channels (LCC) in the sarcolemmal membrane, particularly in T-tubule (TT) invaginations, resulting in a relatively small Ca^{2+} influx. Next, cardiac ryanodine receptors (RyR) are activated in the sarcoplasmic reticulum (SR) via Ca^{2+}-induced Ca^{2+} release **(red horizontal arrow)**. This systolic Ca^{2+} release activates the troponin complex (TnC, TnT, TnI), cross-bridge cycling in sarcomeres, and cardiac contraction. For diastolic relaxation to occur, the relatively large amount of Ca^{2+} released via RyR channels must be pumped back into the SR store by SERCA2a ATPases, which are tonically inhibited by phospholamban (PLN). While catecholaminergic PLN phosphorylation (P) at Ser-16 and Thr-17 significantly increases SR Ca^{2+} uptake **(red vertical arrow)** and cardiac force generation, dephosphorylation by phosphatases (PP1 and PP2Ce) limits the inotropic response through a negative feedback mechanism. A novel role of PP2Ce in normal hearts versus heart failure is described by Akaike et al. (3). NCX = Na^+/Ca^{2+} exchanger.

failure. Cardiac gene targeting through overexpression of SERCA2a showed promising effects in early preclinical and clinical trials (2). Although a recent SERCA2a gene therapy trial (phase 2b) failed to improve cardiac function in patients with advanced HF, alternative RyR2-associated targeting strategies based on junctophilin-2 are emerging (7). Therefore, careful dissection of the complex differential mechanisms of SR Ca^{2+} regulation, including trials in large animal models, might be necessary for successful translation.

Here, Akaike et al. (3) provide important new information regarding distinct roles of PLN regulation. The authors investigated protein phosphatase 2Ce (PP2Ce), showing that this SR-located enzyme may specifically dephosphorylate PLN at Thr-17, the Ca^{2+} calmodulin-dependent kinase site (**Figure 1**). Notably, phosphorylation of other myocyte regulatory proteins such as RyR2, PKA, and troponin I were not affected by PP2Ce. Furthermore, both phosphatase activity and SR membrane localization were required for PP2Ce-dependent PLN dephosphorylation.

PP2Ce-dependent PLN phosphorylation was particularly relevant during increased ß-adrenergic signaling (3). Physiologically relevant, the inhibitory PLN to SERCA2a ratio differs substantially between ventricular and atrial myocytes, which can explain fundamental differences in adrenergic regulation (8). In the ventricle, PP2Ce diminished isoproterenol (ISO)-induced PLN-phosphorylation mainly through Thr-17 dephosphorylation (3). Reversing ISO effects, PP2Ce decreased the amplitude and prolonged the decay of intracellular Ca^{2+} transients, associated with a remarkably blunted inotropic effect. Antagonistic effects via PLN dephosphorylation seem to be potentiated by ISO-induced, proteosomally mediated increases of PP2Ce protein levels as early as 2 h after ß-adrenergic stimulation (3).

PP2Ce-mediated PLN dephosphorylation also resulted in significant adverse effects. Following ischemia/reperfusion, the authors found impaired cardiac relaxation and increased infarct sizes attributed to increased apoptosis (3). These effects may be further enhanced through oxidative cell stress. Indeed, cleaved caspase 9 was detected in transgenic hearts with increased PP2Ce expression, indicating mitochondrially mediated cell death. Taken together, PP2Ce may function as a PLN-specific protein phosphatase that mediates adverse effects in heart failure and ischemia/reperfusion through attenuated beta-adrenergic PLN effects. PP2Ce may be of relevance in humans, because expression was increased in samples from patients with ischemic and dilated cardiomyopathy (3).

How does the new phosphatase concept fit into our current understanding of PLN function?

Cardiac SERCA2a function depends on a multimeric protein complex, with PLN being the major regulatory molecule of the Ca^{2+} pump (4). Although PLN exists in an equilibrium between monomeric and oligomeric forms, the dephosphorylated PLN monomer inhibits SERCA2a as opposed to PLN phosphorylation inducing oligomer formation. SERCA2a inhibitory PLN dephosphorylation occurs through protein phosphatase 1 (PP1), an ubiquitous enzyme with multiple substrates including RyR2 channels (9). Although PP1 has been described as a negative modulator of cardiac function, PP1 expression is significantly increased in failing human hearts (10), as was PP2Ce expression in human ischemic and dilated cardiomyopathy samples (3). But how does PLN regulation by PP1 and PP2Ce differ?

PP1 is endogenously regulated by inhibitory peptides I-1 and I-2 (4). Upon PKA phosphorylation of I-1, PP1 phosphatase activity is attenuated, which amplifies ß-adrenergic signaling. Interestingly, I-1

was proposed for therapeutic targeting in heart failure (11). Recently, miR-765, which is increased in human failing hearts, was shown to suppress I-1 expression, which may depress cardiac function through enhanced PP1 activity (12). In this regard, the new finding that PP2Ce regulates PLN differs considerably from PP1 because: 1) PLN seems to be a specific substrate of PP2Ce in the myocardium; and 2) PP2Ce was up-regulated following ISO stimulation, and therefore, may constitute an inhibitory feedback loop. These aspects not only add important new insights about PLN regulation in general, because increased PP2Ce expression was associated with human heart failure and cardiac-specific PP2Ce overexpression increased ischemia/reperfusion injury (3), but may contribute to new opportunities for PLN-targeted, SERCA2a-modulatory therapeutic approaches.

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