Is Phospholamban or Troponin I the "Prima Donna" in \(\beta\)-Adrenergic Induced Lusitropy?

Genaro A. Ramirez-Correa, Anne M. Murphy

In normal myocardium, acute \(\beta\)-adrenergic stimulation augments both systolic and diastolic performance via protein kinase A (PKA)–mediated phosphorylation of key proteins governing \(\text{Ca}^{2+}\) handling and the contractile machinery. Twenty-five years ago Kranias and Solaro identified troponin I (TnI) and phospholamban (PLN) as the 2 major cardiac proteins which were nearly simultaneously phosphorylated by \(\beta\)-adrenergic stimulation of the beating heart in synchrony with the agonist effect of augmenting contractility (inotropy) and rate of relaxation (lusitropy).\(^1\) Numerous subsequent studies have mechanistically examined the role of these proteins in inotropy and lusitropy. It is well established that enhanced \(\text{Ca}^{2+}\) availability during systole is the major, though perhaps not the only, driver of enhanced inotropy with \(\beta\)-adrenergic stimulation.\(^2\)–\(^4\) The effect on \(\text{Ca}^{2+}\) dynamics is primarily mediated by phosphorylation of PLN, though increased ionic current through the L-type calcium channel contributes to \(\text{Ca}^{2+}\) loading. When PLN is phosphorylated by PKA, the “brake” imposed by PLN on sarcoplasmic reticulum (SR) \(\text{Ca}^{2+}\) ATPase is relieved, resulting in an increase in the activity of the latter that leads to a faster sequestration of \(\text{Ca}^{2+}\) into the SR, enhancing cardiac relaxation and re-loading the SR with \(\text{Ca}^{2+}\) to increase \(\text{Ca}^{2+}\) release in subsequent beats. Yet, PLN phosphorylation by PKA is not the sole mechanism implicated in enhancing relaxation. In particular, phosphorylation of TnI by PKA has long been proposed to have a role in diastole because it desensitizes the myofilament to \(\text{Ca}^{2+}\), increases the off rate of \(\text{Ca}^{2+}\) from troponin, and speeds cross-bridge cycling (reviewed in\(^5\)), although PKA phosphorylation of myosin binding protein C may also contribute to these effects. In the past decade a more detailed set of experiments to further establish that inotropic and lusitropic effects of PKA are mediated by phosphorylation at PKA sites of TnI and PLN were nearly simultaneously phosphorylated (Ser 23/24) and PLN phosphorylation.\(^6\)\(^,\)\(^7\) The relative role of PKA-mediated induced cardiac TnI phosphorylation on lusitropy is mechanistically and practically important, particularly with respect to congestive heart failure (CHF) because the TnI PKA sites (Ser 23/24) are hypophosphorylated in failing human hearts.\(^8\) However, because regulation of \(\text{Ca}^{2+}\) dynamics via SR is also altered in heart failure (reviewed in\(^9\)), understanding the relative contribution of phosphorylation of these two proteins is crucial.

In this issue of Circulation Research, Yasuda et al describe a detailed set of experiments to further establish that in addition to PLN phosphorylation, TnI phosphorylation is a major player in the lusitropic effects of \(\beta\)-adrenergic stimulation.\(^10\) The first set of these experiments takes advantage of a transgenic line in which native cardiac TnI is essentially completely replaced by a version in which the 2 Serines targeted for \(\beta\)-adrenergic mediated phosphorylation are mutated to Aspartic Acids (cTnI S23/24D) thus mimicking maximal phosphorylation. Yasuda et al demonstrate that in cardiac myocytes from cTnI S23/24D mice, twitch relaxation is faster at baseline with minimal further enhancement by \(\beta\)-adrenergic stimulation. Similarly, there is no further decrease in pCa50 (rightward shift) with the addition of PKA during \(\text{Ca}^{2+}\) sensitivity studies. It is worth noting that peak sarcomeric shortening and peak isometric twitch tension are increased by a similar magnitude in transgenic cTnI S23/24D and nontransgenic control mice on challenge with isoproterenol, indicating that the positive inotropic response is unchanged in the cTnI S23/24D mice. Along the same lines, stimulation with the \(\beta\)-agonist increased the peak \(\text{Ca}^{2+}\) transient and its rate of decay to the same degree in both cTnI S23/24D and the nontransgenic control groups. These studies indicate a significant contribution of TnI phosphorylation to \(\beta\)-adrenergically mediated lusitropy. To further consolidate their findings, the authors conducted a second set of experiments in which they used rat cardiac myocytes with adenoviral mediated expression of the pseudo phosphorylated TnI (cTnI S23/24D). For negative controls, they transduced expression of the nonphosphorylatable slow skeletal TnI vector was also used. They focus their observations on the lack of isoproterenol speeding of twitch relaxation after cTnI S23/24A gene transfer. They argue that the lack of enhanced twitch relaxation in response to isoproterenol in the nonphos-
phosphorylatable negative control (cTnI S23/24A) and the fact that Ca\(^{2+}\) transient decay rate is accelerated similarly in both groups (cTnI and cTnI S23/24A), supports the role of TnI in \(\beta\)-adrenergic mediated enhanced relaxation. The use of both chronic and acute manipulation of TnI phosphorylation is one of the strengths of the experimental approach in this article, for although compensatory changes in PLN have not been noted in this transgenic model, subtle changes can occur with such long-term models.

The authors conclude their studies with a quantitative analysis of the relative contribution of TnI and PLN phosphorylation to lusitropy. Indeed, they use a similar analytical approach as Li et al used to demonstrate the primacy of PLN phosphorylation to the lusitropic effect. Li et al concluded that over 80% of lusitropy was determined by PLN whereas Yasuda et al conclude that nearly 80% of lusitropy (as measured by shortening of time to 75% relaxation), is determined by TnI. Why the discrepancy? In part this may be attributable to differences in baseline TnI phosphorylation in the PLN knockout mice as pointed out in the discussion of Yasuda et al. However, the experiments of Li et al were also conducted at room temperature, whereas those of Yasuda et al at physiologic temperature. As noted by Janssen et al, myofilaments appear to be the rate limiting step in cardiac muscle relaxation at physiologic temperature.

How are these findings relevant to cardiac disease? To date, many gene transfer based strategies aimed to treat heart failure have been based on modifying calcium handling proteins such as SR Ca\(^{2+}\) ATPase and PLN (reviewed in14), following the mainstream notion that defective Ca\(^{2+}\) handling is cause rather than consequence of failing cardiac myocytes. However, others have suggested that myofilament dysfunction is a central player in the physiopathology of heart failure. Recently, Day et al have shown that gain of function strategies by genetically engineering the myofilaments, specifically a Histidine mutant of cardiac TnI, is feasible and effective in both chronic and acute animal models. Would a mutant TnI S23/24D gene transfer be capable of increasing cardiac relaxation in vivo in a failing heart? Certainly this and other studies suggest this is the case. This is notable because diastolic heart failure is a particularly prevalent in heart failure syndromes (\(\approx\)40% to 50% of cases), yet therapies for diastolic failure are lacking.

In summary, the study of Yasuda et al reports important new information reassessing the relative contribution of PLN and TnI to \(\beta\)-adrenergic–induced lusitropy in loaded and unloaded cardiac myocytes at physiological temperature. In doing so, they suggest that TnI may indeed be the “Prima Dona” of \(\beta\)-adrenergic induced lusitropy, and as such TnI is a valid target for future targeted therapies of diastolic heart failure.

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None.

### References


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