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Protein Kinase A as A Promising Target for Heart Failure Drug Development

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Abstract

Heart failure (HF) is a clinical syndrome characterized by impaired ability of the heart to fill or eject blood. HF is rather prevalent and it represents the foremost reason of hospitalization in the United States. The costs linked to HF overrun those of all other causes of disabilities, and death in the United States and all over the developed as well as the developing countries which amplify the supreme significance of its prevention. Protein kinase (PK) A plays multiple roles in heart functions including, contraction, metabolism, ion fluxes, and gene transcription. Altered PKA activity is likely to cause the progression to cardiomyopathy and HF. Thus, this review is intended to focus on the roles of PKA and PKA-mediated signal transduction in the healthy heart as well as during the development of HF. Furthermore, the impact of cardiac PKA inhibition/activation will be highlighted to identify PKA as a potential target for the HF drug development.

Keywords

Heart failure; Cardiac hypertrophy; Cardiac muscle; PKA; PKA inhibitors

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Disclosures

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Introduction

The heart is no bigger than the size of a human adult fist, yet, this small organ beats more than two and a half billion times in an average lifetime without ever pausing to rest, in order to provide the contractile power needed for life. This remarkable muscle can remodel histologically, and cope functionally through various regulatory mechanisms. Often only when these mechanisms are no longer sufficiently adapting, the cardiac output that is sufficient to fulfill the body's need for oxygen, the disease manifests and the patient is diagnosed with HF. Many different underlying pathological conditions can result in HF including hypertension, ischemia, diabetes, idiopathic cardiomyopathy, congenital cardiovascular defects, and valvular diseases. However, the most common etiologies of HF are the coronary artery diseases and myocardial infarction (MI) (1).

HF affects more than 5 million adult Americans and this number is expected to increase to be > 8 million in 2030 (2,3). Fifty percent of patients with HF are readmitted to the hospital within 6 months of discharge (4) and half of them die within 5 years of diagnosis (2). In 2011, 1 in 9 death certificates (284,388 deaths) in the United States mentioned HF. HF was the underlying cause in 58,309 of those deaths. The total estimated cost for HF in 2012 was \$30.7 billion, and of this total 68% was attributable to direct medical costs (5). Estimates show that by 2030 the total cost of HF will increase almost 127% from 2012 to roughly be \$69.7 billion, which means approximately \$244 for every American adult (5). In fact, the costs linked to HF overrun those of all other causes of disabilities and death in the United States and all over the developed as well as the developing countries which amplify the supreme significance of its prevention (6).

Among the molecular alterations occurring during HF development that result in aggravation of the disease are the changes in protein kinase (PK) activities. Autophosphorylation, oxidative modifications, and interaction with non-enzymatic proteins are examples of these changes that can occur specifically during HF, as reviewed by Lorenz K, et al. (7). This makes the PKs an attractive target for therapeutic intervention.

One of these kinases is the PKA (cAMP-dependent protein kinase), a kinase that is strongly implicated in the progression of HF and other cardiac diseases. PKA is a serine/threonine kinase that is activated by cyclic adenosine monophosphate (cAMP). It is composed of two regulatory subunits and two catalytic subunits. There are 4 isoforms of the regulatory subunits (RI α , RI β , RII α , RII β) and 3 isoforms of the catalytic subunits (C α , C β , C γ), each of which has different patterns of tissue expression and subcellular localization (8,9). PKA is considered to be the most common downstream effector system for cAMP. In the absence of cAMP PKA is a heterotetramer of two identical catalytic subunits (PKA-C) and two identical regulatory subunits (PKA-R). However, in the presence of cAMP the regulatory subunits bind to cAMP and the catalytic subunits are released from the holoenzyme, allowing phosphorylation of target substrates. This process involves binding of an extracellular ligand to a G protein-coupled receptor, which through G proteins regulates one of several isoforms of the adenylyl cyclase leading to the generation of cAMP (Figure 1).

PKA plays multiple roles in heart function regulation including, contraction, metabolism, ion fluxes, and gene transcription. Altered PKA activity is likely to cause the progression to cardiomyopathy and HF. Thus, this review is intended to focus on the roles of PKA, and PKA-mediated signal transduction in the healthy heart as well as during the development of HF. Furthermore, the impact of cardiac PKA inhibition/activation will be highlighted to identify PKA as a potential target for the HF drug development.

Protein Kinase A (PKA) in Healthy and Failing Heart

Normally, PKA plays multiple roles in the heart function. Phosphorylation of PKA in cardiomyocytes regulates many processes including metabolism, transcription of numerous genes, ion fluxes, and contraction (Figure 2) (10). Regarding metabolism and energy production in muscle cells, both phosphorylation of phosphorylase kinase by PKA as well as calcium ions released from the sarcoplasmic reticulum during muscle contraction (11–13) play an important role in stimulating glycogen breakdown into free glucose. Muscle cells generally breakdown glycogen to provide energy during bursts of activity (14). In the meanwhile, phosphorylation of glycogen synthase, a key enzyme in glycogenesis, by PKA (15,16) leads to its deactivation and inhibits conversion of glucose into glycogen. Also, binding of norepinephrine to the β -adrenergic receptors (β AR) in the heart activates the cAMP-PKA pathway, leading to the phosphorylation of multiple target proteins (17,18). PKA phosphorylates and activates the transcription factor cAMP regulatory element binding proteins (CREB), which subsequently stimulates gene transcription (19,20). This allows interaction with the co-activator CREB binding protein, which in turn binds to components of the basal transcriptional machinery and affects the transcriptional function (21–23). Additionally, PKA has several substrates in the cardiomyocytes that influence contractility in response to activated β AR signaling. Activation of PKA-RII α by β AR signaling phosphorylates many of the target proteins involved in the excitation-contraction (E–C) coupling mechanism such as, cardiac troponin I (cTnI), cardiac myosin binding protein C (cMyBPC), phospholamban (PLB), L-type calcium channel, phosphodiesterase (PDE)4D3, CREB and the ryanodine receptor (RyR2) to modify their function and/or activity (24–26). PKA increases the Ca^{2+} current, SR Ca^{2+} uptake and release, SR Ca^{2+} content, and the dissociation of Ca^{2+} from the myofilaments which facilitate the contraction and relaxation of the heart through phosphorylation of these and other related substrates (Figure 3) (27,28). In this context, It has been documented that abnormal handling of Ca^{2+} at any of these steps could lead to cardiac dysfunction and HF (29).

In HF, deregulated Ca^{2+} fluxes as well as Ca^{2+} utilization due to altered PKA have been reported (30–32). For example, several reports have shown that in failing human hearts PLB phosphorylation as well as the SR Ca^{2+} were reduced resulting in decreased Ca^{2+} affinity of the SR Ca^{2+} pump and calcium current I_{Ca} -triggered Ca^{2+} -induced Ca^{2+} -release from the SR Ca^{2+} stores (33–38). Since PLB is phosphorylated by PKA (39,40), PKA-PLB interaction is proposed to be a potential target for drug development in the treatment of HF (41). In addition, phosphorylation of cMyBP, a downstream target of PKA, has been shown to be decreased in patients with atrial fibrillation, hypertrophic cardiomyopathy, and HF (42–44). Likewise, decreased expression of PKA regulatory subunits RI and RII in HF decreases the affinity of the troponin complex for Ca^{2+} , increases baseline myofibrillar Ca^{2+} sensitivity

resulting in cardiomyopathy in humans, due to reduced phosphorylation of cTnI and other targets (31).

On the other hand, activation of PKA has been shown to have a synergistic effect on PKC-induced stimulation of Raf-1 and mitogen-activated protein kinases in rat cardiomyocytes (45,46), which are involved in the development of cardiac hypertrophy (47). Moreover, alterations in muscle-specific A-kinase anchoring protein (AKAP) was found to affect the onset of cardiac hypertrophy (48). In view of the fact that AKAP binds to PKA regulatory subunits such as RII β in order to regulate its interactions and its downstream targets, it is more likely support the involvement of PKA in the development of cardiac hypertrophy. Consistent with that finding, PKA under the influence of AKAP-Lbc, an AKAP, phosphorylates the protein tyrosine phosphatase Shp2 (PTPN11) at Thr73 and Ser189, which was found to be contributing to β -AR-induced cardiomyocyte hypertrophy (49). Also, Wang J, et al. (30) have shown that the activity and protein level of PKA in HF is significantly increased compared to non-failing hearts. Increased PKA activity could lead to hyperphosphorylation of downstream targets such as RyRs resulting in loss of E-C coupling gain, both in failing human hearts (50) and in transgenic mice with dilated cardiomyopathy, arrhythmia, and sudden death (32). In this latter study, prolonged activation of PKA results also in hyperphosphorylation of PLB (32), yet, there are contradictory reports concerning hyperphosphorylation of PLB as we mentioned above (33–38).

Impact of Activating and Deactivating Protein Kinase A (PKA) Signaling Pathway on the Development of Cardiomyopathy

Table 1 summarizes past studies on the effect of different PKA inhibitors on the heart. Altered PKA signaling has been found to be involved in a number of physiological problems leading to hypertrophy (48). Since hypertrophy increases the risk of HF, prevention or reversal of this maladaptive phenotype has thus been proposed to treat HF (51). In this regard, Enns LC, et al. (52) have found that deletion of RII β regulatory subunit of PKA resulted in a cardio-protective effect against age-related pathologies, including cardiac hypertrophy and cardiac dysfunction. Similarly, C57/BL6J male mice lacking the PKA catalytic C β subunits have been found to resist cardiac hypertrophy induced by angiotensin II (53). In another study, using a PDE4 novel activator, UCR1C, was found to inhibit nuclear PKA activity and attenuate cardiomyocyte hypertrophy (54). Beside, Huang T-S, et al. (55) found that inhibition of PKA activity using H89 significantly inhibited thyrotropin-induced expression of HMG-CoA reductase, a known regulator of the expression of some HF marker proteins, such as β -myosin heavy chain and brain natriuretic peptide (56). Astoundingly, this latter study indicated for the first time the involvement of PKA signaling pathway in the development of HF associated with hypothyroidism (55). Likewise, increased Adrenomedullin levels in patients with HF, hypertension, and MI (57–60) have been linked to increased PKA activation (61). Inhibiting the PKA by KT5720 effectively attenuated the negative inotropic and lusitropic effects of adrenomedullin and improved cardiac function (62). Moreover, HF-induced myocardial depression was suggested to be mediated, at least in part, by inducible nitric oxide (NO) synthase (63–69). KT5720, a PKA inhibitor, has been reported to block the interleukin-1 β -induced NO formation, potentially reducing the effect of damage caused by NO synthase formation and preventing HF (70). Furthermore, both

PKA inhibitors, H89 and KT5720, exhibited a protective effect against NO-induced cytotoxicity in H9c2 cardiac cells (71). It has also been found that H89 partially inhibited the proliferation of T cells when used before stimulation with β_1 -Adrenoreceptor autoimmune antibodies (72). Given that the activation of T lymphocytes as well as increased inflammatory cytokines have been reported to be involved in the development of dilated cardiomyopathy and chronic HF (73,74) it may be concluded that inhibition of PKA could play an important role in attenuation of dilated cardiomyopathy and prevention of HF. Notably, prolonged exposure to PKA activators was found to stimulate the production of intracellular reactive oxygen species, which in turn significantly impaired the HERG K^+ channel function, a critical regulator of cardiac action potential repolarization (75–77), possibly leading to electrical disturbance in failing hearts (78,79). These effects were efficiently prevented by PKA inhibitor, H89 (79). Likewise, cAMP/PKA pathway through stimulation of β_2 -AR was demonstrated to inhibit the rectifier potassium current (I_{kr}) and resulted in action potential prolongation in ventricular myocytes of guinea pigs with HF. These inhibitory effects were fully prevented by intracellular application of Rp-cAMPS, competitive inhibitor of cAMP-dependent protein kinases, and partly attenuated by PKA inhibitor (80). Furthermore, PKA inhibition has been revealed to decrease cell death occurring in I/R and HF (81–86), and hence exerts protective effect against I/R injury (87–90). In an experimentally-induced ischemia model in rabbits, PKA inhibitor, H89, reversed the phosphorylation and kinetic properties of cytochrome C oxidase and thus protected the heart against ischemic injury (91). Inhibition of PKA has also been shown to have a great impact on improving cardiac function after MI. A PKA specific inhibition gene named PKI-GFP was reported to block the β -adrenergic agonist-induced myocyte death and improve cardiac function after MI (92). Interestingly, inhibition of PKA in this latter study was superior to a β_1 -blocker, metoprolol, in improving cardiac function, which suggests that selective inhibition of PKA could be an effective therapy in HF.

At variance with the above mentioned studies, Ha CH, et al. (93) reported that PKA attenuates angiotensin II-induced rat cardiomyocyte hypertrophy through inhibiting histone deacetylase 5 pathway. In addition, NO-induced cardiomyocytes apoptosis was suggested to be protected by PDE4 inhibitor, roflumilast, via dual activation of the PKA and Epac pathways (88). Finally, PKA inhibitors were shown to prevent the growth hormone releasing hormone-induced protective benefits on cardiac performance in I/R injury (87). Similarly, PKA has also been shown to enhance the phosphorylation and assembly of the proteasome *in-vivo*, and this effect was blunted by administration of PKA inhibitor, H89 (94). Of note, it has been reported that impairment of proteasome activity may contribute to the progression of cardiac dysfunction and I/R (95,96). Our own recent data on ex vivo human cardiac trabeculae showed that PKA inhibition by the H-89 did not significantly affect the rate of tension redevelopment (k_{tr}) at either L_{90} (90% of optimal length) or at L_{opt} (optimal length) (97). Not only that, inhibition of PKA also could not significantly affect either contractile force or kinetics parameters of isolated muscles, despite the fact that this inhibitor was used at a concentration higher than the reported IC_{50} s and K_i s. However, several factors such as selectivity, concentration, and treatment time, which are related to this PKA inhibitor that is used in our experiments according to previous studies still require further exploration (98).

Conclusion and Future Directions

In conclusion, PKA is a key regulator of heart function in health and disease. Although inconsistency exists in literature about the exact role of PKA in the development of cardiomyopathy most of these reports show PKA inhibition as a potential target in the treatment of cardiac hypertrophy, cardiac dilation, I/R, MI and HF. Yet, the vast majority of studies have been done in isolated cardiomyocytes or under other *in-vitro* settings (Table 1). Therefore, it is required to validate the results of these studies *in-vivo* in both small and large animal models. Further validation of the effect of PKA inhibitors in human heart diseases through clinical trials is a prerequisite before final approval of these drugs as therapies in HF. Now, this can be feasibly accomplished through taking advantage of new development in drug delivery system with the practicability of selective and direct drug targeting to the heart (99).

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1. Heart failure represents the foremost reason of hospitalization in the United States.
2. PKA is a key regulator of heart function in health and disease.
3. Most of the studies show PKA as a promising target for heart failure drug development.
4. It is required to validate the results of all studies *in-vivo* in both animal and human.
5. Selectivity, concentration, and treatment time of PKA inhibitors should be considered.

Figure 1.

Activation and inactivation mechanism of cAMP-dependent protein kinase A (PKA). When a G protein-coupled receptor is activated by its extracellular ligand, a conformational change is induced in the receptor, catalyzes the conversion of ATP into cyclic adenosine monophosphate (cAMP) increasing cAMP levels. Four cAMP molecules are required to activate a single PKA enzyme. This is done by two cAMP molecules binding to each of the two regulatory subunits (R) on a PKA enzyme causing the subunits to detach exposing the two (now activated) catalytic subunits (C). Next the catalytic subunits can go on to phosphorylate other proteins.

Figure 2.

Role of protein kinase A (PKA) in normal heart function. Phosphorylation of PKA in cardiomyocytes regulates many processes including metabolism, transcription of numerous genes such as transcription factor cAMP regulatory element binding proteins (CREB), ion fluxes and contraction.

Figure 3.

Role of activated Protein kinase A (PKA) in the excitation-coupling mechanism and cardiac contraction and relaxation. When PKA is activated by β -adrenergic receptors (β AR) signaling it phosphorylates many components increasing intracellular Ca^{2+} such as L-type calcium channel, ryanodine receptor (RyR2), and cardiac myosin binding protein (cMyBP). These phosphorylated components in turn accelerate the kinetics of cross-bridge cycling and the heart contracts faster and stronger. Whereas, phosphorylation of phospholamban (PLB) by PKA increases sarcoplasmic reticulum (SR) Ca^{2+} uptake and typically results in enhanced

cardiac relaxation. Similarly, PKA phosphorylates troponin I (cTnI), which decreases myofilaments Ca^{2+} sensitivity and accelerates relaxation as well.

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Table 1.

Different protein kinase A (PKA) inhibitors and their effects on heart function

Inhibitor	Model	Pathway/Target	Outcomes	Reference
H89	Humans (The IgG fractions from β_1 -AA - positive DCM patients and corresponding receptor agonists were added to rats myocytes) (RV trabeculae muscles from non-failing and failing hearts)	Inhibit proliferation of T cells induced by β_1 -AA	May attenuates development of HF due to dilated DCM	(72)
	(LV trabeculae muscles from non-failing and failing hearts)	Determine the <i>ex-vivo</i> effect of PKA inhibitor on k_{tr}	No significant effect	(97)
H89	Isolated Rabbit hearts (Langendorff preparation)	Investigate the <i>ex-vivo</i> effect of PKA inhibitor on developed force and kinetics	No significant effect	(98)
		Reverse the phosphorylation and kinetic properties of cytochrome C oxidase	Protects heart against ischemic heart injury	(91)
H89	Rats (<i>In vivo</i> treatment then ventricle was isolated) Mice (<i>In vivo</i> treatment then ventricle was isolated) H9c2 cardiac cells	Inhibit TSH-induced expression of HMGCR and BNP	May attenuate TSH-induced HF	(55)
H89	HEK293 cells	Inhibited ROS production by PKA	Reserve the HERG K ⁺ channel function which plays a critical role in cardiac AP repolarization	(79)
H89	Isolated rat hearts Rats myocytes H9c2 cardiac cells	Blunt the phosphorylation and assembly of the proteasome	Impairment of proteasome activity may contribute to the progression of cardiac dysfunction and I/R	(94)
H89 and KT5720	H9c2 cardiac cells	Block the IL-1 induced NO-formation	Protective effect against NO-induced cytotoxicity	(71)
KT5720	Rabbit papillary muscles	Inhibit the negative inotropic and lusitropic effects of ADM	Protect the heart against the deleterious effects of highly activated PKA by ADM in case of HF, hypertension, and MI	(62)
KT5720 and Rp-cAMPs	Guinea pig ventricular myocytes	Prevent the inhibitory effect of β_2 -AR stimulation on Ikr	Prevent delay in cardiac repolarization	(80)
KT5720	Isolated rat hearts Rat myocytes H9c2 cardiac cells	Inhibit GHRH	Prevent GHRH induced protective benefits on cardiac performance in I/R injury	(87)
PKI-GFP	Mice (<i>In vivo</i> treatment) AFVMs	Inhibit β -A agonists-induced myocyte death	Improve cardiac function after MI	(92)
UCR1C	Neonatal rat ventricular myocytes	Inhibit nuclear PKA activity	Attenuate cardiomyocyte hypertrophy	(54)

β_1 - ADM, adrenomedullin; ADM, Adrenomedullin; AFVMs, Adult feline ventricular myocytes; AP, Action potential; β_1 -AA, β_1 - Adrenoreceptor autoimmune antibodies; β_2 -AR, β_2 adrenergic receptor; BNP, Brain natriuretic peptide; DCM, Diastolic cardiomyopathy; GHRH, Growth hormone releasing hormone; HERG K⁺, Human ether-a-go-go-related gene potassium; HF, heart failure; HMGCR, 3-hydroxy-3-methyl-glutaryl-CoA reductase; Ikr, Rectifier K⁺ channel; IL-1, interleukin-1; I/R, Ischemic/Reperfusion; Ikr, rate of tension redevelopment MI, myocardial infarction; NO, nitric oxide; PKA, protein kinase A; ROS, Reactive oxygen species; TSH, Thyrotropin.