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Role of protein phosphatase-1 inhibitor-1 in cardiac physiology and pathophysiology

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Abstract

The type 1 protein phosphatase (PP1) is a critical negative regulator of Ca²⁺ cycling and contractility in the cardiomyocyte. In particular, it mediates restoration of cardiac function to basal levels, after β -adrenergic stimulation, by dephosphorylating key phospho-proteins. PP1 is a holoenzyme comprised of its catalytic and auxiliary subunits. These regulatory proteins dictate PP1's subcellular localization, substrate specificity and activity. Amongst them, inhibitor-1 is of particular importance since it has been implicated as an integrator of multiple neurohormonal pathways, which finely regulate PP1 activity, at the level of the sarcoplasmic reticulum (SR). In fact, perturbations in the regulation of PP1 by inhibitor-1 have been implicated in the pathogenesis of heart failure, suggesting that inhibitor-1-based therapeutic interventions may ameliorate cardiac dysfunction and remodeling in the failing heart. This review will discuss the current views on the role of inhibitor-1 in cardiac physiology, its possible contribution to cardiac disease and its potential as a novel therapeutic strategy.

Keywords

protein phosphatase-1; inhibitor-1; phospholamban; heart failure; sarcoplasmic reticulum; Ca²⁺ cycling

1. Introduction

Although major strides have been made in unraveling the mechanisms, which contribute to disease etiology in the heart, cardiovascular disease still remains the leading cause of morbidity and mortality in the United States, with an estimated 80.7 million American adults having been diagnosed with one or more types of cardiovascular disease. Amongst them, heart failure represents the fastest growing subcategory over the past ten years [1]. In fact, it has been reported that 5.3 million Americans were afflicted with heart failure in 2005. Even though current treatments, such as β -blockers and Angiotensin-converting enzyme (ACE) inhibitors

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have improved the overall prognosis of heart failure, the 5-year mortality rate still remains at an alarming 50%. Furthermore, 80% of men and 70% of women under the age of 65, suffering from heart failure, appear to die within eight years [2]. In light of the growing prevalence and detrimental effects of heart failure, the identification of contributing subcellular mechanisms has been the focus of intense research, in the hope of identifying more efficacious therapeutic targets. Amongst the causes of this multifactorial disease, disturbed Ca^{2+} homeostasis [3] and depressed Ca^{2+} cycling [4,5] have been reported as central contributors to cardiac dysfunction. Since the sarcoplasmic reticulum (SR) is a critical regulator of Ca^{2+} handling in the cardiomyocyte, therapeutic interventions have been targeted at rectifying Ca^{2+} homeostasis at the level of this organelle.

1.1. SR Ca^{2+} cycling and excitation-contraction coupling in the cardiomyocyte

The SR is a specialized network of endoplasmic reticulum in the heart, which regulates Ca^{2+} dynamics on a beat-to-beat basis, through Ca^{2+} release during systole and Ca^{2+} sequestration during relaxation (diastole). The cellular events governing these processes are described below. Upon depolarization, a small amount of Ca^{2+} enters the cardiac cell through the L-type Ca^{2+} channels (LTCC), which are localized on special invaginations of the plasma membrane, namely the transverse tubules (t-tubules). Since the t-tubules are in close proximity to the SR, LTCC-mediated Ca^{2+} entry causes a localized increase in Ca^{2+} in the cleft between the sarcolemma and the SR, which activates the Ca^{2+} -release channel on the SR membrane or ryanodine receptor (RyR) to release Ca^{2+} into the cytosol. This process is termed Calcium-induced-Calcium-release (CICR) [6]. The Ca^{2+} subsequently diffuses to the myofilaments, where it binds to troponin C (TnC), enabling actomyosin complex formation and contraction. This process is termed excitation-contraction coupling, since it facilitates the transduction of an electrical stimulus to mechanical function. Relaxation is initiated by the uptake of Ca^{2+} into the SR by the Ca^{2+} -ATPase, SERCA2a, which is under the regulation of the small phospho-protein phospholamban (PLN), and to a lesser extent by the Na^{+} - Ca^{2+} exchanger (NCX), the sarcolemmal Ca^{2+} -ATPase and a mitochondrial Ca^{2+} uniporter.

During sympathetic stimulation, human cardiac output can increase by as much as 5-fold, to accommodate increased demands. This is attributed to stimulation of the β -adrenergic neurohormonal axis, which increases the rate and amplitude of Ca^{2+} cycling, with concomitant increases in contractility. In particular, catecholamine-mediated activation of adenylase cyclase leads to increases in intracellular cAMP levels, which activate protein kinase A (PKA) [7]. In turn, PKA phosphorylates a set of key, regulatory phospho-proteins, including LTCCs, RyR, troponin I (TnI), Myosin Binding Protein C (MyBP-C) and PLN, which effectively enhance contractile function. Restoration of cardiac performance to basal levels is mediated by dephosphorylation of these substrates by protein phosphatases. In the heart, the majority of phosphatase activity is attributed to type 1 protein phosphatase PP1, and type 2 phosphatases, PP2A and PP2B. Among these phosphatases, PP1 is of particular importance since it has been implicated as an important negative regulator of cardiac function. In fact, alterations in its levels and activity have been reported to play a major role in the pathogenesis of heart disease. Intriguingly, this aberrant activity has been, at least partially, attributed to disrupted regulation by its endogenous modulator, inhibitor-1 (I-1). This review will summarize the current views on the fine regulation of PP1 by I-1, its possible contribution to heart disease and the potential of I-1 as a novel therapeutic target.

2. Protein Phosphatase-1 (PP1) And Inhibitor-1 (I-1)

The intricate regulation of phosphorylation, which exists in the cell, is governed by the balance between kinase and phosphatase activities. It has been reported that the human genome encodes for as many as 500 protein kinases, which enables high diversity and fine-tuned regulation of protein phosphorylation. Conversely, only ~ 150 phosphatases, of which ~ 40 are Ser/Thr

phosphatases, are encoded by the human genome [8]. Therefore, these enzymes rely on combinatorial mechanisms to achieve the high versatility exhibited by the large number of kinases. Consistent with this paradigm, PP1 is a holoenzyme comprised of a catalytic subunit, with phosphatase activity, complexed with as many as 100 putative or established auxiliary proteins [9,10]. These proteins target PP1 to appropriate subcellular compartments, supposedly dictate substrate specificity and modulate its catalytic activity. The following sections will discuss the role of PP1 in the heart and its critical regulation by the endogenous phosphoprotein, I-1.

2.1. The role of PP1 in cardiac contractility

Early studies, in SR-enriched preparations, indicated that PP1 was the main phosphatase responsible for dephosphorylating PLN [11,12]. Based on the major role of PLN in cardiac function, it was postulated that PP1 may also be a critical regulator of contractility. As mentioned above, PLN regulates the activity of the Ca^{2+} -ATPase pump on the SR. Specifically, dephosphorylated PLN exhibits an inhibitory effect on SERCA2a. PKA- and CaMKII-dependent phosphorylation of PLN at Ser16 and Thr17, respectively, relieves the inhibition and allows for increased Ca^{2+} pumping into the SR. Extensive investigations, by the generation and characterization of genetically altered models, established PLN as a key regulator of both basal contractility and the heart's β -agonist responses (reviewed in 13-16). As such, the identification of PP1 as a putative regulator of PLN phosphorylation was of great importance. Indeed, subsequent studies indicated that PP1 is a key regulator of cardiac performance.

Early studies, utilizing non-specific inhibitors, which inhibit both PP1 and PP2A at low concentrations, provided the first evidence for the potential physiological significance of PP1 in the heart. In particular, two independent investigations showed that treatment of isolated guinea-pig ventricular muscle with okadaic acid effectively enhanced contractility [17,18]. Furthermore, calyculin A or cantharidin administration also increased contractility in isolated guinea pig ventricular myocytes and in human cardiac preparations [19,20,21]. These positive inotropic effects were associated with increases in the phosphorylation of key phosphoproteins including PLN, TnI, myosin light chain 2 (MLC2), C-protein and the LTCC. However, the interpretation of these results was confounded by the lack of specificity of these inhibitors. Carr and colleagues were the first to provide direct evidence into the role of PP1 *in vivo*, though the generation and characterization of mice with cardiac-specific overexpression of PP1c- α [22]. These hearts had depressed basal contractile function and a blunted β -adrenergic response. The inhibitory effects were attributed to increases in PP1 activity (almost three-fold) and diminished PLN phosphorylation. Further insights into the role of this phosphatase were provided by genetic manipulations of its endogenous inhibitors, I-1 and I-2. The detailed role of I-1 is discussed in section 2.2 below. The role of I-2 was elucidated by Kirchhefer and colleagues, who reported that cardiac-specific expression of a truncated (AA: 1-140) and constitutively active form of I-2 (I-2*) depressed PP1 activity, enhanced contractile parameters and increased Ca^{2+} transient kinetics [23]. Interestingly, the authors demonstrated that these effects were associated with increased PLN phosphorylation at Ser16 but not at Thr17, suggesting that the PP1c/I-2* complex may preferentially dephosphorylate the PKA site in PLN. Further studies indicated that the phenotype of PP1c- α transgenic mice was rescued by co-expression of I-2*, thus supporting an important role of I-2 in the regulation of PP1 [24]. Collectively, these results indicated that PP1 is a critical negative modulator of cardiac function.

2.2. The role of I-1 in cardiac function

Inhibitor-1 (I-1) was the first recognized endogenous inhibitor of PP1 [25]. Early studies indicated that it has unusual properties in that it has little ordered structure [26,27] and is extremely stable to heat, low pH, organic solvents and detergents [25,27,28]. Mechanistic

insights into the regulation of PP1c by I-1 and other auxiliary proteins came from extensive crystallographic and biochemical studies. Goldberg and colleagues were the first to resolve the crystal structure of PP1c, complexed with the inhibitory toxin microcystin [29]. This study identified three surface grooves projected from a central scaffold in PP1c, as potential binding sites for regulatory proteins. Furthermore, these authors identified the hydrophobic groove in the β 12- β 13 loop, which is close to the active site, as the site of interaction with inhibitory toxins. Further studies indicated that alterations in this loop attenuated inhibition by toxins, phosphatase inhibitors and endogenous regulatory proteins, including I-1 [30-32], suggesting that this region is important for the interaction between PP1c and I-1. In addition, it has been reported that I-1 contains an "RVXF" motif sequence, similarly to other PP1c-binding proteins, which facilitates its interaction with PP1c [33]. It would be of great interest to further identify the structural elements in I-1 that dictate its specificity for PP1c, which may lead to the design of more specific and potent PP1c inhibitors, with potential clinical application.

I-1 is expressed in all mammalian tissues. In fact, its significance has been studied extensively in the brain and skeletal muscle, where it has been implicated in neuronal plasticity and glycogen metabolism, respectively [34-37]. However, its role in the heart has remained obscure and only recently have its effects begun to be deciphered. Biochemical studies indicated that I-1 potently inhibits PP1 activity (IC_{50} : 1 nM), when phosphorylated at Thr35 by the cAMP-dependent kinase, PKA [25,38]. The presence of I-1 in the heart was initially documented in guinea pig ventricles [39,40] and in rat heart slices [41]. Further studies indicated that I-1 is found in isolated ventricular cardiomyocytes [42]. Importantly, these studies showed that I-1 is hormonally regulated and that isoproterenol treatment resulted in I-1 phosphorylation, associated with decreased PP1 activity, in these cardiac preparations [40-42]. These lines of evidence suggested that I-1 may be an important mediator of the heart's β -adrenergic responses, through modulation of PP1's catalytic activity. The characterization of a knock-out (KO) mouse model with I-1 ablation provided the first evidence for the physiological significance of I-1 in the heart *in vivo*. Echocardiographic assessment indicated that ablation of I-1 was associated with depressed basal cardiac function, which did not progress further upon aging to 15 months. In work-performing hearts, I-1 ablation was associated with a mild decrease ($\sim 10\%$; $P > 0.05$) in basal contractile parameters, a significantly blunted β -adrenergic response and enhanced PP1 activity [22]. Interestingly, these effects were attributed to depressed PLN phosphorylation at both Ser16 and Thr17, while the LTCC and TnI phosphorylation were not affected, suggesting that I-1 may preferentially regulate the SR-coupled PP1 activity [22]. Collectively, these results indicated that I-1 may be an important regulator of both basal and β -adrenergic-mediated contractile function. However, another study found that I-1 null hearts displayed normal basal contractility and a preserved β -adrenergic response, assessed by echocardiographic and catheterization analysis [43]. However, isoprenaline was less potent in I-1 deficient left atria, indicating that I-1 ablation induced a mild β -adrenergic desensitization [43]. Even though the reasons for these discrepancies remain elusive, it is possible that differential experimental conditions, including the extent of anesthesia and catecholaminergic stimulation may account for the different effects. Nonetheless both studies substantiated a role for I-1 in the heart's contractile response.

Additional lines of evidence further corroborated an important role for I-1 in the heart. It has been reported that adenoviral-mediated overexpression of I-1 in engineered heart tissue as well as in neonatal and adult rat cardiomyocytes increased contractile function, associated with decreased PP1 activity and increased pSer16-PLN phosphorylation, upon stimulation with isoprenaline [44]. Conversely, cardiac-specific overexpression of I-1 *in vivo* (by ~ 200 -fold) was associated with contractile dysfunction and cardiac hypertrophy [43]. Even though, no differences were observed in PLN phosphorylation *in vivo*, I-1 TG hearts exhibited depressed pSer16-PLN phosphorylation *ex vivo*, associated with enhanced PP1 activity. These results may be explained by the observed compensatory increases in PP1c levels. In agreement with

the amplifier role of I-1 in β -adrenergic signaling, PLN phosphorylation at Ser16 was enhanced upon isoprenaline stimulation *ex vivo*. The interpretation of these results is limited by the exceedingly high overexpression levels of I-1 and confounding increases in PP1c levels.

Conversely to the effects noted with overexpression of full-length I-1, transgenic expression of a truncated (AA: 1-65), constitutively active (T35D) form of I-1 (I-1c) *in vivo*, was associated with decreased SR-coupled PP1 activity and enhanced contractility both basally and after β -adrenergic stimulation [45]. Interestingly, these effects were specifically associated with enhanced PLN phosphorylation at its PKA- (Ser16) and CaMKII- (Thr17) dependent sites, while phosphorylation of the RyR at Ser2808 and TnI phosphorylation at Ser22/Ser23 were unaffected, in agreement with the specific effects of I-1 observed in the I-1 KO mouse model by the same group. However, the apparent specificity of I-1 for PLN in these studies was challenged by the fact that chronic ablation or expression results in compensatory alterations, arising during early development, which may confound the resultant phenotype. As such, these studies were extended to an inducible transgenic model, which allowed for expression of I-1c in the adult heart [46]. The findings indicated that expression of I-1c in the adult heart augmented basal cardiac performance. Immunoblotting analysis revealed that these effects were associated with preferential enhancement of PLN phosphorylation at Ser16 and Thr17, since phosphorylation of the RyR at Ser2808, TnI phosphorylation at Ser22/Ser23 and phosphorylation of Ser282 in MyBP-C were unaltered by expression of I-1c. Importantly, these authors employed a phospho-proteomics approach to identify other potential I-1c-regulated phospho-substrates. Interestingly, only proteins involved in energy production and protein synthesis were altered, possibly to accommodate the increased metabolic demands in these hearts. Collectively, these experiments suggested that I-1 may be acting as a molecular inotrope by suppressing PP1 activity and allowing for unopposed increases in the phosphorylation of PLN, which amplifies the β -agonist response. Crucial for the regulation of I-1 is its inactivation by dephosphorylation at Thr35, which relieves PP1 inhibition and allows for restoration of function to basal levels. El Armouche and colleagues have shown that I-1 becomes dephosphorylated at Thr35 by PP2A and PP2B in neonatal rat cardiomyocytes [47]. These results suggest that the cross-talk between the cAMP and Ca^{2+} signaling pathways may be partly mediated through I-1 (Figure 1A).

Although it is now generally accepted that phosphorylation at Thr35 plays an amplifier role in β -adrenergic neurohormonal stimulation, the role of other identified phosphorylation sites in I-1 is less clear. As such, recent studies have focused on elucidating the role of these phosphorylation sites on I-1 activity. Aitken and colleagues were the first to report that I-1 could also be phosphorylated at another distinct site, namely Ser67 in skeletal muscle. These authors further reported that Ser67 could also be phosphorylated by PKA *in vitro*, but at a much slower rate than Thr35, with no effects on PP1 activity [26]. Conversely, a subsequent study indicated that Ser67 was phosphorylated by neuronal cdc2-like protein kinase (NCLK) in brain extracts, resulting in potent PP1 inhibition, similar to phosphorylation at Thr35 [48]. Further experimental evidence by Bibb and colleagues indicated that Ser67 showed poor specificity *in vitro* and could be phosphorylated by several kinases, including cyclin-dependent kinase 1 (Cdk1), cyclin-dependent kinase 5 (Cdk5) and mitogen-activated protein kinase. However, this site was only phosphorylated by Cdk5 in striatal brain tissue *in vivo* [49]. Similarly to the study by Aitken and collaborators, these authors also reported that I-1 activity was unaltered by phosphorylation at Ser67. Intriguingly, they did find that phosphorylation at Ser67 makes I-1 a poor substrate for phosphorylation at the Thr35 PKA site, suggesting that this site may play an important regulatory role on I-1 *in vivo*. A subsequent study by the same group identified a PKC phosphorylation site in the rat I-1 (Accession #: NP_073167.1) at Ser65 [50]. Interestingly, phosphorylation at this site also prevented efficient phosphorylation at Thr35, similarly to their reported results with Ser67. However, Ser65 is substituted by aspartic acid in humans (Accession #: NP_006732.3), limiting the significance of this site in human

physiology. The first evidence for the functional role of phosphorylation of I-1 at Ser67 in the heart was shown in a study by Braz and colleagues [51]. These authors reported that ablation of PKC- α was associated with depressed phosphorylation of I-1 at Ser67, decreased PP1 activity, enhanced PLN phosphorylation and augmented contractile function. Conversely, cardiac-specific overexpression of PKC- α was associated with enhanced phosphorylation at Ser67, increased PP1 activity, decreased PLN phosphorylation and depressed contractility. In contrast to previous studies, phosphorylation of I-1 at the Ser67 site did not affect Thr35 phosphorylation but altered the formation of the PP1c/I-1 complex, providing yet another possible mechanism for the effects of phosphorylation at this residue [51]. Collectively, these results suggested that Ser67 may be phosphorylated by PKC- α *in vivo* and positively regulate PP1 activity (Figure 1A).

An additional PKC- α phosphorylation site on human I-1, Thr75, has recently been identified [52]. Phosphorylation at this site was also associated with enhanced PP1 activity, decreased PLN phosphorylation and depressed contractile function in isolated cardiomyocytes [52]. A more recent study compared the effects of the Thr75 site to those of the Ser67 site and showed that phosphorylation at Ser67 and/or Thr75 depressed contractile function to a similar extent in isolated cardiomyocytes [53]. Furthermore, activation of the cAMP pathway, using forskolin, could not completely reverse the depressed mechanical parameters, which may be due to inefficient phosphorylation of Thr35. Overall, these data suggest that I-1 may be an important mediator of the crosstalk between the PKA and PKC pathways in the heart (Figure 1A). As such, I-1 appears to be a more complex regulator than previously thought and may modulate PP1's activity according to differential cellular conditions, which activate these signaling pathways.

3. The Role Of Pp-1 And I-1 In The Failing Heart

The fine equilibrium of protein phosphorylation, which is enacted by protein kinases and phosphatases in the cardiomyocyte, is disrupted in the failing heart. This disruption has been partially attributed to attenuation of the β -adrenergic cascade due to receptor desensitization, receptor downregulation and uncoupling, which occurs during disease progression [54,55]. However, altered phosphatase activation may also play an important role in heart disease pathogenesis. Indeed, accumulating evidence has indicated that PP1 activity is increased both in the human failing heart and in experimental models of heart failure. Neumann and colleagues were the first to demonstrate that PP1 mRNA levels and activity were increased in patients with end-stage heart failure [56]. Mishra and colleagues further validated these findings, when they reported that protein phosphatase activity, attributed both to PP1 and PP2A, was augmented in idiopathic dilated cardiomyopathic patients [57]. Subsequent studies reported that PP1 activity was enhanced in rat models of myocardial infarction [58,59], β -adrenergic-induced cardiac hypertrophy [60] and congestive heart failure induced by chronic renal hypertension [61]. Importantly, Gupta and colleagues extended these studies and showed that SR-coupled PP1 activity was specifically increased in a canine model of heart failure [62]. Collectively, this experimental evidence suggests that enhanced PP1 activity may be a universal characteristic of heart failure, associated with cardiac dysfunction. Indeed, overexpression of PP1c- α , in mouse hearts, to levels similar to those observed in human failing hearts (3-fold) depressed cardiac function and led to dilated cardiomyopathy, with premature mortality [22]. Overall, these data implicate PP1 as an important contributing factor to cardiac etiopathogenesis.

The observed increase in PP1 activity in heart failure raised the possibility that regulation of this phosphatase via its endogenous regulator I-1 may be impaired, which may account for the alterations in the enzyme's activity. Indeed, two independent studies showed that phosphorylation of I-1 at its Thr35 site was depressed in human failing hearts [22,63]. A

subsequent study demonstrated that phosphorylation of I-1 at Thr35 was depressed in a rat heart failure model induced by renal hypertension [61]. These results may be a reflection of attenuated PKA activity and increased calcineurin activity observed in the failing heart [64]. Furthermore, it has been reported that phosphorylation of I-1 at Ser67 is enhanced in failing human hearts [51], which may be attributed to enhanced PKC- α activity [65]. The depressed phosphorylation at Thr35 and enhanced Ser67 phosphorylation in I-1 are consistent with a decrease in its inhibitory activity and enhanced PP1 activity observed in heart failure (Figure 1B). Decreased I-1 protein levels have also been observed in human failing hearts [63], in a canine heart failure model [62] and a rat model subjected to prolonged β -adrenergic stimulation [66], thereby providing another mechanism by which PP1 activity may be enhanced under this pathophysiological setting [22]. More recently, a human polymorphism has been identified in the human I-1 gene (G147D), which was associated with blunted β -adrenergic responses and depressed PLN phosphorylation in isolated cardiomyocytes [67], suggesting that this transversion may contribute to depressed SR Ca^{2+} cycling and functional deterioration in the failing heart. In addition, alterations in PP1 activity have been reported in chronic atrial fibrillation (cAF) in humans [68]. Total PP1 activity was increased in cAF, associated with depressed phosphorylation of pSer282-MyBP-C. Paradoxically, phosphorylation of I-1 at Thr35 and consequently PLN phosphorylation were found to be enhanced, demonstrating differential compartmentalization and regulation of kinases and phosphatases in the cell. The authors postulated that enhanced PLN phosphorylation in the atria may further enhance leakiness of the RyR and trigger arrhythmogenic activity. Collectively, these reports demonstrated that regulation of PP1 is altered in heart disease, suggesting that restoration of proper PP1 activity by I-1 may be a novel therapeutic strategy to rectify the disturbed Ca^{2+} homeostasis and Ca^{2+} cycling in the failing heart.

4. I-1: A Potential New Therapeutic Target

There is a need for new therapies to reverse the course of ventricular dysfunction during the progression of heart failure. Heart failure induced by genetic mutations, coronary artery disease, myocardial infarction, hypertension, diabetes, infection or inflammation results in a myocardium with a mixture of replacement fibrosis as well as dysfunctional and normal myocytes. The normal myocytes that remain are under continuous stress from hormonal and physical stimuli that can induce apoptosis and cell death or render them dysfunctional. Although current treatments have improved survival in this patient population, these therapies do not arrest further cardiac deterioration and death, with the 5-year survival rates at less than 50% in the heart failure population and in end-stage heart failure, the 1-year survival may be as low as 25% [1]. Reversing the contractile failure of cardiac myocytes with the use of standard pharmacological inotropic agents has been controversial, most likely due to the pleiotropic intracellular effects of the targeted molecules [69]. As such, it has been suggested that a targeted approach may be more effective in alleviating the depressed function. In this respect, I-1 is an interesting molecule, since several studies have indicated that it may preferentially regulate the PP1 associated with the SR. Therefore, recent studies have focused on its potential use as a therapeutic agent or target.

Carr and colleagues [22] were the first to demonstrate a potential therapeutic strategy, utilizing the constitutively active and truncated form of I-1 described above (I-1c), which lacks the PKC- α sites, which mitigate I-1's beneficial effects. Adenoviral-mediated expression of I-1c enhanced the contractile parameters and Ca^{2+} kinetics of human failing cardiomyocytes, upon maximal (100 nM) isoproterenol stimulation [22]. The potential benefits of I-1c were further assessed *in vivo*, through the generation of a transgenic mouse model with cardiac-specific expression of this protein [45]. Upon trans-aortic constriction, I-1c hearts maintained their enhanced cardiac performance. Importantly, these mice exhibited an attenuated progression to heart failure, characterized by a diminished extent of cardiac hypertrophy and fibrosis. In

addition, adenoviral-mediated expression of I-1c in a rat model of pressure overload-induced heart failure restored contractility to non-failing levels and halted the progression of cardiac dysfunction and decompensation. These beneficial effects were associated with enhanced PLN phosphorylation at Ser16. No alterations were observed at Thr17 phosphorylation, perhaps due to the enhanced CaMKII activity observed in the failing heart [70,71]. Furthermore, in agreement with previous data, no alterations were detected in phosphorylation of the RyR at Ser2808. This may have important implications since increased RyR phosphorylation may potentially lead to diastolic leakiness and arrhythmogenic activity [72,73]. Inducible expression of I-1c in the adult heart protects against ischemia/reperfusion (I/R) injury [46]. In particular, expression of I-1c ameliorated contractile dysfunction and attenuated cellular damage post-I/R, suggesting that I-1c may be a plausible therapeutic strategy not only in heart failure but also in myocardial infarction. These results in rodent models have been confirmed in large animal models of heart failure whereby acute and chronic gene transfer of I-1c by viral gene transfer induced both short-term and long-term beneficial hemodynamic effects (our unpublished data).

In recent studies, El Armouche and colleagues have also investigated the effects of I-1 ablation on isoprenaline-induced arrhythmias and cardiac hypertrophy [43]. The I-1 KO mice exhibited less susceptibility to acute isoprenaline-induced deaths, associated with a lesser extent of ventricular arrhythmias. I-1 ablation also protected against chronic isoprenaline-induced hypertrophy, dilatation and fibrosis. Furthermore, the I-1 KO mice showed a partially preserved β -adrenergic response. These effects were associated with decreased pSer16-PLN and pSer2815-RyR phosphorylation levels, while there were no significant changes in pThr17-PLN phosphorylation or the phosphorylation of RyR at Ser2808. Since phosphorylation of Ser2815 in RyR has been associated with diastolic Ca^{2+} leak and arrhythmogenic activity [74,75], it was postulated that I-1 ablation may diminish the extent of ventricular arrhythmias by decreasing RyR phosphorylation at this site. Future studies may be designed to further investigate pSer2815-RyR as a potential I-1 substrate and the associated consequences *in vivo*.

Although the findings on the beneficial effects of I-1 ablation against catecholamine-induced arrhythmias and remodeling may appear contradictory to the equally beneficial effects of increased I-1c expression against heart failure progression and post-ischemic injury, it should be noted that the studies on I-1c utilized a constitutively active form of I-1 (T35D), which lacked the detrimental PKC sites. Thus, it is possible that phosphorylation of the PKC sites has detrimental consequences during isoprenaline administration and ablation of I-1 is overall beneficial, under these conditions. Collectively, although the studies on I-1c point to the benefits associated with this molecule in the treatment of heart failure [22,45,46], further studies are required to address its therapeutic potential.

5. Conclusion And Perspective

In summary, several lines of experimental evidence indicate that proper cardiac function is maintained, in part, through the intricate regulation of I-1. Indeed, I-1 may be a nodal point in the cross-talk between Ca^{2+} -, PKA- and PKC-mediated signaling pathways, which coordinately regulate PP1 activity, SR- Ca^{2+} cycling and cardiac performance. In fact, disturbances in the fine regulation of I-1 have been implicated as important contributors to depressed cardiac function and remodeling in the failing heart. As such, targeting I-1 appears beneficial in alleviating the detrimental effects of heart failure, through specific modulation of the SR-coupled PP1.

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Abbreviations

PP1	type 1 protein phosphatase
Ca	Calcium
PP1c	type 1 protein phosphatase catalytic subunit
I-1	inhibitor-1
I-2	inhibitor-2
SR	sarcoplasmic reticulum
LTCC	L-type Ca channel
RyR	ryanodine receptor
SERCA2a	SR/ER-Ca ATPase 2a
PLN	phospholamban
NCX	sodium-calcium exchanger
cAMP	3'-5'-cyclic-AMP
PKA	protein kinase A
TnI	troponin I
MyBP-C	myosin binding protein C
PP2A	type 2 protein phosphatase A
PP2B	type 2 protein phosphatase B

CaMKII	calmodulin kinase 2
MLC2a	myosin light chain 2a
PKC	protein kinase C
Cdk1	cyclin dependent kinase 1
Cdk5	cyclin dependent kinase 5

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Figure 1.

Proposed role of I-1 in cardiac function and dysfunction. **A.** Phosphorylation of I-1 at Thr35, suppresses PP1 activity, resulting in enhanced contractility. Furthermore, phosphorylation of I-1 at Ser67 and/or Thr75 results in increases in PP1 activity, which concomitantly depresses contractility. **B.** The failing heart is characterized by enhanced PP1 activity and depressed cardiac performance. This is at least partially attributed to decreased phosphorylation of I-1 at Thr35, which could be due to altered PKA and/or PP2B activity in the failing heart. In addition, enhanced phosphorylation at Ser67, possibly due to increased PKC- α activity, also contributes to enhanced PP1 activity. I-1: Inhibitor-1; PP1/2B: protein phosphatase-1/2B; PKA: protein kinase A; PKC- α : protein kinase C- α .