ESC European Society of Cardiology

Cardiovascular Research (2022) **118**, 386–398
doi:10.1093/cvr/cvab008

REVIEW

Physiological and pathological roles of protein kinase A in the heart

Yuening Liu, Jingrui Chen, Shayne K. Fontes, Erika N. Bautista, and Zhaokang Cheng *

Department of Pharmaceutical Sciences, Washington State University, PBS 423, 412 E. Spokane Falls Blvd, Spokane, WA 99202-2131, USA

Received 9 September 2020; revised 30 November 2020; editorial decision 19 December 2020; accepted 8 January 2021; online publish-ahead-of-print 23 January 2021

Abstract

Protein kinase A (PKA) is a central regulator of cardiac performance and morphology. Myocardial PKA activation is induced by a variety of hormones, neurotransmitters, and stress signals, most notably catecholamines secreted by the sympathetic nervous system. Catecholamines bind β-adrenergic receptors to stimulate cAMP-dependent PKA activation in cardiomyocytes. Elevated PKA activity enhances Ca^{2+} cycling and increases cardiac muscle contractility. Dynamic control of PKA is essential for cardiac homeostasis, as dysregulation of PKA signalling is associated with a broad range of heart diseases. Specifically, abnormal PKA activation or inactivation contributes to the pathogenesis of myocardial ischaemia, hypertrophy, heart failure, as well as diabetic, takotsubo, or anthracycline cardiomyopathies. PKA may also determine sex-dependent differences in contractile function and heart disease predisposition. Here, we describe the recent advances regarding the roles of PKA in cardiac physiology and pathology, highlighting previous study limitations and future research directions. Moreover, we discuss the therapeutic strategies and molecular mechanisms associated with cardiac PKA biology. In summary, PKA could serve as a promising drug target for cardioprotection. Depending on disease types and mechanisms, therapeutic intervention may require either inhibition or activation of PKA. Therefore, specific PKA inhibitors or activators may represent valuable drug candidates for the treatment of heart diseases.

Keywords Norepinephrine • Isoproterenol • Adenylyl cyclase • Phosphodiesterase • AKAP

1. Introduction of the PKA signalling system

Protein kinases play fundamental roles in nearly every aspect of cell biology and physiology, and thus their malfunctions are frequently associated with diseases. The 3',5'-cyclic adenosine monophosphate (cAMP)-dependent protein kinase [protein kinase A (PKA)], first discovered in 1968,[1] has been viewed as the prototype for all protein kinases.[2] PKA belongs to the AGC [named after the representative members PKA, 3′,5′-cyclic guanosine monophosphate (cGMP)-dependent protein kinase (PKG) and protein kinase C (PKC)] kinase family, which contains >60 serine/threonine protein kinases. PKA is evolutionarily conserved from fungi to humans and is ubiquitously expressed in all mammalian cell types.[2] The PKA holoenzyme, composed of two regulatory (R) and two catalytic (C) subunits, remains in its inactive state as a PKA tetramer (R_2C_2) in the absence of cAMP. The PKA regulatory (PKA-R) subunits have four isoforms, R1α, R1β, R2α, and R2β (encoded by *PRKAR1A, PRKAR1B, PRKAR2A*, and *PRKAR2B*, respectively), whereas three isoforms of the PKA catalytic (PKA-C) subunits have been identified, Cα, Cβ, and Cγ (encoded by *PRKACA, PRKACB*, and *PRKACG*, respectively).[3] These isoforms differ in their expression patterns and levels and may exhibit alternative splicing. Depending on the R subunit, PKA holoenzyme is divided into type I and II PKA, which bind to R1 and R2, respectively. In general, R1α and Cα1 are the most abundant and ubiquitously expressed PKA-R and PKA-C subunits, respectively.[3]

The canonical PKA signalling pathway is essential for the cardiac actions of many hormones and neurotransmitters, particularly the catecholamines including norepinephrine secreted by cardiac sympathetic nerve terminals, and epinephrine released by the adrenal medulla (*Figure 1*).[4] Catecholamines bind the transmembrane β-adrenergic receptors (β-ARs), major G-protein-coupled receptors in the heart, leading to release of the stimulatory G-protein α subunit ($G\alpha_s$) inside the target cells. $G\alpha_s$ then binds and activates adenylyl cyclases (ACs), which convert ATP into cAMP, resulting in a rapid increase in intracellular cAMP levels. The cAMP molecules bind the PKA-R subunits to induce dissociation of the tetrameric PKA holoenzyme, leading to release of the free PKA-C subunits and subsequent PKA activation. PKA phosphorylates a plethora of substrates to regulate cellular activities. It is worth

* Corresponding author. Tel: +1 509 358 7741; Fax: +1 509 368 6561, E-mail: zhaokang.cheng@wsu.edu

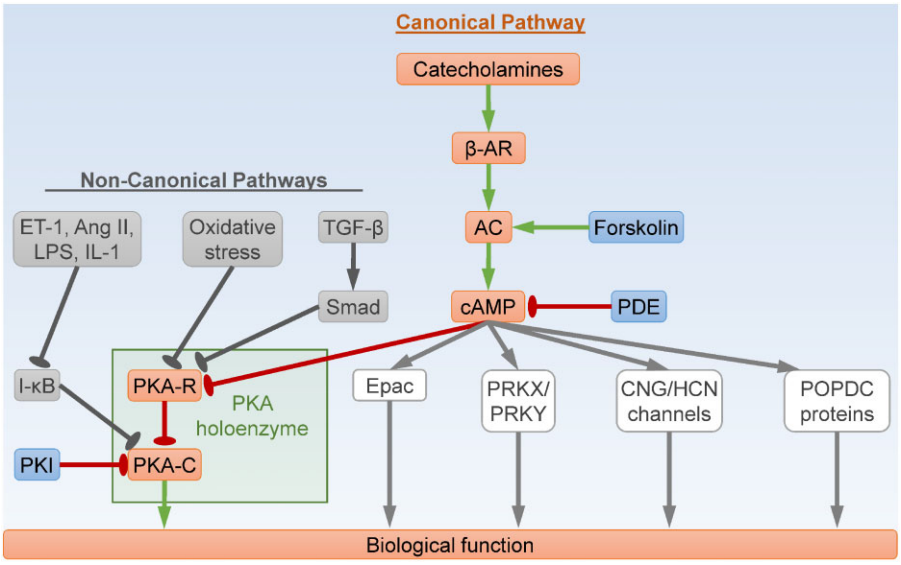

Figure 1 PKA signalling pathway. The canonical cAMP-dependent PKA pathway is initiated by catecholamines, which stimulate β-adrenergic receptor (β-AR) to induce adenylyl cyclase (AC)-mediated cAMP synthesis. The second messenger cAMP then binds the PKA regulatory (PKA-R) subunits, leading to release of the PKA catalytic (PKA-C) subunits and subsequent kinase activation. The non-canonical pathways of PKA activation are cAMP-independent. For example, oxidative stress or TGF-β induces PKA activation through blocking PKA-R-mediated sequestration of PKA-C, whereas ET-1, Ang II, LPS, or IL-1 derepresses I-κB-mediated inhibition of PKA-C. On the other hand, cAMP also exerts its biological functions through direct binding with other effectors beyond PKA, including Epac, PRKX, PRKY, CNG ion channels, HCN ion channels, and POPDC proteins. Ang II, angiotensin II; cAMP, 3',5'-cyclic adenosine monophosphate; CNG, cyclic nucleotide-gated; Epac, exchange protein directly activated by cAMP; ET-1, endothelin-1; HCN, hyperpolarization-activated cyclic nucleotide-gated; I-κB, inhibitor of κB; IL-1, interleukin-1; LPS, lipopolysaccharides; PKA, cAMP-dependent protein kinase, or protein

noting that holoenzyme dissociation may not be necessary for PKA activation under certain circumstances.[5] PKA can also be activated through non-canonical pathways in a cAMP-independent manner (*Figure 1*), by a variety of stimuli including reactive oxygen species (ROS),[6–8] lipopolysaccharides (LPS)/interleukin-1 (IL-1),[9] endothelin-1 (ET-1)/angiotensin II (Ang II),[10] transforming growth factor-β (TGF-β),[11] sphingosine,[12] and peroxynitrite.[13] For example, R1α can serve as a redox sensor and undergo oxidant-induced protein degradation, leading to PKA holoenzyme dissociation and kinase activation.[6–8] In addition, the PKA-C subunits are also sequestered by the inhibitor of κB (I-κB) proteins within the I-κB-PKA-C complex, and can be activated following I-κB degradation.[9,10]

Cellular cAMP levels are determined by the balance between the enzymatic activities of ACs and cyclic nucleotide phosphodiesterases (PDEs). In contrast to ACs, PDEs hydrolyze cAMP to 5′-AMP, thereby reducing cAMP levels and resulting in a decrease in PKA activity (*Figure 1*). In addition to cAMP, some PDEs also hydrolyze cGMP.[14] PKA activity is inhibited by the endogenous PKI protein, a pseudosubstrate of PKA that is capable of sequestering and inactivating the PKA-C subunits.[2] Unlike PKA-R, the interaction between PKI and PKA-C is not modulated by the levels of cAMP. A-kinase anchoring proteins (AKAPs) tether PKA holoenzyme to certain subcellular locations, allowing for rapid and precise control of its substrates within the specific compartments.[15] For example, short AKAP7 (also known as AKAP15) anchors PKA at the plasma membrane to regulate L-type Ca^{2+} channel (LTCC, $Ca_v1.2$) activity, whereas muscle AKAP (mAKAP, AKAP6) and long AKAP7 (AKAP18) tether PKA at the sarcoplasmic reticulum (SR) to modulate the functions of ryanodine receptors (RyRs) and sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA), respectively.[15]

PKA is a key regulator of cardiac function, structure, and remodelling. Recent studies have uncovered novel roles of PKA in normal and diseased heart, and have provided new insights on the underlying mechanisms. Here, we review our current knowledge of PKA in cardiac physiology and pathophysiology.

2. PKA and cardiac contraction/relaxation

Cardiac muscle contraction and relaxation determine systolic and diastolic functions of the heart. Contraction is initiated by electrical excitation (i.e. membrane depolarization through the action potential), a process termed excitation–contraction coupling.[16] Depolarization of the sarcolemma induces cellular Ca^{2+} entry through LTCC. Elevated cytosolic Ca^{2+} level triggers Ca^{2+} release from SR through RyR2 channel, further raising intracellular Ca^{2+} concentration. Ca^{2+} binds to cardiac troponin C (cTnC), relieves cardiac troponin I (cTnI)-mediated inhibition of actin-myosin filament interaction, leading to contraction. During cardiac relaxation, Ca^{2+} is dissociated from cTnC, transported back into the SR via SERCA, and to the extracellular space via the Na^+/Ca^{2+} exchanger (NCX) as well as the sarcolemmal Ca^{2+}-ATPase.

The force (inotropy), rate (chronotropy) of cardiac contraction, and the ability of relaxation (lusitropy) are modulated by the sympathetic (or adrenergic) nervous system through secretion of catecholamines. In the fight-or-flight response, rapid release of catecholamines markedly increases heart rate and contractility through β-AR-dependent

Table 1 Myocardial PKA substrates and their biological functions

Substrate	Phosphorylation site(s)	Molecular/cellular function	Physiological/pathological function
$Ca_v1.2$	Ser1700	$Ca_v1.2$ activation, Ca^{2+} influx, Ca^{2+} overload, necrosis	Inotropy,[18,19] chronotropy,[21,22] heart failure[23,24]
Rad	Ser25, Ser38, Ser272, Ser300	$Ca_v1.2$ activation, Ca^{2+} influx	Inotropy[20]
PLN	Ser16	SERCA activation, SR Ca^{2+} uptake	Inotropy,[25] chronotropy,[21,22,26,27] lusitropy,[25] heart failure[28,29]
RyR2	Ser2808	SR Ca^{2+} release, Ca^{2+} overload, necrosis	Inotropy,[30–32] heart failure[28,29,33]
cMyBP-C	Ser273, Ser282, Ser302	Myosin activation, actin-myosin cross-bridge detachment	Inotropy,[34–36] lusitropy[37,38]
cTnI	Ser23, Ser24	Ca^{2+}-cTnC dissociation	Lusitropy[39–41]
CcO		CcO activity↓, ROS generation, necrosis	I/R injury[42]
HSP20	Ser16	Autophagy↑, apoptosis↓, necrosis↓	I/R injury↓[43]
eNOS	Ser633, Ser1177	eNOS activation	I/R injury↓[44,45]
CREB	Ser133	CREB-mediated transcription of genes involved in hypertrophy (foetal genes) and apoptosis (ICER,[46,47] Bim,[48] Bcl-2[49])	Hypertrophy,[49,50] I/R injury→[51]
GSK-3β	Ser9	NFAT-mediated transcription of hypertrophic genes↑	Hypertrophy[52,53]
NFAT	Ser245, Ser269, Ser294	NFAT-mediated transcription of hypertrophic genes↓	Hypertrophy↓[54–56]
Drp1	Ser637	Mitochondrial fission↓	Hypertrophy↓[57]
HDAC5	Ser279	MEF2-mediated transcription of hypertrophic genes↓	Hypertrophy↓[58,59]
HDAC4		MEF2-mediated transcription of hypertrophic genes↓	Hypertrophy↓[60]
Titin, N2B element	Ser4185, Ser4010	Titin compliance↑, cardiomyocyte stiffness↓	Diastolic function↑, HFpEF↓[61–64]
RPN6	Ser14	Proteasome activation, clearance of misfolded proteins↑	Proteinopathy-related HFpEF↓[65]

CcO, cytochrome *c* oxidase; cMyBP-C, cardiac myosin-binding protein C; CREB, cAMP-response element binding protein; cTnC, cardiac troponin C; cTnI, cardiac troponin I; eNOS, endothelial nitric oxide synthase; GSK-3β, glycogen synthase kinase-3β; HDAC4/5, histone deacetylase 4/5; HFpEF, heart failure with preserved ejection fraction; HSP20, heat shock protein 20; ICER, inducible cAMP early repressor; I/R, ischaemia/reperfusion; MEF2, myocyte enhancer factor 2; NFAT, nuclear factor of activated T cells; PLN, phospholamban; ROS, reactive oxygen species; RyR2, ryanodine receptor 2; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase; SR, sarcoplasmic reticulum; ↑, increase; ↓, decrease; →, no change.

activation of the cAMP/PKA signalling pathway. Particularly, catecholamines may bind β-ARs at the sarcolemma and the SR to induce local PKA activation.[17] PKA, in turn, phosphorylates target proteins involved in the regulation of contraction/relaxation, including $Ca_v1.2$, RyR2, phospholamban (PLN), cTnI, and cardiac myosin-binding protein C (cMyBP-C).[16]

PKA positively regulates inotropy through phosphorylation of $Ca_v1.2$, PLN, and cMyBP-C. Phosphorylation of $Ca_v1.2$ or PLN increases Ca^{2+} availability, whereas phosphorylation of cMyBP-C improves responsiveness of the contractile apparatus to Ca^{2+}. In response to β-adrenergic stimulation, PKA phosphorylates $Ca_v1.2$ at S1700, resulting in increased LTCC activity and Ca^{2+} influx, thereby enhancing contraction.[18] The above model is supported by studies in the knock-in mice expressing a non-phosphorylatable mutant of endogenous $Ca_v1.2$ (S1700A), which show decreased basal and β-adrenergic-stimulated calcium currents, as well as reduced cardiomyocyte contractility.[18] Surprisingly, overexpression of the same $Ca_v1.2$ mutant (S1700A) using a transgenic approach does not interfere with Ca^{2+} current, arguing against a role for S1700 phosphorylation.[19] A most recent study further reveals that PKA augments $Ca_v1.2$ channel activity indirectly, through phosphorylation and depletion of the $Ca_v1.2$ inhibitor Rad.[20] Despite the ongoing debate about the mechanisms, there is consensus that LTCC is essential for PKA-dependent Ca^{2+} influx and cardiac contraction (*Table 1*). PKA-mediated phosphorylation of PLN at S16 relieves PLN-dependent inhibition of SERCA, resulting in increased SR Ca^{2+} uptake from the cytosol and consequently increased SR Ca^{2+} load.[25] Therefore, higher levels of Ca^{2+} can be released from SR for binding to the contractile proteins, leading to more forceful contraction. The physiological significance of PKA-dependent RyR2 phosphorylation has been controversial. Although there is evidence that phosphorylation of RyR2 at S2808 by PKA is necessary for β-adrenergic-induced SR Ca^{2+} release and cardiac contraction, contradictory findings have been reported.[30,31] The discrepancy may be due to the fact that either maximal or minimal RyR2 phosphorylation by PKA favours SR Ca^{2+} leak.[32] Phosphorylation of cMyBP-C at S273, S282, or S302 by multiple kinases including PKA increases myocardial contractility through weakening cMyBP-C-mediated inhibition of myosin,[34] increasing force-producing myosin heads,[35] or accelerating cross-bridge cycling,[36] respectively. Thus PKA-mediated cMyBP-C phosphorylation is also a key regulator of cardiac inotropy.

PKA drives cardiac chronotropy primarily through phosphorylation of SR Ca^{2+} cycling proteins in cardiac pacemaker cells (i.e. sinoatrial nodal cells, a group of specialized cardiac myocytes).[21] Pacemaker cells are unique in their high basal cAMP level and PKA activity, which are necessary and sufficient for the generation of rhythmic internal Ca^{2+} store oscillations and spontaneous beating, even in the absence of β-adrenergic stimulation. In response to β-AR stimulation, however, PKA-mediated phosphorylation of PLN at S16 speeds up SR Ca^{2+} uptake and contributes to the acceleration of heart rate.[21] PKA-dependent generation of rhythmic action potentials also requires coupling between spontaneous local Ca^{2+} release and the membrane ion channels including NCX and hyperpolarization-activated cyclic nucleotide-gated (HCN) channels.[66] Cardiac-specific overexpression of AC8 increases AC activity in the sinoatrial node tissue, which is accompanied by a marked increase in heart rate.[67] Pharmacological inhibition of PDE3A and PDE4B,

the major PDE subtypes expressed in pacemaker cells, synergistically increase the spontaneous beating rate.[26] PDE2 inhibition with Bay 60–7550 also accelerates heart rate.[22] Conversely, cardiac-specific PDE2 overexpression results in a decrease in phospho-PLN(S16) level, which is associated with reduced resting and maximal heart rate.[22] Cardiac-specific PKI transgenic mice show lower PKA activity, reduced PLN phosphorylation at S16, and slower heart rate following β-AR stimulation.[27] Intriguingly, basal contractility is enhanced in the transgenic heart overexpressing either PDE2 or PKI, likely due to reduced SR Ca^{2+} leak, increased myocyte Ca^{2+} transient and enhanced myofilament Ca^{2+} sensitivity.[22,27] Aberrant PKA activation can cause Ca^{2+} cycling protein dysfunction and lead to cardiac arrhythmias. For example, overexpression of PDE2 decreases arrhythmia occurrence following myocardial infarction,[22] but PDE4D gene deficiency induces PKA-dependent RyR2 hyperphosphorylation, which enhances SR Ca^{2+} leak and promotes arrhythmias.[68]

PKA enhances cardiac lusitropy through phosphorylation of PLN, cTnI, and cMyBP-C. Myocardial relaxation depends on efficient cytosolic Ca^{2+} removal. Phosphorylation of PLN by PKA accelerates SERCA-mediated transport of cytosolic Ca^{2+} into SR, resulting in faster relaxation.[25] PKA-dependent phosphorylation of cTnI and cMyBP-C augments lusitropy primarily through reducing Ca^{2+} sensitivity. The N-terminal region of cTnI binds cTnC to increase its Ca^{2+} affinity, which determines myofibrillar Ca^{2+} sensitivity and contractile activation.[39] Phosphorylation of cTnI at the N-terminal residues S23/24 by PKA weakens cTnI–cTnC interaction, thereby promoting dissociation of Ca^{2+} from cTnC and accelerating myofibril relaxation.[40] Importantly, monophosphorylation at S24 is sufficient to reduce Ca^{2+} sensitivity.[41] Phosphorylation of cMyBP-C also represents a major lusitropic mechanism during β-adrenergic stimulation. Concurrent non-phosphorylatable mutations of all PKA target sites on cMyBP-C (S273A, S282A, and S302A) impair diastolic function without altering intracellular Ca^{2+} handling, suggesting that cMyBP-C phosphorylation enhances relaxation likely through increasing the rates of actin-myosin cross-bridge detachment.[37,38] Conversely, phosphomimetic mutations of all three serine residues of cMyBP-C (S273D, S282D, and S302D) increase peak myocardial relaxation velocity, indicating enhanced lusitropy.[37] However, the specific role of each individual site remains to be determined.

Although required by the positive inotropic, chronotropic, and lusitropic effects of β-adrenergic stimulation, PKA activity is not necessary for the maintenance of basal heart function.[27] Thus, pharmacological PKA inhibition might be a safe and viable strategy for clinical use. Since PKA inhibition with PKI preferentially reduces PKA activity in the SR and myofilament but not the sarcolemma and nuclei, it is possible that basal heart function is maintained by sarcolemmal or nuclear PKA. Nonetheless, long-term PKA inhibition reduces cardiac reserve and impairs exercise capability.[27]

In summary, PKA plays an essential role in neurohormonal regulation of cardiac function, through phosphorylation of its substrates including $Ca_v1.2$, RyR2, PLN, cTnI, and cMyBP-C. Although loss of PKA activity does not diminish cardiac function at resting conditions, it can limit the heart's ability to cope with stress.

3. PKA and ischaemic heart disease

Ischaemic heart disease, also known as coronary artery disease, occurs when blood flow to the heart muscle is reduced or blocked, most commonly due to atherosclerosis. The biological functions of PKA in atherosclerosis pathogenesis have been described elsewhere.[69] This review will summarize our current knowledge of PKA in myocardial ischaemia, with a particular focus on cardiac myocytes. Myocardial ischaemia is characterized by tissue hypoxia (i.e. oxygen deprivation due to insufficient blood supply). Restoration of blood supply by reperfusion results in tissue re-oxygenation but paradoxically also causes ischaemia/reperfusion (I/R) injury. During myocardial ischaemia, interstitial catecholamine concentration dramatically increases by 100–1000 fold (*Figure 2*),[70,71] due to hyperactivation of the sympathetic nervous system.[72] Accordingly, both cAMP level[73,74] and PKA activity[75,76] are increased in the ischaemic myocardium. Following reperfusion, myocardial catecholamine level rapidly declines and returns to baseline within 120 min.[70,71] However, cardiac PKA activity remains elevated after reperfusion,[76,77] due at least in part to R1α loss as a result of oxidation and dimerization.[8,77]

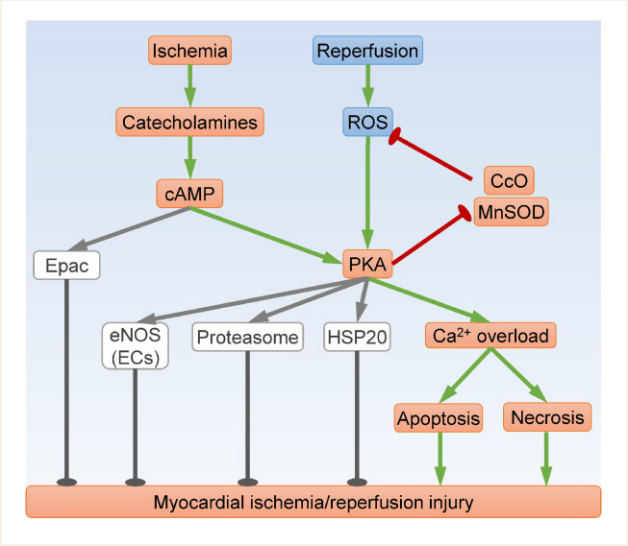

Figure 2 PKA signalling in myocardial ischaemia/reperfusion injury. Both ischaemia and reperfusion induce PKA activation, which can contribute to oxidative stress and Ca^{2+} overload, leading to cardiac cell death and myocardial damage. However, cAMP-induced activation of Epac, eNOS, proteasome, and HSP20 appear to be cardioprotective. CcO, cytochrome *c* oxidase; ECs, endothelial cells; eNOS, endothelial nitric oxide synthase; Epac, exchange protein directly activated by cAMP; HSP20, heat shock protein 20; MnSOD, manganese superoxide

At physiological levels, catecholamines mediate the fight-or-flight response to rapidly increase contractility and heart rate. At extremely high concentrations, however, catecholamines can cause myocardial tissue damage. Administration of a single, high dose of the synthetic catecholamine isoproterenol in rats induces PKA-dependent RyR2 hyperphosphorylation, leading to SR Ca^{2+} leakage and subsequent myocyte death via apoptosis or necrosis (*Figure 2*).[33] Moreover, isoproterenol induces PKA-mediated phosphorylation of cAMP-response element binding protein (CREB) at S133, leading to enhanced binding of CREB with the cAMP-response element in the promoter region, and subsequent transcription of pro-apoptotic genes including inducible cAMP early repressor (ICER)[46,47] and Bim.[48] It is noteworthy that CREB deficiency has no impact on apoptosis following I/R injury,[51] likely because CREB also mediates transcription of the anti-apoptotic gene Bcl-2 in certain context.[49] In addition, acute β-adrenergic stimulation with isoproterenol in mice transiently increases myocyte membrane permeability, an important marker of necrosis.[78] Intriguingly, acute catecholamine injury

appears to be fully reversible.[33,78] By contrast, sustained activation of the PKA substrate LTCC in mice results in cardiac dysfunction and premature death, which is exacerbated by isoproterenol infusion.[23] Mechanistically, isoproterenol exposure augments LTCC activity and enhances Ca^{2+} influx, leading to Ca^{2+} overload and myocyte necrosis.[23]

Activation of β-AR/cAMP signalling during the ischaemic phase contributes to myocardial I/R injury. Overexpression of PDE3A1 reduces myocardial cAMP levels and attenuates I/R-induced myocyte apoptosis, possibly due to decreased ICER and increased Bcl-2 expression.[79] Moreover, treatment with the β_1-AR inhibitor CGP-20712A[80] or the PKA inhibitor H89/PKI [42,81] diminishes I/R-induced myocardial necrosis. In the ischaemic heart, excessive cAMP induces PKA-dependent phosphorylation and inhibition of cytochrome *c* oxidase (CcO), resulting in augmented ROS production.[42] In turn, oxidative stress prompts R1α loss to activate PKA,[8,77] leading to further CcO inhibition and ROS generation.[7] Therefore, a rapid rise in cAMP level may trigger a vicious cycle of oxidative stress through activation of PKA (*Figure 2*).

Reduced cAMP level during ischaemia, caused by ischaemic preconditioning, is associated with marked protection against myocardial I/R injury.[73,74] Interestingly, ischaemic preconditioning initially increases cAMP level during intermittent ischaemia (i.e. the preconditioning phase), but later reduces cAMP accumulation during sustained ischaemia (i.e. the main ischaemia phase) possibly due to β-AR desensitization or compensatory activation of PDEs.[73,74] Myocardial I/R injury is attenuated by pre-ischaemic cAMP up-regulation using isoproterenol (a β-AR agonist), forskolin (an AC agonist),[74,81–83] milrinone/olprinone/amrinone (PDE3 inhibitors),[84,85] trapidil (a non-selective PDE inhibitor),[86] or dibutyryl-cAMP (a cell-permeable cAMP analogue).[75,84] Germline ablation of PDE3B increases cardiac cAMP level at baseline, reduces infarct size, and improves cardiac function following I/R.[87] The cardioprotective effect of PDE3B ablation is blocked by treatment with KT5720, a PKA inhibitor.[87] Intramyocardial injection of PKA-Cα siRNAs before ischaemia increases infarct size and impairs systolic function after I/R, possibly due to NF-κB activation and superoxide production.[88] Together these studies suggest that pre-ischaemic cAMP/PKA activation represses PKA activity during ischaemia, thereby leading to cardioprotection.

Emerging evidence suggests that PKA could be a better drug target than β-AR, because PKI is more effective than the β_1-AR blocker metoprolol in alleviating myocyte apoptosis and cardiac dysfunction following myocardial infarction.[89] Mechanistically, β-AR and cAMP induce PKA activation to cause cardiotoxicity but stimulate exchange protein directly activated by cAMP (Epac) to mediate cardioprotection (*Figure 2*).[89] In this regard, cardiac cell death following simulated I/R is exaggerated by inhibition of type 10 soluble AC (sAC) with gene silencing, KH7 treatment, or bicarbonate withdrawal, but mitigated by overexpression of sAC or treatment with the PDE2 inhibitor Bay 60–7550.[90] Moreover, incubation with the cAMP inhibitor Rp-cAMP abolishes glucagon-like peptide 1 (GLP-1)-induced protection against I/R Injury.[91] Interestingly, the beneficial effects of GLP-1 are mediated by sAC-dependent cAMP/PKA activation, and subsequent endothelial nitric oxide synthase (eNOS) phosphorylation.[44,45] PKA has also been shown to protect against I/R injury through proteasome activation,[92] as well as phosphorylation of heat shock protein 20.[43] Therefore, although believed to be detrimental during myocardial I/R injury, cAMP and PKA may also activate certain protective molecules in some contexts.

In summary, the majority of studies suggest that activation of PKA during ischaemia contributes to I/R injury through aggravating cardiomyocyte apoptosis and/or necrosis. Ischaemic preconditioning reduces PKA activity during ischaemia and thus attenuates I/R injury. Notably, elevated cAMP levels during ischaemia may also activate protective mechanisms in a PKA-dependent or independent manner.

4. PKA and cardiac hypertrophy

It is well established that chronic stimulation of β_1-AR induces cardiac hypertrophy.[4] However, whether PKA contributes to β_1-AR-mediated hypertrophy remains elusive. Earlier studies reveal that cardiac-specific overexpression of β_1-AR,[93] $G\alpha_s$,[94] or PKA-Cα,[28] markedly increases cardiomyocyte size, but only moderately increases heart weight due to concurrent apoptosis or necrosis. Notably, β-adrenergic stimulation is sufficient to cause apoptosis in adult cardiomyocytes, in a PKA-dependent manner.[95] Overexpression of type 5 AC (AC5), a major cardiac isoform, exacerbates apoptosis and only marginally increases cardiomyocyte size following chronic infusion of isoproterenol.[96] Mechanistically, AC5-mediated cAMP/PKA activation augments oxidative stress through repression of manganese superoxide dismutase (MnSOD) expression (*Figure 2*).[96] Cardiac-specific transgenic expression of AC6, another major cardiac isoform, does not alter heart weight in mice with ischaemic cardiomyopathy.[97] Moreover, overexpression of AC8 fails to increase heart weight, despite four-fold higher PKA activity.[98] These studies suggest that PKA activation may primarily provoke myocyte loss, which can eventually lead to compensatory hypertrophy. Given the essential role of PKA in regulating cardiac contractility, it is not surprising that interfering with PKA-dependent phosphorylation can disrupt normal heart function, which may also result in hypertrophic remodelling to compensate for reduced cardiac output. Indeed, mice with non-phosphorylatable mutations of $Ca_v1.2$,[18,24] PLN,[99] cTnI,[100] or cMyBP-C[37,38] at their PKA sites exhibit cardiac dysfunction and hypertrophy. However, myocardial PKA inactivation by overexpression of PKI does not induce cardiac dysfunction or hypertrophy in mice up to 12 months of age.[27] Germline deletion of AC5 in mice does not blunt physiological hypertrophy during postnatal heart development (up to 3–6 months of age),[101] or attenuate pathological hypertrophy induced by pressure overload.[102] Again, disruption of AC5 primarily suppresses apoptosis, due in part to up-regulation of the anti-oxidant protein MnSOD and the anti-apoptotic molecule Bcl-2.[101,102] Collectively, the above *in vivo* studies do not support a major, direct role of the AC/cAMP/PKA signalling in hypertrophy.

Myocardial hypertrophic growth is not associated with a change in total PKA activity.[103] However, *in vitro* real-time imaging reveals that hypertrophy is accompanied by relocation of PKA and PDE activity within myocytes, resulting in altered cAMP/PKA compartmentalization.[104] Upon induction of hypertrophy, expression, and activity of several cAMP-hydrolyzing PDEs, including the pro-hypertrophic PDE1C,[105] PDE2,[54] and PDE10A[106] are increased, whereas PDE3 and PDE4 are down-regulated.[107,108] Interestingly, PDE3 or PDE4 appears to be anti-hypertrophic as PDE3/4 inhibition causes spontaneous hypertrophy,[54] rendering myocytes insensitive to catecholamine stimulation *in vitro*[54] or pressure overload *in vivo*.[109] Mechanistically, PDE4 suppresses cardiomyocyte hypertrophy through inhibition of nuclear PKA activity.[47,50] Since PDEs differ in their subcellular distribution,[14] their opposing roles in hypertrophy could be due to differential modulation of PKA activity within specific subcellular compartments.

Indeed, nuclear PKA activation is associated with augmented cardiac hypertrophy (*Figure 3*). Overexpression of nuclear-targeted PKA-C increases cardiomyocyte size, whereas overexpression of cytosolic-targeted PKA-C enhances Ca^{2+} transients and cardiac contractile force

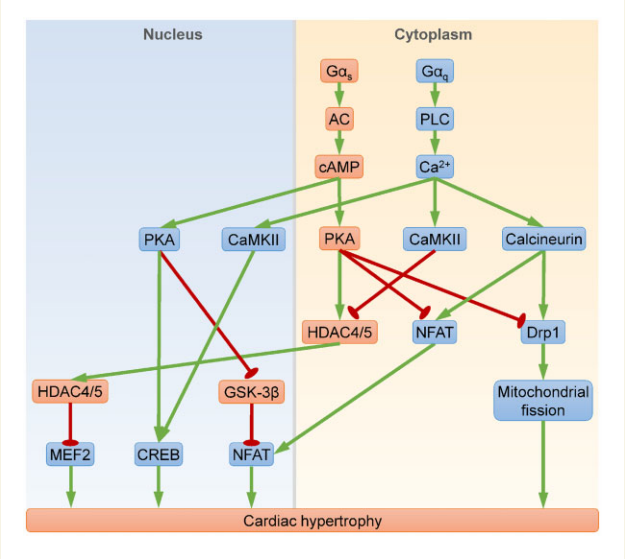

Figure 3 PKA signalling in cardiac hypertrophy. Key molecular events contributing to cardiac hypertrophy include MEF2-, CREB-, NFAT-mediated gene transcription, or Drp1-mediated mitochondrial fission. $G\alpha_s$ stimulates synthesis of the second messenger cAMP, which activates nuclear PKA to induce hypertrophy and cytoplasmic PKA to inhibit hypertrophy. Nuclear PKA promotes hypertrophy through: (i) activating CREB; and (ii) alleviating GSK-3β-mediated NFAT repression. Cytoplasmic PKA inhibits hypertrophy through: (i) invoking HDAC4/5-mediated MEF2 repression; (ii) inactivating NFAT; and (iii) suppressing Drp1. By contrast, $G\alpha_q$ acts through the second messenger Ca^{2+} to provoke Ca^{2+}/CaMKII- and calcineurin-mediated hypertrophy. CaMKII activates MEF2 and CREB, whereas calcineurin activates NFAT and Drp1. Nuclear PKA may act cooperatively with CaMKII and calcineurin to induce hypertrophy, but cytoplasmic PKA antagonizes CaMKII- and calcineurin-dependent hypertrophy. AC, adenylyl cyclase; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase type II; CREB, cAMP-response element binding protein; GSK-3β, glycogen synthase kinase-3β; HDAC4/5, histone deacetylase 4/5; MEF2, myocyte enhancer factor 2;

without inducing hypertrophy.[110] β-Adrenergic stimulation or pressure overload-induced hypertrophy requires sAC, which is known to mediate nuclear cAMP synthesis.[111] Interestingly, activation of β_1-AR increases nuclear PKA activity,[47] and results in hypertrophy.[93] By contrast, stimulation of β_2-AR does not induce nuclear PKA activation,[47] and fails to cause hypertrophy.[112] Mechanistically, PKA phosphorylates CREB at S133 in the nuclei of cardiomyocytes, thereby initiating CREB-mediated transcription of hypertrophy-related genes.[49,50] Since β_1-AR-mediated nuclear PKA activation is abolished by PKI,[47] cardiac-specific PKI transgenic mice are resistant to isoproterenol-induced CREB phosphorylation at S133[27] and subsequent pathological hypertrophy.[89] Moreover, overexpression of a non-phosphorylatable CREB mutant (S133A) attenuates isoproterenol-induced hypertrophy.[46] Collectively, these studies suggest that CREB-mediated transcription is a critical pro-hypertrophic mechanism downstream of nuclear PKA. β-AR-mediated nuclear PKA activation requires the scaffold protein mAKAP.[47] Interestingly, mAKAP facilitates calcineurin-dependent activation of the pro-hypertrophic transcription factor nuclear factor of activated T cells (NFAT), which binds GATA4 or myocyte enhancer factor 2 (MEF2) to stimulate expression of hypertrophy-related genes.[113] Glycogen synthase kinase-3β (GSK-3β), which phosphorylates NFAT to trigger its nuclear export and inactivation,[52] is repressed by PKA via PKA-dependent phosphorylation at S9.[53] Therefore, nuclear PKA may promote hypertrophy in a synergistic manner with calcineurin, through relieving GSK-3β-mediated inhibition of NFAT (*Figure 3*). In line with these findings, global PKA-Cβ null mice are resistant to Ang II-induced cardiac hypertrophy.[114] Intriguingly, overexpression of PKA-Cα in cardiac fibroblasts also induces ventricular dilation and cardiomyocyte hypertrophy, possibly via a paracrine mechanism.[115] Taken together, PKA mediates cardiac hypertrophy while localized in the nuclei of cardiomyocytes or in cardiac fibroblasts.

In contrast, accumulating evidence suggests that cytoplasmic PKA may inhibit hypertrophy. In response to PDE2 inhibition, an increased local pool of cAMP induces PKA-dependent phosphorylation of NFAT at S245/S269/S294, resulting in cytoplasmic retention of NFAT and inhibition of hypertrophy.[54] Cytoplasmic NFAT retention requires mitochondrial localization of PKA, as disruption of AKAP1 (also known as AKAP121, which targets PKA to mitochondria) reduces mitochondrial PKA activity, leading to augmented nuclear NFAT accumulation and aggravated hypertrophy both *in vitro* and *in vivo*.[55,56] These results suggest that cytoplasmic PKA may directly repress NFAT (*Figure 3*). Since cytoplasmic PKA activity is restricted by R1α,[116] we recently delete the R1α-encoding gene *PRKAR1A* in mouse heart to assess the impact of cytoplasmic PKA activation on physiological cardiac hypertrophy during development.[57] Because homozygous *PRKAR1A* ablation inhibits cardiomyocyte proliferation and results in embryonic lethality,[117] we use a heterozygous deletion approach and show that *PRKAR1A* deficiency suppresses hypertrophic heart growth, likely through inhibition of mitochondrial fission via PKA-dependent Drp1 phosphorylation at S637 (*Figure 3*). Importantly, *PRKAR1A* mutations/deletions in humans are also associated with reduced left ventricular mass.[57] In addition, germline *PRKAR2B* ablation attenuates age-related cardiac hypertrophy, possibly through a non-cardiomyocyte-autonomous mechanism because R2β is predominantly expressed in non-cardiac tissues.[118]

Although stimulation of either $G\alpha_s$-coupled receptors (e.g. β_1-AR) or $G\alpha_q$-coupled receptors (e.g. α_1-AR, Ang II type 1 receptor, ET A receptor) can lead to cardiomyocyte hypertrophy, $G\alpha_s$ signalling appears to antagonize $G\alpha_q$-mediated hypertrophy. For example, treatment with forskolin (an AC agonist),[57] or cAMP[58] attenuates α_1-AR-mediated hypertrophy *in vitro*. Moreover, $G\alpha_q$-mediated cardiac foetal gene expression, a common hypertrophy marker, is blocked by isoproterenol, forskolin, or a selective PKA activator 8-CPT-6-Phe-cAMP, but augmented by a PKA inhibitor H89.[119] Mechanistically, $G\alpha_s$-dependent PKA activation induces histone deacetylase 5 (HDAC5) phosphorylation at S279 [58,59] or dephosphorylation at S259/S498,[120] resulting in translocation of HDAC5 from the cytoplasm to the nucleus, where it represses MEF2-mediated transcription of the hypertrophic gene program (*Figure 3*). PKA also induces nuclear import of HDAC4 and subsequent MEF2 repression.[60] By contrast, $G\alpha_q$ stimulates phospholipase C to increase intracellular Ca^{2+} level, leading to activation of Ca^{2+}/calmodulin-dependent protein kinase type II (CaMKII) and calcineurin (i.e. protein phosphatase 2B). Intriguingly, CaMKII induces HDAC4 nuclear export to provoke MEF2-mediated pathological cardiac hypertrophy.[121] On the other hand, calcineurin dephosphorylates NFAT at S245/S269/S294 and Drp1 at S637, the same sites phosphorylated by cytoplasmic PKA.[122,123] Therefore, cytoplasmic PKA may counteract CaMKII- and calcineurin-mediated hypertrophy (*Figure 3*). It is worth noting that CaMKII also phosphorylates CREB at S133,[124] suggesting that CaMKII and nuclear PKA may promote CREB-dependent hypertrophy in a redundant manner.

In summary, PKA differentially modulates cardiac hypertrophy depending on its subcellular localization. Nuclear PKA induces cardiomyocyte hypertrophy through activation of CREB and NFAT-mediated transcription of hypertrophic genes, whereas cytoplasmic PKA inhibits hypertrophy via repression of MEF2 and NFAT-mediated transcription, as well as inhibition of Drp1-mediated mitochondrial fission. In cardiomyocytes, Ca^{2+} and cAMP are major second messengers downstream of $G\alpha_q$ and $G\alpha_s$, respectively. Ca^{2+}-dependent activation of CaMKII or calcineurin provokes hypertrophy, which is likely augmented by cAMP-dependent activation of nuclear PKA, but attenuated by cytoplasmic PKA. These points need to be further investigated with approaches that specifically target PKA in various intracellular compartments.

5. PKA and heart failure

Heart failure occurs when the heart is unable to supply adequate blood to meet the metabolic needs of the body. Decreased cardiac output induces compensatory activation of the sympathetic nervous system, which increases circulating catecholamines in an attempt to restore heart function. Although acute neurohormonal activation maintains cardiac output, chronic β-AR stimulation impairs myocardial β-AR responsiveness through receptor down-regulation (via β-arrestin-mediated internalization) and desensitization (via uncoupling from G-proteins).[4] Decreased β-AR responsiveness blunts catecholamine-induced PKA activation, thereby reducing myocardial contractility. Thus β-AR antagonism with β-blockers has been a mainstay of heart failure therapy.

Human heart failure is associated with diminished phosphorylation of PLN,[125] cTnI,[126–128] and cMyBP-C.[128,129] Although the cause of discrepancy remains an unsettled debate,[130] animal models of heart failure exhibit increased cTnI phosphorylation.[131,132] In failing rabbit cardiomyocytes, β-AR-induced PKA activation is diminished at the SR and sarcolemma, but enhanced at the myofilaments, indicating intracellular PKA activity redistribution.[132] Accordingly, PKA-mediated phosphorylation of the SR protein PLN is decreased, but phosphorylation of the myofilament protein cTnI is increased in failing myocytes. Myofilament PKA activation in heart failure is due to reduced local PDE activity and enhanced β_2-AR signalling. Mechanistically, heart failure is associated with a redistribution of β_2-AR and PKA from t-tubules to other sarcolemmal areas owing to reduced expression of caveolin-3, a major structural protein of caveolae.[132–134] It is worth noting that total PKA activity following cAMP stimulation is comparable between failing and non-failing human hearts, suggesting that heart failure alters PKA signalling primarily at the level of β-AR.[135] Nonetheless, failing human hearts express lower level of R1α,[126,136] but opposite findings have also been reported.[128]

Proper functioning of the heart relies on efficient Ca^{2+} cycling, which is controlled by PKA-dependent phosphorylation of the Ca^{2+} handling proteins in a highly dynamic manner. Therefore, prolonged activation or inhibition of PKA as well as its substrates can cause heart failure. For example, either gain-[99,137] or loss-of-function PLN mutations[138] induce lethal dilated cardiomyopathy. Chronic LTCC activation provokes Ca^{2+} overload-mediated cardiomyocyte necrosis, eventually leading to heart failure.[23] On the other hand, permanent LTCC inactivation also results in heart failure and premature death.[24] Constitutive PKA activation induces PLN and RyR2 hyperphosphorylation, leading to reduced contractility, dilated cardiomyopathy and sudden death.[28] Although persistent PKA inhibition in mice does not cause functional or morphological abnormalities, depressed PKA activity impairs cardiac adaptation to stress, and may contribute to heart failure morbidity.[27]

The role of PKA in heart failure is modulated by AKAPs. Failing human hearts exhibit decreased interaction between PKA and AKAP1.[136] Ablation of AKAP1 impairs mitochondrial PKA signalling and accelerates heart failure development after pressure overload.[55] In addition, heart failure progression is accompanied by myocardial AKAP5 (also known as AKAP150, or AKAP79) down-regulation.[29] Loss of AKAP5 abolishes PKA-mediated phosphorylation of PLN and RyR2, disrupts Ca^{2+} cycling, and predisposes to heart failure.[29]

Heart failure differentially affects expression of the PDE family members. Human heart failure is associated with decreased levels of PDE3A and PDE4D, which are sufficient to provoke SR Ca^{2+} leak and cardiomyocyte apoptosis.[68,107] Interestingly, failing human hearts express higher levels of PDE1C,[105] PDE2,[139] and PDE10A,[106] which hydrolyze cAMP and may contribute to decreased contractile response. Inhibition of PDE1C augments adenosine A_2 receptor (A_2R)-dependent cAMP synthesis, thereby providing acute inotropic, lusitropic, and vasodilatory effects in tachypacing-induced heart failure.[140] Similarly, inhibition of PDE2[141] or PDE10A[106] protects against pressure overload-induced heart failure. Since inhibition of PDE1C,[105] PDE2,[141] or PDE10A[106] also increases cardiac cGMP level, their cardioprotective effects are likely mediated by both cAMP-dependent and cAMP-independent mechanisms.

Conventional animal models of heart failure, such as pressure/volume overload or ischaemic/toxic injury, typically develop heart failure with reduced ejection fraction (HFrEF).[142] The most common form of heart failure, however, is heart failure with preserved ejection fraction (HFpEF), which is characterized by myocardial stiffness and diastolic dysfunction. At the molecular level, myofilament stiffness is associated with reduced phosphorylation of titin's N2B element at the PKA/PKG sites S4185/S4010/S4099.[61,62] Stimulation with PKA-C reduces passive stiffness of human HFpEF cardiomyocytes *in vitro*.[63] In an HFpEF-like mouse model, administration of metformin augments N2B phosphorylation at the PKA-specific site S4010, leading to increased titin compliance and improved diastolic function.[64] In another HFpEF-like model, inhibition of PDE1 enhances PKA-mediated phosphorylation of RPN6 at S14, which augments proteasomal degradation of misfolded proteins and improves diastolic function.[65] A new animal model that recapitulates many features of human HFpEF has been developed recently,[143] and will likely advance our knowledge of PKA in HFpEF pathogenesis.

In summary, the dynamic, nearly instantaneous control of cardiac contractility by PKA is compromised in heart failure. Although acute PKA activation improves cardiac performance, chronic PKA activation or inhibition can lead to heart failure.

6. PKA and cardiomyopathies

6.1 Diabetic cardiomyopathy

Emerging evidence suggests that catecholamines antagonize insulin-induced cardiac glucose transport.[144] Sustained catecholamine stimulation not only contributes to heart failure progression, but also leads to insulin resistance in the heart.[145] Mechanistically, overstimulation of β_2-AR in cardiomyocytes inhibits insulin-induced translocation of glucose transporter 4 (GLUT4) to the plasma membrane, resulting in reduced glucose uptake in a PKA-dependent manner.[145] Treatment with the β-blockers propranolol or metoprolol prevents catecholamine-induced cardiac insulin resistance.[145] In adipocytes, catecholamines induce PKA-dependent lipolysis, which blocks insulin-induced, phosphoinositide 3-

kinase/Akt/mammalian target of rapamycin-dependent GLUT4 translocation, resulting in decreased glucose uptake.[146]

Insulin resistance is a common feature of type 2 diabetes mellitus, which can cause cardiac dysfunction through PDE4D-mediated decline in total PKA activity.[147] In type 2 diabetes, insulin excess induces PDE4D expression via G-protein-coupled receptor kinase 2 (GRK2)-dependent transactivation of β_2-AR/β-arrestin2/extracellular signal-regulated kinase pathway.[147] Therefore, treatment with the GRK2 inhibitor paroxetine or the β-blocker carvedilol diminishes PDE4D expression, normalizes cAMP/PKA activity, and prevents type 2 diabetes-associated cardiac dysfunction.[147] Interestingly, type 1 diabetes is also associated with reduced cardiac contractility due to decreased PKA activity.[148] Since type 1 diabetes is characterized by insulin deficiency, physiological level of insulin may be necessary for myocardial PKA activation. Together, these studies suggest that either excess or deficiency of insulin can diminish myocardial PKA activity and cause diabetic cardiomyopathy.

6.2 Takotsubo cardiomyopathy

Takotsubo cardiomyopathy (also known as stress cardiomyopathy, broken heart syndrome, or apical ballooning syndrome) is a form of acute, transient heart failure that typically occurs after physical or emotional stress. The incidence of takotsubo cardiomyopathy is markedly increased during the coronavirus disease 2019 pandemic, due to either coronavirus infection[149] or the pandemic-related psychological stress.[150] Although heart function usually recovers within a few weeks, takotsubo cardiomyopathy can cause long-term structural, metabolic changes and a heart failure phenotype.[151] Takotsubo cardiomyopathy is associated with elevated circulating and myocardial catecholamine levels, which can be higher than after acute myocardial infarction.[152] Excessive catecholamines directly contribute to myocardial contraction band necrosis, a pathological hallmark of takotsubo cardiomyopathy.[152] Moreover, catecholamines induce severe lipid accumulation in the heart, resulting in lipotoxicity.[153] There is evidence that catecholamine hypersensitivity caused by genetic abnormalities may increase the susceptibility to takotsubo cardiomyopathy, owing to PKA-mediated hyperphosphorylation of RyR2, PLN, cTnI, and $Ca_v1.2$.[154] Therefore, a genetic predisposition for takotsubo cardiomyopathy is possible, but remains to be further characterized.

6.3 Anthracycline cardiomyopathy

The anthracycline family anticancer drugs, such as doxorubicin, are frequently used in the treatment of various cancers. Unfortunately, their uses are limited by irreversible and dose-dependent cardiotoxicity, which may eventually progress into heart failure. Doxorubicin-induced reduction in cardiac contractility is correlated with decreased Ca^{2+} transients, likely due to increased RyR2 phosphorylation by PKA.[155] Consistent with this, blockade of the β-AR/cAMP/PKA signalling attenuates anthracycline-related cardiotoxicity in cancer patients.[156] Interestingly, enhancing PKA-mediated PLN phosphorylation with the PDE3 inhibitor levosimendan maintains cardiac contractility following doxorubicin administration.[157] Levosimendan improves cardiomyocyte viability through activation of PKA, as the protective effect is abolished by the PKA inhibitor H89. In addition, PDE1 inhibition with IC86340 or via gene deletion attenuates doxorubicin-induced cardiomyocyte apoptosis, through augmenting A_2R-mediated cAMP synthesis.[158] Since β-AR-mediated cAMP production provokes apoptosis,[95] A_2R-derived cAMP likely resides in a different subcellular compartment to protect against apoptosis.[158]

7. PKA and the sex differences in cardiac health or disease

Biological sex has a profound impact on the heart. Baseline heart function is better in premenopausal women, who are also at lower risk of heart disease compared with age-matched men. Better heart function in females is associated with higher basal PKA activity and distinct gene expression profiles in cardiomyocytes, indicating a fundamental difference between female and male cardiomyocytes.[159] Moreover, the female sex hormone oestrogen can activate cAMP/PKA and enhance cardiac contractility under basal conditions.[160,161] In response to increasing demand (e.g. exercise, stress, and disease), however, female hearts and myocytes exhibit smaller contractions than males. A possible explanation is that oestrogen antagonizes the function of catecholamines,[162] thereby reducing intracellular cAMP levels and suppressing SR Ca^{2+} release.[163,164] Based on the above findings, we speculate that oestrogen counteracts catecholamine-mediated fight-or-flight response via up-regulating cAMP within different intracellular compartments. However, this possibility needs to be confirmed in future studies.

8. Study limitations

Our current knowledge of PKA has been largely based on studies of cAMP, PDEs, AKAPs, and pharmacological PKA inhibitors. Potential limitations of these approaches are summarized below.

8.1 cAMP ≠ PKA

In addition to PKA, cAMP activates other effectors including Epac,[165,166] protein kinase X-linked (PRKX)/protein kinase Y-linked (PRKY),[167] cyclic nucleotide-regulated cation channels [i.e. cyclic nucleotide-gated (CNG) channels and HCN channels],[168,169] and the popeye domain containing (POPDC) proteins (*Figure 1*).[170] Thus cAMP may produce PKA-independent responses.

On the other hand, PKA can also undergo cAMP-independent activation through diverse mechanisms including R1α oxidation,[6–8] I-κB degradation,[9,10] Smad3/4-PKA-R complex formation,[11] and sphingosine-PKA holoenzyme interaction (*Figure 1*).[12] Therefore, cAMP and PKA can be involved in completely different biological processes, and can have functions distinct from each other.

8.2 PDEs may modulate both cAMP and cGMP levels

The role of PKA in myocardial biology has also been investigated using genetic and pharmacological approaches to alter PDE activity. PDEs are divided into 11 super families (PDE1–11), comprising more than 80 different isoforms that modulate the levels of cAMP and/or cGMP.[14] As a second messenger, cGMP regulates many aspects of cardiovascular homeostasis and pathophysiology through activation of PKG. Hence, PDEs may have cAMP/PKA-independent biological functions.

8.3 AKAPs may anchor other proteins not related with PKA

As scaffold proteins, AKAPs target PKA to subcellular locations and modulate local substrate function. However, AKAPs also anchor other kinases and regulatory proteins not related with PKA.[171] Therefore, AKAP deficiency may cause phenotypes in a PKA-independent manner.

8.4 Specificity of PKA inhibitors

Many previous studies have relied on small molecule inhibitors to control PKA activity. Since pharmacological PKA inhibitors have off-target effects and interfere with a wide range of cellular activities,[172] this approach may not accurately demonstrate the role of PKA.

9. Conclusions and perspectives

Preclinical studies over the past decades have greatly advanced our understanding of PKA in the heart. PKA regulates cardiac muscle contraction, relaxation and heart rate through modulating Ca^{2+} dynamics in cardiac myocytes. Myocardial PKA can be activated by the canonical and the non-canonical pathways. In the canonical PKA pathway, β-AR stimulation by catecholamines induces cAMP-dependent PKA activation. The non-canonical pathways prompt PKA activation in a cAMP-independent manner. Abnormal PKA activity has been observed in a wide range of cardiac pathologies. Therefore, PKA has the potential to serve as a drug target for the treatment of heart diseases.

Despite the great progress in recent years, the precise roles of PKA in heart disease pathogenesis remain not fully understood. Critical areas that warrant further investigation include:

- Spatiotemporal regulation of PKA activity in cardiac health and disease
- Proteins that determine PKA compartmentalization
- Control of cardiomyocyte morphology, fate, and function by PKA
- Signalling pathways that regulate apoptosis/necrosis downstream of PKA
- Connections between PKA and oxidative stress
- Additional PKA substrates in the heart
- Role of PKA in the sex differences of heart disease
- Heart disease types that require PKA-activating or inhibiting interventions
- Adverse effects of PKA activation or inhibition
- Development of potent and specific PKA activators and inhibitors.

Our current knowledge of cardiac PKA is mostly obtained using indirect approaches, which have limitations as described above. Strategies that directly alter the PKA holoenzyme should be considered in future research. Specific PKA inhibitors, such as PKI are also valuable tools for studying the biological functions of PKA in cardiac physiology and pathophysiology. Development of specific PKA activators or inhibitors with a satisfactory pharmacokinetic profile, good efficacy, and tolerability would greatly benefit future preclinical and clinical studies.

Acknowledgements

The authors would like to thank Dr Yang K. Xiang (University of California Davis) for critical reading of the manuscript.

Conflict of interest: none declared.

Funding

This work was supported by WSU College of Pharmacy and Pharmaceutical Sciences. Z.C. was supported by the National Heart, Lung, and Blood Institute (NHLBI, R00HL119605, R56HL145034), National Institutes of Health.

References

1. Walsh DA, Perkins JP, Krebs EG. An adenosine 3',5'-monophosphate-dependant protein kinase from rabbit skeletal muscle. *J Biol Chem* 1968;**243**:3763–3765.
2. Taylor SS, Ilouz R, Zhang P, Kornev AP. Assembly of allosteric macromolecular switches: lessons from PKA. *Nat Rev Mol Cell Biol* 2012;**13**:646–658.
3. Kirschner LS, Yin Z, Jones GN, Mahoney E. Mouse models of altered protein kinase A signaling. *Endocr Relat Cancer* 2009;**16**:773–793.
4. Wang J, Gareri C, Rockman HA. G-protein-coupled receptors in heart disease. *Circ Res* 2018;**123**:716–735.
5. Smith FD, Esseltine JL, Nygren PJ, Veesler D, Byrne DP, Vonderach M, Strashnov I, Eyers CE, Eyers PA, Langeberg LK, Scott JD. Local protein kinase A action proceeds through intact holoenzymes. *Science* 2017;**356**:1288–1293.
6. Brennan JP, Bardswell SC, Burgoyne JR, Fuller W, Schroder E, Wait R, Begum S, Kentish JC, Eaton P. Oxidant-induced activation of type I protein kinase A is mediated by RI subunit interprotein disulfide bond formation. *J Biol Chem* 2006;**281**:21827–21836.
7. Srinivasan S, Spear J, Chandran K, Joseph J, Kalyanaraman B, Avadhani NG. Oxidative stress induced mitochondrial protein kinase A mediates cytochrome c oxidase dysfunction. *PLoS One* 2013;**8**:e77129.
8. Haushalter KJ, Schilling JM, Song Y, Sastri M, Perkins GA, Strack S, Taylor SS, Patel HH. Cardiac ischemia-reperfusion injury induces ROS-dependent loss of PKA regulatory subunit RIalpha. *Am J Physiol Heart Circ Physiol* 2019;**317**:H1231–H1242.
9. Zhong H, SuYang H, Erdjument-Bromage H, Tempst P, Ghosh S. The transcriptional activity of NF-kappaB is regulated by the IkappaB-associated PKAc subunit through a cyclic AMP-independent mechanism. *Cell* 1997;**89**:413–424.
10. Dulin NO, Niu J, Browning DD, Ye RD, Voyno-Yasenetskaya T. Cyclic AMP-independent activation of protein kinase A by vasoactive peptides. *J Biol Chem* 2001;**276**:20827–20830.
11. Zhang L, Duan CJ, Binkley C, Li G, Uhler MD, Logsdon CD, Simeone DM. A transforming growth factor beta-induced Smad3/Smad4 complex directly activates protein kinase A. *Mol Cell Biol* 2004;**24**:2169–2180.
12. Ma Y, Pitson S, Hercus T, Murphy J, Lopez A, Woodcock J. Sphingosine activates protein kinase A type II by a novel cAMP-independent mechanism. *J Biol Chem* 2005;**280**:26011–26017.
13. Kohr MJ, Traynham CJ, Roof SR, Davis JP, Ziolo MT. cAMP-independent activation of protein kinase A by the peroxynitrite generator SIN-1 elicits positive inotropic effects in cardiomyocytes. *J Mol Cell Cardiol* 2010;**48**:645–648.
14. Stangherlin A, Zaccolo M. Phosphodiesterases and subcellular compartmentalized cAMP signaling in the cardiovascular system. *Am J Physiol Heart Circ Physiol* 2012;**302**:H379–H390.
15. Ghigo A, Mika D. cAMP/PKA signaling compartmentalization in cardiomyocytes: lessons from FRET-based biosensors. *J Mol Cell Cardiol* 2019;**131**:112–121.
16. Bers DM. Cardiac excitation-contraction coupling. *Nature* 2002;**415**:198–205.
17. Wang Y, Shi Q, Li M, Zhao M, Raghavender Reddy G, Teoh JP, Xu B, Zhu C, Ireton KE, Srinivasan S, Chen SL, Gasser PJ, Bossuyt J, Hell JW, Bers DM, Xiang YK. Intracellular beta1-adrenergic receptors and organic cation transporter 3 mediate phospholamban phosphorylation to enhance cardiac contractility. *Circ Res* 2021;**128**:246–261.
18. Fu Y, Westenbroek RE, Scheuer T, Catterall WA. Basal and beta-adrenergic regulation of the cardiac calcium channel CaV1.2 requires phosphorylation of serine 1700. *Proc Natl Acad Sci USA* 2014;**111**:16598–16603.
19. Yang L, Katchman A, Samad T, Morrow J, Weinberg R, Marx SO. Beta-adrenergic regulation of the L-type Ca2+ channel does not require phosphorylation of alpha1C Ser1700. *Circ Res* 2013;**113**:871–880.
20. Liu G, Papa A, Katchman AN, Zakharov SI, Roybal D, Hennessey JA, Kushner J, Yang L, Chen BX, Kushnir A, Dangas K, Gygi SP, Pitt GS, Colecraft HM, Ben-Johny M, Kalocsay M, Marx SO. Mechanism of adrenergic CaV1.2 stimulation revealed by proximity proteomics. *Nature* 2020;**577**:695–700.
21. Vinogradova TM, Lyashkov AE, Zhu W, Ruknudin AM, Sirenko S, Yang D, Deo S, Barlow M, Johnson S, Caffrey JL, Zhou YY, Xiao RP, Cheng H, Stern MD, Maltsev VA, Lakatta EG. High basal protein kinase A-dependent phosphorylation drives rhythmic internal Ca2+ store oscillations and spontaneous beating of cardiac pacemaker cells. *Circ Res* 2006;**98**:505–514.
22. Vettel C, Lindner M, Dewenter M, Lorenz K, Schanbacher C, Riedel M, Lammle S, Meinecke S, Mason FE, Sossalla S, Geerts A, Hoffmann M, Wunder F, Brunner FJ, Wieland T, Mehel H, Karam S, Lechene P, Leroy J, Vandecasteele G, Wagner M, Fischmeister R, El-Armouche A. Phosphodiesterase 2 protects against catecholamine-induced arrhythmia and preserves contractile function after myocardial infarction. *Circ Res* 2017;**120**:120–132.
23. Nakayama H, Chen X, Baines CP, Klevitsky R, Zhang X, Zhang H, Jaleel N, Chua BH, Hewett TE, Robbins J, Houser SR, Molkentin JD. Ca2+- and mitochondrial-dependent cardiomyocyte necrosis as a primary mediator of heart failure. *J Clin Invest* 2007;**117**:2431–2444.
24. Yang L, Dai DF, Yuan C, Westenbroek RE, Yu H, West N, de la Iglesia HO, Catterall WA. Loss of beta-adrenergic-stimulated phosphorylation of CaV1.2 channels on Ser1700 leads to heart failure. *Proc Natl Acad Sci USA* 2016;**113**:E7976–E7985.

25. Kranias EG, Hajjar RJ. Modulation of cardiac contractility by the phospholamban/SERCA2a regulatome. *Circ Res* 2012;**110**:1646–1660.
26. Vinogradova TM, Sirenko S, Lukyanenko YO, Yang D, Tarasov KV, Lyashkov AE, Varghese NJ, Li Y, Chakir K, Ziman B, Lakatta EG. Basal spontaneous firing of rabbit sinoatrial node cells is regulated by dual activation of PDEs (phosphodiesterases) 3 and 4. *Circ Arrhythm Electrophysiol* 2018;**11**:e005896.
27. Zhang Y, Wang WE, Zhang X, Li Y, Chen B, Liu C, Ai X, Zhang X, Tian Y, Zhang C, Tang M, Szeto C, Hua X, Xie M, Zeng C, Wu Y, Zhou L, Zhu W, Yu D, Houser SR, Chen X. Cardiomyocyte PKA ablation enhances basal contractility while eliminates cardiac beta-adrenergic response without adverse effects on the heart. *Circ Res* 2019;**124**:1760–1777.
28. Antos CL, Frey N, Marx SO, Reiken S, Gaburjakova M, Richardson JA, Marks AR, Olson EN. Dilated cardiomyopathy and sudden death resulting from constitutive activation of protein kinase A. *Circ Res* 2001;**89**:997–1004.
29. Li L, Li J, Drum BM, Chen Y, Yin H, Guo X, Luckey SW, Gilbert ML, McKnight GS, Scott JD, Santana LF, Liu Q. Loss of AKAP150 promotes pathological remodelling and heart failure propensity by disrupting calcium cycling and contractile reserve. *Cardiovasc Res* 2017;**113**:147–159.
30. Dobrev D, Wehrens XH. Role of RyR2 phosphorylation in heart failure and arrhythmias: controversies around ryanodine receptor phosphorylation in cardiac disease. *Circ Res* 2014;**114**:1311–1319.
31. Houser SR. Role of RyR2 phosphorylation in heart failure and arrhythmias: protein kinase A-mediated hyperphosphorylation of the ryanodine receptor at serine 2808 does not alter cardiac contractility or cause heart failure and arrhythmias. *Circ Res* 2014;**114**:1320–1327.
32. Bovo E, Huke S, Blatter LA, Zima AV. The effect of PKA-mediated phosphorylation of ryanodine receptor on SR Ca(2+) leak in ventricular myocytes. *J Mol Cell Cardiol* 2017;**104**:9–16.
33. Ellison GM, Torella D, Karakikes I, Purushothaman S, Curcio A, Gasparri C, Indolfi C, Cable NT, Goldspink DF, Nadal-Ginard B. Acute beta-adrenergic overload produces myocyte damage through calcium leakage from the ryanodine receptor 2 but spares cardiac stem cells. *J Biol Chem* 2007;**282**:11397–11409.
34. Ponnam S, Sevrieva I, Sun YB, Irving M, Kampourakis T. Site-specific phosphorylation of myosin binding protein-C coordinates thin and thick filament activation in cardiac muscle. *Proc Natl Acad Sci USA* 2019;**116**:15485–15494.
35. McNamara JW, Singh RR, Sadayappan S. Cardiac myosin binding protein-C phosphorylation regulates the super-relaxed state of myosin. *Proc Natl Acad Sci USA* 2019;**116**:11731–11736.
36. Mamidi R, Gresham KS, Li J, Stelzer JE. Cardiac myosin binding protein-C Ser(302) phosphorylation regulates cardiac beta-adrenergic reserve. *Sci Adv* 2017;**3**:e1602445.
37. Rosas PC, Liu Y, Abdalla MI, Thomas CM, Kidwell DT, Dusio GF, Mukhopadhyay D, Kumar R, Baker KM, Mitchell BM, Powers PA, Fitzsimons DP, Patel BG, Warren CM, Solaro RJ, Moss RL, Tong CW. Phosphorylation of cardiac myosin-binding protein-C is a critical mediator of diastolic function. *Circ Heart Fail* 2015;**8**:582–594.
38. Gresham KS, Stelzer JE. The contributions of cardiac myosin binding protein C and troponin I phosphorylation to beta-adrenergic enhancement of in vivo cardiac function. *J Physiol* 2016;**594**:669–686.
39. Hwang PM, Cai F, Pineda-Sanabria SE, Corson DC, Sykes BD. The cardiac-specific N-terminal region of troponin I positions the regulatory domain of troponin C. *Proc Natl Acad Sci USA* 2014;**111**:14412–14417.
40. Rao V, Cheng Y, Lindert S, Wang D, Oxenford L, McCulloch AD, McCammon JA, Regnier M. PKA phosphorylation of cardiac troponin I modulates activation and relaxation kinetics of ventricular myofibrils. *Biophys J* 2014;**107**:1196–1204.
41. Martin-Garrido A, Biesiadecki BJ, Salhi HE, Shaifta Y, Dos Remedios CG, Ayaz-Guner S, Cai W, Ge Y, Avkiran M, Kentish JC. Monophosphorylation of cardiac troponin-I at Ser-23/24 is sufficient to regulate cardiac myofibrillar Ca(2+) sensitivity and calpain-induced proteolysis. *J Biol Chem* 2018;**293**:8588–8599.
42. Prabu SK, Anandatheerthavarada HK, Raza H, Srinivasan S, Spear JF, Avadhani NG. Protein kinase A-mediated phosphorylation modulates cytochrome c oxidase function and augments hypoxia and myocardial ischemia-related injury. *J Biol Chem* 2006;**281**:2061–2070.
43. Qian J, Ren X, Wang X, Zhang P, Jones WK, Molkentin JD, Fan GC, Kranias EG. Blockade of Hsp20 phosphorylation exacerbates cardiac ischemia/reperfusion injury by suppressed autophagy and increased cell death. *Circ Res* 2009;**105**:1223–1231.
44. Ye Y, Keyes KT, Zhang C, Perez-Polo JR, Lin Y, Birnbaum Y. The myocardial infarct size-limiting effect of sitagliptin is PKA-dependent, whereas the protective effect of pioglitazone is partially dependent on PKA. *Am J Physiol Heart Circ Physiol* 2010;**298**:H1454–H1465.
45. Siraj MA, Mundil D, Beca S, Momen A, Shikatani EA, Afroze T, Sun X, Liu Y, Ghaffari S, Lee W, Wheeler MB, Keller G, Backx P, Husain M. Cardioprotective GLP-1 metabolite prevents ischemic cardiac injury by inhibiting mitochondrial trifunctional protein-alpha. *J Clin Invest* 2020;**130**:1392–1404.
46. Tomita H, Nazmy M, Kajimoto K, Yehia G, Molina CA, Sadoshima J. Inducible cAMP early repressor (ICER) is a negative-feedback regulator of cardiac hypertrophy and an important mediator of cardiac myocyte apoptosis in response to beta-adrenergic receptor stimulation. *Circ Res* 2003;**93**:12–22.
47. Bedioune I, Lefebvre F, Lechene P, Varin A, Domergue V, Kapiloff MS, Fischmeister R, Vandecasteele G. PDE4 and mAKAPbeta are nodal organizers of beta2-ARs nuclear PKA signalling in cardiac myocytes. *Cardiovasc Res* 2018;**114**:1499–1511.
48. Lee YY, Moujalled D, Doerflinger M, Gangoda L, Weston R, Rahimi A, de Alboran I, Herold M, Bouillet P, Xu Q, Gao X, Du XJ, Puthalakath H. CREB-binding protein (CBP) regulates beta-adrenoceptor (beta-AR)-mediated apoptosis. *Cell Death Differ* 2013;**20**:941–952.
49. Watson PA, Reusch JE, McCune SA, Leinwand LA, Luckey SW, Konhilas JP, Brown DA, Chicco AJ, Sparagna GC, Long CS, Moore RL. Restoration of CREB function is linked to completion and stabilization of adaptive cardiac hypertrophy in response to exercise. *Am J Physiol Heart Circ Physiol* 2007;**293**:H246–H259.
50. Wang L, Burmeister BT, Johnson KR, Baillie GS, Karginov AV, Skidgel RA, O'Bryan JP, Carnegie GK. UCR1C is a novel activator of phosphodiesterase 4 (PDE4) long isoforms and attenuates cardiomyocyte hypertrophy. *Cell Signal* 2015;**27**:908–922.
51. Matus M, Lewin G, Stumpel F, Buchwalow IB, Schneider MD, Schutz G, Schmitz W, Muller FU. Cardiomyocyte-specific inactivation of transcription factor CREB in mice. *Faseb J* 2007;**21**:1884–1892.
52. Antos CL, McKinsey TA, Frey N, Kutschke W, McAnally J, Shelton JM, Richardson JA, Hill JA, Olson EN. Activated glycogen synthase-3 beta suppresses cardiac hypertrophy in vivo. *Proc Natl Acad Sci USA* 2002;**99**:907–912.
53. Fang X, Yu SX, Lu Y, Bast RC Jr, Woodgett JR, Mills GB. Phosphorylation and inactivation of glycogen synthase kinase 3 by protein kinase A. *Proc Natl Acad Sci USA* 2000;**97**:11960–11965.
54. Zoccarato A, Surdo NC, Aronsen JM, Fields LA, Mancuso L, Dodoni G, Stangherlin A, Livie C, Jiang H, Sin YY, Gesellchen F, Terrin A, Baillie GS, Nicklin SA, Graham D, Szabo-Fresnais N, Krall J, Vandeput F, Movsesian M, Furlan L, Corsetti V, Hamilton G, Lefkimmiatis K, Sjaastad I, Zaccolo M. Cardiac hypertrophy is inhibited by a local pool of cAMP regulated by phosphodiesterase 2. *Circ Res* 2015;**117**:707–719.
55. Schiattarella GG, Boccella N, Paolillo R, Cattaneo F, Trimarco V, Franzone A, D'Apice S, Giugliano G, Rinaldi L, Borzacchiello D, Gentile A, Lombardi A, Feliciello A, Esposito G, Perrino C. Loss of Akap1 exacerbates pressure overload-induced cardiac hypertrophy and heart failure. *Front Physiol* 2018;**9**:558.
56. Abrenica B, AlShaaban M, Czubryt MP. The A-kinase anchor protein AKAP121 is a negative regulator of cardiomyocyte hypertrophy. *J Mol Cell Cardiol* 2009;**46**:674–681.
57. Liu Y, Xia P, Chen J, Bandettini WP, Kirschner LS, Stratakis CA, Cheng Z. PRKAR1A deficiency impedes hypertrophy and reduces heart size. *Physiol Rep* 2020;**8**:e14405.
58. Ha CH, Kim JY, Zhao J, Wang W, Jhun BS, Wong C, Jin ZG. PKA phosphorylates histone deacetylase 5 and prevents its nuclear export, leading to the inhibition of gene transcription and cardiomyocyte hypertrophy. *Proc Natl Acad Sci USA* 2010;**107**:15467–15472.
59. Chang CW, Lee L, Yu D, Dao K, Bossuyt J, Bers DM. Acute beta-adrenergic activation triggers nuclear import of histone deacetylase 5 and delays G(q)-induced transcriptional activation. *J Biol Chem* 2013;**288**:192–204.
60. Backs J, Worst BC, Lehmann LH, Patrick DM, Jebessa Z, Kreusser MM, Sun Q, Chen L, Heft C, Katus HA, Olson EN. Selective repression of MEF2 activity by PKA-dependent proteolysis of HDAC4. *J Cell Biol* 2011;**195**:403–415.
61. Zile MR, Baicu CF, S. Ikonomidis J, Stroud RE, Nietert PJ, Bradshaw AD, Slater R, Palmer BM, Van Buren P, Meyer M, M. Redfield M, A. Bull D, L. Granzier H, LeWinter MM. Myocardial stiffness in patients with heart failure and a preserved ejection fraction: contributions of collagen and titin. *Circulation* 2015;**131**:1247–1259.
62. Kötter S, Gout L, Von Frieling-Salewsky M, Müller AE, Helling S, Marcus K, Dos Remedios C, Linke WA, Krüger M. Differential changes in titin domain phosphorylation increase myofilament stiffness in failing human hearts. *Cardiovasc Res* 2013;**99**:648–656.
63. Borbély A, Falcao-Pires I, van Heerebeek L, Hamdani N, Édes I, Gavina C, Leite-Moreira AF, Bronzwaer JGF, Papp Z, van der Velden J, Stienen GJM, Paulus WJ, Hypophosphorylation of the Stiff N2B titin isoform raises cardiomyocyte resting tension in failing human myocardium. *Circ Res* 2009;**104**:780–786.
64. Slater RE, Strom JG, Methawasin M, Liss M, Gotthardt M, Sweitzer N, Granzier HL. Metformin improves diastolic function in an HFpEF-like mouse model by increasing titin compliance. *J Gen Physiol* 2019;**151**:42–52.
65. Zhang H, Pan B, Wu P, Parajuli N, Rekhter MD, Goldberg AL, Wang X. PDE1 inhibition facilitates proteasomal degradation of misfolded proteins and protects against cardiac proteinopathy. *Sci Adv* 2019;**5**:eaaw5870.
66. Tsutsui K, Monfredi OJ, Sirenko-Tagirova SG, Maltseva LA, Bychkov R, Kim MS, Ziman BD, Tarasov KV, Tarasova YS, Zhang J, Wang M, Maltsev AV, Brennan JA, Efimov IR, Stern MD, Maltsev VA, Lakatta EG. A coupled-clock system drives the automaticity of human sinoatrial nodal pacemaker cells. *Sci Signal* 2018;**11**:eaap7608.
67. Moen JM, Matt MG, Ramirez C, Tarasov KV, Chakir K, Tarasova YS, Lukyanenko Y, Tsutsui K, Monfredi O, Morrell CH, Tagirova S, Yaniv Y, Huynh T, Pacak K, Ahmet I Lakatta, EG. Overexpression of a neuronal type adenylyl cyclase (type 8) in sinoatrial node markedly impacts heart rate and rhythm. *Front Neurosci* 2019;**13**:615.
68. Lehnart SE, Wehrens XH, Reiken S, Warrier S, Belevych AE, Harvey RD, Richter W, Jin SL, Conti M, Marks AR. Phosphodiesterase 4D deficiency in the ryanodine-receptor complex promotes heart failure and arrhythmias. *Cell* 2005;**123**:25–35.

69. Hopkins PN. Molecular biology of atherosclerosis. *Physiol Rev* 2013;**93**:1317–1542.
70. Schomig A, Dart AM, Dietz R, Mayer E, Kubler W. Release of endogenous catecholamines in the ischemic myocardium of the rat. Part A: locally mediated release. *Circ Res* 1984;**55**:689–701.
71. Lameris TW, de Zeeuw S, Alberts G, Boomsma F, Duncker DJ, Verdouw PD, Veld AJ, van Den Meiracker AH. Time course and mechanism of myocardial catecholamine release during transient ischemia in vivo. *Circulation* 2000;**101**:2645–2650.
72. Graham LN, Smith PA, Stoker JB, Mackintosh AF, Mary DA. Time course of sympathetic neural hyperactivity after uncomplicated acute myocardial infarction. *Circulation* 2002;**106**:793–797.
73. Sandhu R, Thomas U, Diaz RJ, Wilson GJ. Effect of ischemic preconditioning of the myocardium on cAMP. *Circ Res* 1996;**78**:137–147.
74. Lochner A, Genade S, Tromp E, Podzuweit T, Moolman JA. Ischemic preconditioning and the beta-adrenergic signal transduction pathway. *Circulation* 1999;**100**:958–966.
75. Sanada S, Asanuma H, Tsukamoto O, Minamino T, Node K, Takashima S, Fukushima T, Ogai A, Shinozaki Y, Fujita M, Hirata A, Okuda H, Shimokawa H, Tomoike H, Hori M, Kitakaze M. Protein kinase A as another mediator of ischemic preconditioning independent of protein kinase C. *Circulation* 2004;**110**:51–57.
76. Li XD, Yang YJ, Geng YJ, Zhao JL, Zhang HT, Cheng YT, Wu YL. Phosphorylation of endothelial NOS contributes to simvastatin protection against myocardial no-reflow and infarction in reperfused swine hearts: partially via the PKA signaling pathway. *Acta Pharmacol Sin* 2012;**33**:879–887.
77. Penela P, Inserte J, Ramos P, Rodriguez-Sinovas A, Garcia-Dorado D, Mayor F Jr. Degradation of GRK2 and AKT is an early and detrimental event in myocardial ischemia/reperfusion. *EBioMedicine* 2019;**48**:605–618.
78. Wallner M, Duran JM, Mohsin S, Troupes CD, Vanhoutte D, Borghetti G, Vagnozzi RJ, Gross P, Yu D, Trappanese DM, Kubo H, Toib A, Sharp TE, Harper SC, Volkert MA, Starosta T, Feldsott EA, Berretta RM, Wang T, Barbe MF, Molkentin JD, Houser SR. Acute catecholamine exposure causes reversible myocyte injury without cardiac regeneration. *Circ Res* 2016;**119**:865–879.
79. Oikawa M, Wu M, Lim S, Knight WE, Miller CL, Cai Y, Lu Y, Blaxall BC, Takeishi Y, Abe J, Yan C. Cyclic nucleotide phosphodiesterase 3A1 protects the heart against ischemia-reperfusion injury. *J Mol Cell Cardiol* 2013;**64**:11–19.
80. Spear JF, Prabu SK, Galati D, Raza H, Anandatheerthavarada HK, Avadhani NG. beta1-Adrenoreceptor activation contributes to ischemia-reperfusion damage as well as playing a role in ischemic preconditioning. *Am J Physiol Heart Circ Physiol* 2007;**292**:H2459–H2466.
81. Makaula S, Lochner A, Genade S, Sack MN, Awan MM, Opie LH. H-89, a non-specific inhibitor of protein kinase A, promotes post-ischemic cardiac contractile recovery and reduces infarct size. *J Cardiovasc Pharmacol* 2005;**45**:341–347.
82. Mieno S, Horimoto H, Watanabe F, Nakai Y, Furuya E, Sasaki S. Potent adenylate cyclase agonist forskolin restores myoprotective effects of ischemic preconditioning in rat hearts after myocardial infarction. *Ann Thorac Surg* 2002;**74**:1213–1218.
83. Heinen A, Strothoff M, Schmidt A, Stracke N, Behmenburg F, Bauer I, Hollmann MW, Huhn R. Pharmacological options to protect the aged heart from ischemia and reperfusion injury by targeting the PKA-BK(Ca) signaling pathway. *Exp Gerontol* 2014;**56**:99–105.
84. Sanada S, Kitakaze M, Papst PJ, Asanuma H, Node K, Takashima S, Asakura M, Ogita H, Liao Y, Sakata Y, Ogai A, Fukushima T, Yamada J, Shinozaki Y, Kuzuya T, Mori H, Terada N, Hori M. Cardioprotective effect afforded by transient exposure to phosphodiesterase III inhibitors: the role of protein kinase A and p38 mitogen-activated protein kinase. *Circulation* 2001;**104**:705–710.
85. Rechtman MP, Van der Zypp A, Majewski H. Amrinone reduces ischaemia-reperfusion injury in rat heart. *Eur J Pharmacol* 2000;**402**:255–262.
86. Sichelschmidt OJ, Hahnefeld C, Hohlfeld T, Herberg FW, Schror K. Trapidil protects ischemic hearts from reperfusion injury by stimulating PKAII activity. *Cardiovasc Res* 2003;**58**:602–610.
87. Chung YW, Lagranha C, Chen Y, Sun J, Tong G, Hockman SC, Ahmad F, Esfahani SG, Bae DH, Polidovitch N, Wu J, Rhee DK, Lee BS, Gucek M, Daniels MP, Brantner CA, Backx PH, Murphy E, Manganiello VC. Targeted disruption of PDE3B, but not PDE3A, protects murine heart from ischemia/reperfusion injury. *Proc Natl Acad Sci USA* 2015;**112**:E2253–E2262.
88. Zhang Y, Wang XL, Zhao J, Wang YJ, Lau WB, Yuan YX, Gao EH, Koch WJ, Ma XL. Adiponectin inhibits oxidative/nitrative stress during myocardial ischemia and reperfusion via PKA signaling. *Am J Physiol Endocrinol Metab* 2013;**305**:E1436–E1443.
89. Zhang X, Szeto C, Gao E, Tang M, Jin J, Fu Q, Makarewich C, Ai X, Li Y, Tang A, Wang J, Gao H, Wang F, Ge XJ, Kunapuli SP, Zhou L, Zeng C, Xiang KY, Chen X. Cardiotoxic and cardioprotective features of chronic beta-adrenergic signaling. *Circ Res* 2013;**112**:498–509.
90. Rinaldi L, Pozdniakova S, Jayarajan V, Troidl C, Abdallah Y, Aslam M, Ladilov Y. Protective role of soluble adenylyl cyclase against reperfusion-induced injury of cardiac cells. *Biochim Biophys Acta Mol Basis Dis* 2019;**1865**:252–260.
91. Bose AK, Mocanu MM, Carr RD, Brand CL, Yellon DM. Glucagon-like peptide 1 can directly protect the heart against ischemia/reperfusion injury. *Diabetes* 2005;**54**:146–151.
92. Asai M, Tsukamoto O, Minamino T, Asanuma H, Fujita M, Asano Y, Takahama H, Sasaki H, Higo S, Asakura M, Takashima S, Hori M, Kitakaze M. PKA rapidly enhances proteasome assembly and activity in in vivo canine hearts. *J Mol Cell Cardiol* 2009;**46**:452–462.
93. Engelhardt S, Hein L, Wiesmann F, Lohse MJ. Progressive hypertrophy and heart failure in beta1-adrenergic receptor transgenic mice. *Proc Natl Acad Sci USA* 1999;**96**:7059–7064.
94. Iwase M, Bishop SP, Uechi M, Vatner DE, Shannon RP, Kudej RK, Wight DC, Wagner TE, Ishikawa Y, Homcy CJ, Vatner SF. Adverse effects of chronic endogenous sympathetic drive induced by cardiac GS alpha overexpression. *Circ Res* 1996;**78**:517–524.
95. Communal C, Singh K, Pimentel DR, Colucci WS. Norepinephrine stimulates apoptosis in adult rat ventricular myocytes by activation of the beta-adrenergic pathway. *Circulation* 1998;**98**:1329–1334.
96. Lai L, Yan L, Gao S, Hu CL, Ge H, Davidow A, Park M, Bravo C, Iwatsubo K, Ishikawa Y, Auwerx J, Sinclair DA, Vatner SF, Vatner DE. Type 5 adenylyl cyclase increases oxidative stress by transcriptional regulation of manganese superoxide dismutase via the SIRT1/FoxO3a pathway. *Circulation* 2013;**127**:1692–1701.
97. Lai NC, Tang T, Gao MH, Saito M, Takahashi T, Roth DM, Hammond HK. Activation of cardiac adenylyl cyclase expression increases function of the failing ischemic heart in mice. *J Am Coll Cardiol* 2008;**51**:1490–1497.
98. Lipskaia L, Defer N, Esposito G, Hajar I, Garel MC, Rockman HA, Hanoune J. Enhanced cardiac function in transgenic mice expressing a Ca(2+)-stimulated adenylyl cyclase. *Circ Res* 2000;**86**:795–801.
99. Schmitt JP, Kamisago M, Asahi M, Li GH, Ahmad F, Mende U, Kranias EG, MacLennan DH, Seidman JG, Seidman CE. Dilated cardiomyopathy and heart failure caused by a mutation in phospholamban. *Science* 2003;**299**:1410–1413.
100. Dweck D, Sanchez-Gonzalez MA, Chang AN, Dulce RA, Badger CD, Koutnik AP, Ruiz EL, Griffin B, Liang J, Kabbaj M, Fincham FD, Hare JM, Overton JM, Pinto JR. Long term ablation of protein kinase A (PKA)-mediated cardiac troponin I phosphorylation leads to excitation-contraction uncoupling and diastolic dysfunction in a knock-in mouse model of hypertrophic cardiomyopathy. *J Biol Chem* 2014;**289**:23097–23111.
101. Yan L, Vatner DE, O'Connor JP, Ivessa A, Ge H, Chen W, Hirotani S, Ishikawa Y, Sadoshima J, Vatner SF. Type 5 adenylyl cyclase disruption increases longevity and protects against stress. *Cell* 2007;**130**:247–258.
102. Okumura S, Takagi G, Kawabe J, Yang G, Lee MC, Hong C, Liu J, Vatner DE, Sadoshima J, Vatner SF, Ishikawa Y. Disruption of type 5 adenylyl cyclase gene preserves cardiac function against pressure overload. *Proc Natl Acad Sci USA* 2003;**100**:9986–9990.
103. Piddo AM, Sanchez MI, Sapag-Hagar M, Corbalan R, Foncea R, Ebensperger R, Godoy I, Melendez J, Jalil JE, Lavandero S. Cyclic AMP-dependent protein kinase and mechanical heart function in ventricular hypertrophy induced by pressure overload or secondary to myocardial infarction. *J Mol Cell Cardiol* 1996;**28**:1073–1083.
104. Fields LA, Koschinski A, Zaccolo M. Sustained exposure to catecholamines affects cAMP/PKA compartmentalised signalling in adult rat ventricular myocytes. *Cell Signal* 2016;**28**:725–732.
105. Knight WE, Chen S, Zhang Y, Oikawa M, Wu M, Zhou Q, Miller CL, Cai Y, Mickelsen DM, Moravec C, Small EM, Abe J, Yan C. PDE1C deficiency antagonizes pathological cardiac remodeling and dysfunction. *Proc Natl Acad Sci USA* 2016;**113**:E7116–E7125.
106. Chen S, Zhang Y, Lighthouse JK, Mickelsen DM, Wu J, Yao P, Small EM, Yan C. A novel role of cyclic nucleotide phosphodiesterase 10A in pathological cardiac remodeling and dysfunction. *Circulation* 2020;**141**:217–233.
107. Ding B, Abe J, Wei H, Huang Q, Walsh RA, Molina CA, Zhao A, Sadoshima J, Blaxall BC, Berk BC, Yan C. Functional role of phosphodiesterase 3 in cardiomyocyte apoptosis: implication in heart failure. *Circulation* 2005;**111**:2469–2476.
108. Abi-Gerges A, Richter W, Lefebvre F, Mateo P, Varin A, Heymes C, Samuel JL, Lugnier C, Conti M, Fischmeister R, Vandecasteele G. Decreased expression and activity of cAMP phosphodiesterases in cardiac hypertrophy and its impact on beta-adrenergic cAMP signals. *Circ Res* 2009;**105**:784–792.
109. Polidovitch N, Yang S, Sun H, Lakin R, Ahmad F, Gao X, Turnbull PC, Chiarello C, Perry CGR, Manganiello V, Yang P, Backx PH. Phosphodiesterase type 3A (PDE3A), but not type 3B (PDE3B), contributes to the adverse cardiac remodeling induced by pressure overload. *J Mol Cell Cardiol* 2019;**132**:60–70.
110. Yang JH, Polanowska-Grabowska RK, Smith JS, Shields CW, Saucerman JJ. PKA catalytic subunit compartmentation regulates contractile and hypertrophic responses to beta-adrenergic signaling. *J Mol Cell Cardiol* 2014;**66**:83–93.
111. Schirmer I, Bualeong T, Budde H, Cimiotti D, Appukuttan A, Klein N, Steinwascher P, Reusch P, Mugge A, Meyer R, Ladilov Y, Jaquet K. Soluble adenylyl cyclase: a novel player in cardiac hypertrophy induced by isoprenaline or pressure overload. *PLoS One* 2018;**13**:e0192322.
112. Liggett SB, Tepe NM, Lorenz JN, Canning AM, Jantz TD, Mitarai S, Yatani A, Dorn GW. Early and delayed consequences of beta(2)-adrenergic receptor overexpression in mouse hearts: critical role for expression level. *Circulation* 2000;**101**:1707–1714.
113. Pare GC, Bauman AL, McHenry M, Michel JJ, Dodge-Kafka KL, Kapiloff MS. The mAKAP complex participates in the induction of cardiac myocyte hypertrophy by adrenergic receptor signaling. *J Cell Sci* 2005;**118**:5637–5646.

114. Enns LC, Bible KL, Emond MJ, Ladiges WC. Mice lacking the Cbeta subunit of PKA are resistant to angiotensin II-induced cardiac hypertrophy and dysfunction. *BMC Res Notes* 2010;**3**:307.
115. Imaeda A, Tanaka S, Tonegawa K, Fuchigami S, Obana M, Maeda M, Kihara M, Kiyonari H, Conway SJ, Fujio Y, Nakayama H. Myofibroblast beta2 adrenergic signaling amplifies cardiac hypertrophy in mice. *Biochem Biophys Res Commun* 2019;**510**: 149–155.
116. Di Benedetto G, Zoccarato A, Lissandron V, Terrin A, Li X, Houslay MD, Baillie GS, Zaccolo M. Protein kinase A type I and type II define distinct intracellular signaling compartments. *Circ Res* 2008;**103**:836–844.
117. Yin Z, Jones GN, Towns WH, Zhang X, Abel ED, Binkley PF, Jarjoura D, Kirschner LS. Heart-specific ablation of Prkar1a causes failure of heart development and myxomagenesis. *Circulation* 2008;**117**:1414–1422.
118. Enns LC, Morton JF, Treuting PR, Emond MJ, Wolf NS, Dai DF, McKnight GS, Rabinovitch PS, Ladiges WC. Disruption of protein kinase A in mice enhances healthy aging. *PLoS One* 2009;**4**:e5963.
119. Patrizio M, Vago V, Musumeci M, Fecchi K, Sposi NM, Mattei E, Catalano L, Stati T, Marano G. cAMP-mediated beta-adrenergic signaling negatively regulates Gq-coupled receptor-mediated fetal gene response in cardiomyocytes. *J Mol Cell Cardiol* 2008;**45**:761–769.
120. Weeks KL, Ranieri A, Karas A, Bernardo BC, Ashcroft AS, Molenaar C, McMullen JR, Avkiran MB. Adrenergic stimulation induces histone deacetylase 5 (HDAC5) nuclear accumulation in cardiomyocytes by B55alpha-PP2A-mediated dephosphorylation. *J Am Heart Assoc* 2017;**6**:e004861.
121. Backs J, Backs T, Neef S, Kreusser MM, Lehmann LH, Patrick DM, Grueter CE, Qi X, Richardson JA, Hill JA, Katus HA, Bassel-Duby R, Maier LS, Olson EN. The delta isoform of CaM kinase II is required for pathological cardiac hypertrophy and remodeling after pressure overload. *Proc Natl Acad Sci USA* 2009;**106**:2342–2347.
122. Cribbs JT, Strack S. Reversible phosphorylation of Drp1 by cyclic AMP-dependent protein kinase and calcineurin regulates mitochondrial fission and cell death. *EMBO Rep* 2007;**8**:939–944.
123. Sheridan CM, Heist EK, Beals CR, Crabtree GR, Gardner P. Protein kinase A negatively modulates the nuclear accumulation of NF-ATc1 by priming for subsequent phosphorylation by glycogen synthase kinase-3. *J Biol Chem* 2002;**277**:48664–48676.
124. Ichiki T. Role of cAMP response element binding protein in cardiovascular remodeling: good, bad, or both? *ATVB* 2006;**26**:449–455.
125. Dash R, Frank KF, Carr AN, Moravec CS, Kranias EG. Gender influences on sarcoplasmic reticulum Ca2+-handling in failing human myocardium. *J Mol Cell Cardiol* 2001;**33**:1345–1353.
126. Zakhary DR, Moravec CS, Stewart RW, Bond M. Protein kinase A (PKA)-dependent troponin-I phosphorylation and PKA regulatory subunits are decreased in human dilated cardiomyopathy. *Circulation* 1999;**99**:505–510.
127. Messer AE, Jacques AM, Marston SB. Troponin phosphorylation and regulatory function in human heart muscle: dephosphorylation of Ser23/24 on troponin I could account for the contractile defect in end-stage heart failure. *J Mol Cell Cardiol* 2007; **42**:247–259.
128. Han YS, Arroyo J, Ogut O. Human heart failure is accompanied by altered protein kinase A subunit expression and post-translational state. *Arch Biochem Biophys* 2013; **538**:25–33.
129. Jacques AM, Copeland O, Messer AE, Gallon CE, King K, McKenna WJ, Tsang VT, Marston SB. Myosin binding protein C phosphorylation in normal, hypertrophic and failing human heart muscle. *J Mol Cell Cardiol* 2008;**45**:209–216.
130. Marston SB, de Tombe PP. Troponin phosphorylation and myofilament Ca2+-sensitivity in heart failure: increased or decreased? *J Mol Cell Cardiol* 2008;**45**:603–607.
131. Dong X, Sumandea CA, Chen YC, Garcia-Cazarin ML, Zhang J, Balke CW, Sumandea MP, Ge Y. Augmented phosphorylation of cardiac troponin I in hypertensive heart failure. *J Biol Chem* 2012;**287**:848–857.
132. Barbagallo F, Xu B, Reddy GR, West T, Wang Q, Fu Q, Li M, Shi Q, Ginsburg KS, Ferrier W, Isidori AM, Naro F, Patel HH, Bossuyt J, Bers D, Xiang YK. Genetically encoded biosensors reveal PKA hyperphosphorylation on the myofilaments in rabbit heart failure. *Circ Res* 2016;**119**:931–943.
133. Bryant S, Kimura TE, Kong CH, Watson JJ, Chase A, Suleiman MS, James AF, Orchard CH. Stimulation of ICa by basal PKA activity is facilitated by caveolin-3 in cardiac ventricular myocytes. *J Mol Cell Cardiol* 2014;**68**:47–55.
134. Nikolaev VO, Moshkov A, Lyon AR, Miragoli M, Novak P, Paur H, Lohse MJ, Korchev YE, Harding SE, Gorelik J. Beta2-adrenergic receptor redistribution in heart failure changes cAMP compartmentation. *Science* 2010;**327**:1653–1657.
135. Kirchhefer U, Schmitz W, Scholz H, Neumann J. Activity of cAMP-dependent protein kinase and Ca2+/calmodulin-dependent protein kinase in failing and nonfailing human hearts. *Cardiovasc Res* 1999;**42**:254–261.
136. Aye TT, Soni S, van Veen TA, van der Heyden MA, Cappadona S, Varro A, de Weger RA, de Jonge N, Vos MA, Heck AJ, Scholten A. Reorganized PKA-AKAP associations in the failing human heart. *J Mol Cell Cardiol* 2012;**52**:511–518.
137. Haghighi K, Kolokathis F, Gramolini AO, Waggoner JR, Pater L, Lynch RA, Fan G-C, Tsiapras D, Parekh RR, Dorn GW, MacLennan DH, Kremastinos DT, Kranias EG. A mutation in the human phospholamban gene, deleting arginine 14, results in lethal, hereditary cardiomyopathy. *Proc Natl Acad Sci USA* 2006;**103**:1388–1393.
138. Haghighi K, Kolokathis F, Pater L, Lynch RA, Asahi M, Gramolini AO, Fan G-C, Tsiapras D, Hahn HS, Adamopoulos S, Liggett SB, Dorn GW, MacLennan DH, Kremastinos DT, Kranias EG. Human phospholamban null results in lethal dilated cardiomyopathy revealing a critical difference between mouse and human. *J Clin Invest* 2003;**111**:869–876.
139. Mehel H, Emons J, Vettel C, Wittköpper K, Seppelt D, Dewenter M, Lutz S, Sossalla S, Maier LS, Lechêne P, Leroy J, Lefebvre F, Varin A, Eschenhagen T, Nattel S, Dobrev D, Zimmermann W-H, Nikolaev VO, Vandecasteele G, Fischmeister R, El-Armouche A. Phosphodiesterase-2 is up-regulated in human failing hearts and blunts beta-adrenergic responses in cardiomyocytes. *J Am Coll Cardiol* 2013;**62**: 1596–1606.
140. Hashimoto T, Kim GE, Tunin RS, Adesiyun T, Hsu S, Nakagawa R, Zhu G, O'Brien JJ, Hendrick JP, Davis RE, Yao W, Beard D, Hoxie HR, Wennogle LP, Lee DI, Kass DA. Acute enhancement of cardiac function by phosphodiesterase type 1 inhibition. *Circulation* 2018;**138**:1974–1987.
141. Baliga RS, Preedy MEJ, Dukinfield MS, Chu SM, Aubdool AA, Bubb KJ, Moyes AJ, Tones MA, Hobbs AJ. Phosphodiesterase 2 inhibition preferentially promotes NO/guanylyl cyclase/cGMP signaling to reverse the development of heart failure. *Proc Natl Acad Sci USA* 2018;**115**:E7428–E7437.
142. Riehle C, Bauersachs J. Small animal models of heart failure. *Cardiovasc Res* 2019; **115**:1838–1849.
143. Schiattarella GG, Altamirano F, Tong D, French KM, Villalobos E, Kim SY, Luo X, Jiang N, May HI, Wang ZV, Hill TM, Mammen PPA, Huang J, Lee DI, Hahn VS, Sharma K, Kass DA, Lavandero S, Gillette TG, Hill JA. Nitrosative stress drives heart failure with preserved ejection fraction. *Nature* 2019;**568**:351–356.
144. Fu Q, Wang Q, Xiang YK. Insulin and beta adrenergic receptor signaling: crosstalk in heart. *Trends Endocrinol Metab* 2017;**28**:416–427.
145. Mangmool S, Denkaew T, Phosri S, Pinthong D, Parichatikanond W, Shimauchi T, Nishida M. Sustained betaAR stimulation mediates cardiac insulin resistance in a PKA-dependent manner. *Mol Endocrinol* 2016;**30**:118–132.
146. Mullins GR, Wang L, Raje V, Sherwood SG, Grande RC, Boroda S, Eaton JM, Blancquaert S, Roger PP, Leitinger N, Harris TE. Catecholamine-induced lipolysis causes mTOR complex dissociation and inhibits glucose uptake in adipocytes. *Proc Natl Acad Sci USA* 2014;**111**:17450–17455.
147. Wang Q, Liu Y, Fu Q, Xu B, Zhang Y, Kim S, Tan R, Barbagallo F, West T, Anderson E, Wei W, Abel ED, Xiang YK. Inhibiting insulin-mediated beta2-adrenergic receptor activation prevents diabetes-associated cardiac dysfunction. *Circulation* 2017;**135**:73–88.
148. Bockus LB, Humphries KM. cAMP-dependent protein kinase (PKA) signaling is impaired in the diabetic heart. *J Biol Chem* 2015;**290**:29250–29258.
149. Minhas AS, Scheel P, Garibaldi B, Liu G, Horton M, Jennings M, Jones SR, Michos ED, Hays AG. Takotsubo syndrome in the setting of COVID-19. *JACC Case Rep* 2020;**2**:1321–1325.
150. Jabri A, Kalra A, Kumar A, Alameh A, Adroja S, Bashir H, Nowacki AS, Shah R, Khubber S, Kanaa NA, Hedrick DP, Sleik KM, Mehta N, Chung MK, Khot UN, Kapadia SR, Puri R, Reed GW. Incidence of stress cardiomyopathy during the coronavirus disease 2019 pandemic. *JAMA Netw Open* 2020;**3**:e2014780.
151. Scally C, Rudd A, Mezincescu A, Wilson H, Srivanasan J, Horgan G, Broadhurst P, Newby DE, Henning A, Dawson DK. Persistent long-term structural, functional, and metabolic changes after stress-induced (takotsubo) cardiomyopathy. *Circulation* 2018;**137**:1039–1048.
152. Pelliccia F, Kaski JC, Crea F, Camici PG. Pathophysiology of takotsubo syndrome. *Circulation* 2017;**135**:2426–2441.
153. Shao Y, Redfors B, Stahlman M, Tang MS, Miljanovic A, Mollmann H, Troidl C, Szardien S, Hamm C, Nef H, Boren J, Omerovic E. A mouse model reveals an important role for catecholamine-induced lipotoxicity in the pathogenesis of stress-induced cardiomyopathy. *Eur J Heart Fail* 2013;**15**:9–22.
154. Borchert T, Hübscher D, Guessoum CI, Lam T-DD, Ghadri JR, Schellinger IN, Tiburcy M, Liaw NY, Li Y, Haas J, Sossalla S, Huber MA, Cyganek L, Jacobshagen C, Dressel R, Raaz U, Nikolaev VO, Guan K, Thiele H, Meder B, Wollnik B, Zimmermann W-H, Lüscher TF, Hasenfuss G, Templin C, Streckfuss-Bömeke K. Catecholamine-dependent beta-adrenergic signaling in a pluripotent stem cell model of takotsubo cardiomyopathy. *J Am Coll Cardiol* 2017;**70**:975–991.
155. Llach A, Mazevet M, Mateo P, Villejouvert O, Ridoux A, Rucker-Martin C, Ribeiro M, Fischmeister R, Crozatier B, Benitah J-P, Morel E, Gómez AM. Progression of excitation-contraction coupling defects in doxorubicin cardiotoxicity. *J Mol Cell Cardiol* 2019;**126**:129–139.
156. Seicean S, Seicean A, Alan N, Plana JC, Budd GT, Marwick TH. Cardioprotective effect of beta-adrenoceptor blockade in patients with breast cancer undergoing chemotherapy: follow-up study of heart failure. *Circ Heart Fail* 2013;**6**:420–426.
157. Efentakis P, Varela A, Chavdoula E, Sigala F, Sanoudou D, Tenta R, Gioti K, Kostomitsopoulos N, Papapetropoulos A, Tasouli A, Farmakis D, Davos CH, Klinakis A, Suter T, Cokkinos DV, Iliodromitis EK, Wenzel P, Andreadou I. Levosimendan prevents doxorubicin-induced cardiotoxicity in time- and dose-dependent manner: implications for inotropy. *Cardiovasc Res* 2020;**116**:576–591.
158. Zhang Y, Knight W, Chen S, Mohan A, Yan C. Multiprotein complex with TRPC (Transient Receptor Potential-Canonical) channel, PDE1C (Phosphodiesterase 1C), and A2R (Adenosine A2 Receptor) plays a critical role in regulating cardiomyocyte cAMP and survival. *Circulation* 2018;**138**:1988–2002.

159. Trexler CL, Odell AT, Jeong MY, Dowell RD, Leinwand LA. Transcriptome and functional profile of cardiac myocytes is influenced by biological sex. *Circ Cardiovasc Genet* 2017;**10**:e001770.
160. Cao X, Zhou C, Chong J, Fu L, Zhang L, Sun D, Hou H, Zhang Y, Li D, Sun H. Estrogen resisted stress-induced cardiomyopathy through increasing the activity of beta(2)AR-Galphas signal pathway in female rats. *Int J Cardiol* 2015;**187**:377–386.
161. Machuki JO, Zhang HY, Geng J, Fu L, Adzika GK, Wu L, Shang W, Wu J, Kexue L, Zhao Z, Sun H. Estrogen regulation of cardiac cAMP-L-type Ca(2+) channel pathway modulates sex differences in basal contraction and responses to beta2AR-mediated stress in left ventricular apical myocytes. *Cell Commun Signal* 2019;**17**:34.
162. El-Battrawy I, Zhao Z, Lan H, Schunemann JD, Sattler K, Buljubasic F, Patocskai B, Li X, Yucel G, Lang S, Nowak D, Cyganek L, Bieback K, Utikal J, Zimmermann WH, Ravens U, Wieland T, Borggrefe M, Zhou XB, Akin I. Estradiol protection against toxic effects of catecholamine on electrical properties in human-induced pluripotent stem cell derived cardiomyocytes. *Int J Cardiol* 2018;**254**:195–202.
163. Parks RJ, Ray G, Bienvenu LA, Rose RA, Howlett SE. Sex differences in SR Ca(2+) release in murine ventricular myocytes are regulated by the cAMP/PKA pathway. *J Mol Cell Cardiol* 2014;**75**:162–173.
164. Parks RJ, Bogachev O, Mackasey M, Ray G, Rose RA, Howlett SE. The impact of ovariectomy on cardiac excitation-contraction coupling is mediated through cAMP/PKA-dependent mechanisms. *J Mol Cell Cardiol* 2017;**111**:51–60.
165. Lezoualc'h F, Fazal L, Laudette M, Conte C. Cyclic AMP sensor EPAC proteins and their role in cardiovascular function and disease. *Circ Res* 2016;**118**:881–897.
166. Robichaux WG, Cheng X. Intracellular cAMP sensor EPAC: physiology, pathophysiology, and therapeutics development. *Physiol Rev* 2018;**98**:919–1053.
167. Huang S, Li Q, Alberts I, Li X. PRKX, a novel cAMP-dependent protein kinase member, plays an important role in development. *J Cell Biochem* 2016;**117**:566–573.
168. Biel M. Cyclic nucleotide-regulated cation channels. *J Biol Chem* 2009;**284**:9017–9021.
169. James ZM, Borst AJ, Haitin Y, Frenz B, DiMaio F, Zagotta WN, Veesler D. CryoEM structure of a prokaryotic cyclic nucleotide-gated ion channel. *Proc Natl Acad Sci USA* 2017;**114**:4430–4435.
170. Brand T. POPDC proteins and cardiac function. *Biochem Soc Trans* 2019;**47**:1393–1404.
171. Diviani D, Reggi E, Arambasic M, Caso S, Maric D. Emerging roles of A-kinase anchoring proteins in cardiovascular pathophysiology. *Biochim Biophys Acta* 2016;**1863**:1926–1936.
172. Murray AJ. Pharmacological PKA inhibition: all may not be what it seems. *Sci Signal* 2008;**1**:re4.