Inhibitor-1 is Potential Target for Enhancing Sarcoplasmic Reticulum Ca\(^{2+}\) Loading in Failing Hearts

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Dysregulated Ca\(^{2+}\) homeostasis is a hallmark of heart failure that is directly linked to cardiac dysfunction and lethal arrhythmia! It is associated with imbalance of kinases/phosphatase activities in the corresponding intracellular microdomains, leading to either hypophosphorylation of phospholamban (PLN)\(^2\) or hyperphosphorylation of ryanodine receptor (RyR)\(^3,4\) or a combination of both in failing cardiomyocytes. It has been increasingly recognized that dysregulation of protein phosphatase 1 (PP1) signaling is associated with the imbalance of kinase/phosphatase in the failing heart\(^3,4\). Therefore, PP1 inhibition has been thought of as a potential molecular target for restoring impaired Ca\(^{2+}\) cycling mediated by the sarcoplasmic reticulum (SR)\(^3,4\). In this regard, the role of inhibitor-1 (I-1), an endogenous PP1 inhibitor, has been drawing attention, not only for understanding the mechanism of increased PP1 activity in failing hearts, but also for its application as a potential therapeutic target!\(^5\)

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I-1 is a ubiquitously expressed acid- and heat-stable cytosolic protein that is primarily rich in skeletal and cardiac muscle!\(^6\) When I-1’s Thr35 residue is phosphorylated by protein kinase A (PKA), I-1 actively inhibits PP1 activity, potentiates PKA phosphorylation, and enhances cardiac contractility. On the other hand, when the Ser67 or Thr75 residues are phosphorylated by protein kinase C (PKC)\(^7,8\) or the Thr35 residue is dephosphorylated by PP2B\(^9\), I-1 becomes inactive, resulting in an increase in PP1 activity and suppression of cardiac contractility. Therefore, I-1 is also recognized as an important element of \(\beta\)-adrenergic signaling, as well as Gq-receptor coupled signaling (angiotensin II, endothelin, and \(\alpha\)-adrenergic signaling)\(^10\). It has been reported that in heart failure, phosphorylation of I-1 at Thr35 is reduced compared with normal hearts, and its expression level is significantly decreased!\(^11\) thereby contributing to impaired \(\beta\)-adrenergic signaling and hence cardiac dysfunction in heart failure.

In this issue, Kawashima et al\(^12\) report a specific effect of I-1 on the SR Ca\(^{2+}\) cycling in normal saponin-permeabilized rat cardiomyocytes. They found that administration of I-1 significantly potentiated PKA-mediated SR Ca\(^{2+}\) load without affecting Ca\(^{2+}\) release function, as assessed by caffeine-induced Ca\(^{2+}\) transient and Ca\(^{2+}\) spark frequency. The effects of I-1 on SR Ca\(^{2+}\) load and Ca\(^{2+}\) release function were also characterized as similar to nonspecific chemical inhibition of protein phosphatase by calyculin A. Indeed, there are at least 2 possible mechanisms by which Ca\(^{2+}\) spark frequency increased in the permeabilized cardiomyocytes; namely, an increased gating property of the RyR, increased SR Ca\(^{2+}\) loading (SR Ca\(^{2+}\) content) or a combination of both. SR Ca\(^{2+}\) loading seems to be a more important target for I-1 than Ca\(^{2+}\) release, because I-1 had no appreciable effect on Ca\(^{2+}\) spark frequency and caffeine-induced Ca\(^{2+}\) transient after an abrupt inhibition of the SR Ca\(^{2+}\) uptake by cyclopiazonic acid, a sarcoendoplasmic ATPase (SERCA2a) inhibitor (Figure 4 in Reference 12). The fact that I-1 had no significant effect on the correlation between intracellular Ca\(^{2+}\) concentration and Ca\(^{2+}\) spark frequency also suggests that the increase in Ca\(^{2+}\) spark frequency is mostly dependent on the SR Ca\(^{2+}\) content. In this respect, their findings appear to be consistent with the previously reported findings of Pathak et al\(^11\) showing that the constitutive overexpression of I-1 increased cardiac contractility, prevented cardiac hypertrophy and slowed progression of heart failure in a rat pressure-overload-induced heart failure model. Indeed, the constitutive I-1 expression caused an increase in PLN phosphorylation at Ser16, thereby increasing the amplitude of the Ca\(^{2+}\) transient and decreasing its decay time. Thus, I-1 is considered to be a potential agent for optimizing SR Ca\(^{2+}\) handling in the diseased heart.

However, the interpretation should be carefully made with regard to its therapeutic application. Recent findings by Eli-Armouche et al\(^13\) contradict the beneficial effect of I-1 on cardiac function. Namely, cardiac overexpression of the full length I-1 rather caused significant cardiac hypertrophy, whereas I-1 knockout mice were resistant to cardiac hypertrophy during chronic administration of high-dose isoproterenol, and moreover, protected from lethal arrhythmia under certain anesthetic conditions. Although it is unclear why the aforementioned 2 types of experiments of I-1 overexpression caused such diverse phenotypes of cardiac hypertrophy, the differences may be partly attributable to the different levels of I-1 expression, lack of I-1 regulatory property in the constitutive I-1 expressed hearts, or a compensatory increase in PP1 catalytic subunits by 3-fold in the full-length I-1 overexpressed hearts. Further study of the in vivo effect of I-1 on SR Ca\(^{2+}\) loading should be considered. In particular, clarification of I-1’s binding partner may determine the preferential effect of I-1 on SR Ca\(^{2+}\) loading, which would be of interest.

On the other hand, the phosphorylation level of RyR at Ser2815 was found to decrease in the I-1 knockout mouse!\(^13\) suggesting that I-1’s effect is also involved in RyR function,
which seems inconsistent with the results obtained by Kawashima et al. Further investigation may be required in this aspect.

In addition, it is well known that β-adrenergic stimulation displays a diverse response in association with the receptor downregulation mechanism. As I-1 is also an important element of β-adrenergic receptor signaling, the effect of I-1 on SR Ca\(^{2+}\) cycling may differ between the acute and chronic stimulation settings. An experimental design that dissects the acute and chronic effects of β-adrenergic stimulation may be important in future.

In conclusion, Kawashima et al report that I-1 preferentially potentiated the PKA-mediated increase in SR Ca\(^{2+}\) loading without appreciable effects on Ca\(^{2+}\) release. As PP1, a major element of serine/threonine phosphatases, reportedly plays a critical role in dysregulated Ca\(^{2+}\) homeostasis in the failing heart, these findings may provide an important clue to a new therapeutic strategy against heart failure. To suppress PP1 signaling, either small molecular applications or cardiac-specific gene delivery would be effective. The therapeutic usefulness of PP1 inhibition by I-1 or other endogenous inhibitors should be further investigated by dissecting the mechanism and carefully determining potential adverse effects in either heart failure or lethal arrhythmia.

References