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Therapeutic potential of PDE modulation in treating heart disease

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Abstract

Altered cyclic nucleotide-mediated signaling plays a critical role in the development of cardiovascular pathology. By degrading cAMP/cGMP, the action of cyclic nucleotide PDEs is essential for controlling cyclic nucleotide-mediated signaling intensity, duration, and specificity. Altered expression, localization and action of PDEs have all been implicated in causing changes in cyclic nucleotide signaling in cardiovascular disease. Accordingly, pharmacological inhibition of PDEs has gained interest as a treatment strategy and as an area of drug development. While targeting of certain PDEs has the potential to ameliorate cardiovascular disease, inhibition of others might actually worsen it. This review will highlight recent research on the physiopathological role of cyclic nucleotide signaling, especially with regard to PDEs. While the physiological roles and biochemical properties of cardiovascular PDEs will be summarized, the primary emphasis will be pathological. Research into the potential benefits and hazards of PDE inhibition will also be discussed.

Cardiovascular cyclic nucleotide signaling

The cyclic nucleotides, cAMP and cGMP, play critical roles as mediators of signal transduction cascades in numerous cell types throughout the body. In the cardiomyocyte, cAMP and cGMP play numerous, and sometimes antagonistic roles, both physiologically and pathologically. In addition, acute and chronic cyclic nucleotide signaling can have divergent effects. For example, acutely, β -adrenergic produced cAMP increases cardiac contractile force and pacemaking, while activation of certain cGMP pools antagonizes this. Chronic stimulation of β -adrenergic receptors is associated with development of maladaptive cardiac remodeling, fibrosis and cardiac myocyte apoptosis, while chronic cGMP signaling can attenuate these same effects and preserve cardiac function. For this reason, clinical trials focusing on drugs that potentiate cardiac β -adrenergic cAMP signaling have been associated

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with increased incidence of cardiac dysfunction [1] and patient death, while there is currently a high level of interest in agents that potentiate cardiac cGMP [2].

Within cells, cAMP and cGMP are each involved in multiple, independent signal transduction cascades. For example, in the cardiomyocyte, cAMP produced by catecholamines/ β -adrenergic signaling regulates contractility and excitation-contraction coupling [3], yet relaxin-mediated cAMP production appears to have entirely separate effects, such as acting as a vasodilator in the cardiovascular system [4]. To achieve this degree of signaling specificity, signal transduction cascades must be highly regulated, both temporally and spatially. This is believed to be dependent on the existence of multiple divergent macromolecular complexes containing unique anchoring proteins, cyclases, PDEs, kinases and other effector molecules. One mechanism of spatio-temporal cyclic nucleotide regulation is through the action or inhibition of PDEs.

Cardiac PDEs

By degrading cAMP and cGMP, PDEs constitute a major mechanism by which cyclic nucleotide signaling is terminated. PDEs appear to both sequester cyclic nucleotides to certain regions of cells, regulating them spatially, and to function as cyclic nucleotide ‘sinks’ within the cell, terminating cyclic nucleotide signaling temporally. The PDE superfamily contains 11 family members (PDE1–11), each with distinct substrate specificities, enzymatic kinetics, cellular/subcellular localizations, and mechanisms of regulation [5]. All 11 PDE family members contain a highly conserved core catalytic region of approximately 270 amino acids, but are distinguished by significant variation in other regions of the protein, particularly within N-terminal and C-terminal regions [6]. The existence of multiple unique PDE families that are different guarantees heterogeneity in cyclic nucleotide signaling and divergence in biological functions regulated by cyclic nucleotides. Increasing evidence indicates that different PDE isozymes associate with discrete pools of cyclic nucleotides and regulate distinct biological functions. Altered PDE expression/activity and cyclic nucleotide signaling have been reported in a number of types of cardiovascular disease, and pharmacological inhibition of PDEs represents a potential approach to treating some of these diseases. Of the 11 PDE families, PDE1-5 and PDE8 have been reported in the cardiomyocyte [7]. The role of each of these PDEs as well as the tools available to study their functions in cardiomyocytes are summarized in Table 1. Therefore, a very precise understanding of the role of each PDE in cardiac physiology and pathology is essential. Additionally, as a number of highly specific PDE inhibitors have been developed or are under development for treating non-cardiovascular diseases, understanding the targeting of PDEs in cardiovascular biology and disease would help to predict their potential cardiovascular side effects and facilitate the development of new applications of these drugs in treating cardiovascular diseases. This review will attempt to summarize what is known of the regulation and function of each PDE in the myocardium, and how this modulates cyclic nucleotide signaling under physiological and pathological conditions. Potential uses of PDE inhibitors for the treatment of cardiovascular diseases in both research animals and humans, and the potential for development of new PDE inhibitors, or other PDE-modulating drugs, to affect therapeutic strategies in **heart failure**, will be also discussed.

PDE1

PDE1 family members are known as the calcium-calmodulin ($\text{Ca}^{2+}/\text{CaM}$)-stimulated PDEs because they can be activated by Ca^{2+} in the presence of calmodulin *in vitro*. The PDE1 family includes three genes: *PDE1A*, *1B* and *1C*, with multiple splice variants occurring in the N- and C-termini of each gene. All PDE1 variants are capable of hydrolyzing both cAMP and cGMP *in vitro*: PDE1A and 1B have higher affinities for cGMP than cAMP, while PDE1C has similarly high affinities for cAMP and cGMP [7]. Previous PDE assay results have indicated that that PDE1 may represent the primary PDE activity in the normal human myocardium [8,9]. However, the relative expression levels of different PDE1 isoforms appear to vary with species. For example, in normal hearts, PDE1A is similarly expressed in human, mouse, and rat. PDE1C appears to be highly expressed in human and mouse [8,10,11], but is very limited in rat [10]. Under pathological conditions, the expression of PDE1 has been shown to be altered. For example, upregulation of PDE1A expression was found *in vivo* in the heart from various animal models of pathological hypertrophy [10] and in human diseased hearts [12]. PDE1A expression is not only increased in myocytes but also induced in activated fibroblasts in fibrotic regions of hearts undergoing pathological remodeling, which was found in both rodents and human [12], and is supported by findings in isolated rat ventricular myocytes and fibroblasts in response to neurohumoral stimulation. However, one previous study showed that PDE1C protein levels were not significantly changed in a pressure overload mouse model of cardiac remodeling [11]. It is worth noting that the relative PDE1 expression levels reported in different studies may be also influenced by differences in the degree of cardiac dysfunction, tissue/cell preparation, and antibody sources. In accordance with its increased expression in diseased state, administration of a pan-PDE1 inhibitor was shown to decrease isoproterenol-infusion induced cardiac hypertrophy, with decreased myocardial hypertrophy and fibrosis [10]. More specifically, *in vitro* studies in isolated cardiac cells suggest that PDE1A may play an important role in development of cardiac hypertrophy and fibrosis. In neonatal and adult rat ventricular myocytes, phenylephrine-induced myocyte hypertrophic growth and/or hypertrophic marker expression were significantly attenuated by a PDE1 inhibitor or PDE1A siRNA [10]. In cultured **cardiac fibroblasts**, PDE1A inhibition or knockdown significantly reduced fibroblast activation and matrix protein synthesis induced by fibrotic stimuli. While these *in vitro* findings support the potential importance of PDE1A in pathological cardiac remodeling by positively regulating both cardiomyocyte hypertrophy and fibrosis, further studies with cell type-specific PDE1A genetically modified animals are necessary. In addition, many of these previous studies relied on rat cardiac myocytes, which appear to have very little PDE1C expression. Thus, PDE1C function might be underestimated. Given that PDE1C is highly expressed in normal human and mouse heart [10], the biological and pathological functions of PDE1C deserve further investigation. It has also been argued that some of the cardioprotective effects of PDE5 inhibitors are through off-target inhibition of PDE1 [11,13], and further studies will certainly be needed to confirm or deny this idea. Given the potential for PDE1 inhibitors in treating cardiovascular disease, the development of PDE1 inhibitors, specifically isoform specific inhibitors, could present a compelling area of research in the future. As the core catalytic region of PDE1 is highly conserved among isoforms, isoform-specific inhibitors would likely need to interact

with unique N- or C-terminal regions on these proteins. It is also possible that small molecules which mimic the binding of ($\text{Ca}^{2+}/\text{CaM}$) specifically to PDE1 could function as PDE1 activators or inhibitors.

PDE2

PDE2 is able to hydrolyze both cAMP and cGMP. The N-terminal region of PDE2 family members contain two GAF domains to which cGMP can bind, resulting in up to a 30-fold increase in catalytic activity. For this reason, PDE2 is often referred to as the cGMP-stimulated PDE. In the cardiomyocyte, PDE2 appears to be involved in cGMP-mediated regulation of cAMP and cardiac contractility. For example, in adult mouse **cardiomyocytes**, activation of β_3 -adrenergic receptors results in NO/cGMP production, which activates PDE2, resulting in degradation of β_1 - and β_2 -produced cAMP [14]. This PDE2 activation blunts isoproterenol-mediated contractility increases [14]. Similarly, in frog ventricular myocytes, the reduction in isoproterenol-induced L-type Ca^{2+} channel (LTCC) activity observed in response to NO treatment also appears to be dependent on PDE2, as pharmacological inhibition of PDE2 blocks this effect [15]. In addition to ventricular myocytes, PDE2 is also involved in the cGMP-dependent reduction in basal L-type Ca^{2+} channel current ($\text{I}_{\text{Ca, L}}$) observed in human atrial cardiac myocytes [16]. Presumably, all these effects occur due to NO/cGMP-mediated PDE2 activation, which results in cAMP degradation, blunting the isoproterenol response. However, this PDE2-dependent mechanism does not apply to all species or all types of myocytes, as studies in ventricular myocytes from guinea pig, human [17], and rat [18] have found that the cGMP-dependent reduction in LTCC activity is PKG-, but not PDE2-dependent. In addition to its role in regulating cAMP pools, PDE2 was also reported to regulate cGMP. In particular, PDE2 is largely associated with the cGMP pools produced by pGC [19]. Under pathological conditions, it was reported that PDE2 mRNA levels and activity are substantially increased in response to pressure overload in rat heart [20]. In failing human hearts, an increased amount of AKAP-bound PDE2 has been reported. Binding of PDE2 by AKAP may prevent PDE2 from hydrolyzing cyclic nucleotides, which could alter cyclic nucleotide signaling and contribute to the pathology of heart failure [21]. Of course, this mechanism could be further verified by specifically disrupting the interaction between AKAP and PDE2. The role of PDE2 in cardiac biology and disease *in vivo* has not been well characterized, and certainly warrants further investigation. Given its lack of a well established role in cardiac disease, development of more specific inhibitors for PDE2 may not be as critical as for other PDEs, but could allow a more detailed characterization of the role of PDE2 in the heart.

PDE3

PDE3 hydrolyzes cAMP with a much higher V_{max} than cGMP, therefore, cGMP effectively inhibits cAMP hydrolysis. For this reason, PDE3 is often referred to as the cGMP-inhibited PDE [22], as under certain conditions, PDE3 has been shown to play a critical role in cGMP-dependent potentiation of cAMP signaling [23–26]. Two PDE3 genes, *PDE-3A* and *-3B*, have been cloned, with both expressed in the heart [27]. Under physiological conditions, PDE3 is known to be important in regulating cardiac pacemaking and contractility, primarily modulating cAMP/PKA signaling [28,29], as pharmacological

inhibition of PDE3 results in a dramatic increase in heart rate and cardiac contractile function in both humans [30] and in animals [31,32]. However, the PDE3 isoform responsible for the chronotropic and inotropic effects of PDE3 inhibitors was not known until recent studies using *PDE3A* and *PDE3B* knockout mice. For example, the observation that basal heart rate is increased in *PDE3A* but not *PDE3B* knockout mice indicates that PDE3A regulates pacemaker activity and heart rate [31]. In addition, increased cardiac contractility by PDE3 inhibitors was found to be diminished in PDE3A but not PDE3B deficient hearts either measured with an *ex vivo* system [33] or assessed *in vivo* [31]. Consistently, *ex vivo* Langendorff perfused hearts from *PDE3A* knockout mice displayed increased LVDP, heart rate, and dP/dtmax relative to WT and *PDE3B* knockout mice at resting state [33]. These data indicate that PDE3A is the primary isoform involved in regulating cardiac contractile function. This increased contractility in *PDE3A* knockout hearts was associated with larger Ca^{2+} transients, greater sarcoplasmic reticulum (SR) Ca^{2+} loading, and PKA-dependent phosphorylation of PLB and RyR2. PDE3A was found to associate with a multimolecule complex including SERCA2a, phospholamban, PKA inhibitory subunit 2, and protein phosphatase 2A [33]. Emerging evidence indicates that PI3K γ acts as an important regulator of cAMP levels. It does so by modulating PDE activity through its function as a scaffold protein for PDEs and PKA [34]. For example, PI3K γ has been shown to associate with PDE3B and locally regulate cAMP/PKA signaling in a kinase-independent manner [35–37], and PI3K γ -PDE3B was reported to control LTCC activity in cardiomyocytes [38]. However, another study later demonstrated that PI3K γ is essential for PDE4, not PDE3, activity in subcellular compartments that associate with SERCA2 but not RyR2 or LTCCs in cardiomyocytes [39]. Interestingly, a recent study defined a multiprotein complex containing PI3K γ , PDE3A, PDE4A and PDE4B, but not PDE4D, which was shown to regulate PKA-mediated phosphorylation of LTCCs and phospholamban [40]. It is not clear whether PDE3B is also in this complex. Although the findings from these studies strongly indicate that PI3K γ controls local cAMP signaling by regulating PDE action in the signaling complex, it is still not clear whether PI3K γ complexes containing distinct PDE isoforms are present in different subcellular compartments. The characterization of PI3K γ complexes in these studies was largely dependent on co-immunoprecipitation approaches, the results of which might be affected by differences in tissue/cell lysate preparation and antibody source. It is possible that a signaling complex may be dissociated during lysate preparation, and some types of antibodies may not be effective for immunoprecipitation, particularly if they happen to block a binding site. Nevertheless, PI3K γ -mediated regulation of PDE activity and localization appears to play a critical role in precisely controlling cAMP signaling, thus protecting against ventricular arrhythmias [40] and pathological cardiac remodeling [35].

PDE3 inhibition is associated with increased cardiac contractility and cardiac output, as well as reduced peripheral vascular resistance, thereby ameliorating many symptoms of heart failure. Thus, PDE3 inhibitors, including amrinone, milrinone and enoximone, have been used to treat congestive heart failure [41]. Despite these benefits, a number of clinical trials have revealed that chronic treatment with PDE3 inhibitors in heart failure patients is associated with increased mortality due to arrhythmias and sudden death [1,42]. Therefore, PDE3 inhibitors are now used clinically only under certain conditions, including in patients

with severe heart failure resistant to standard regimens and in patients awaiting cardiac transplant [43,44]. Although the mechanism of detrimental effects of chronic PDE3 inhibition in human is still not completely understood, recent findings regarding PDE3 inhibition and cardiomyocyte apoptosis in isolated cardiomyocytes and experimental animals may provide an explanation. For example, in isolated neonatal and adult cardiomyocytes, chronic PDE3 inhibition or PDE3A depletion significantly enhanced cardiomyocyte apoptosis induced by neurohumoral stimuli such as isoproterenol and angiotensin II [45,46]. Chronic inhibition of PDE3A function resulted in sustained induction of the inducible cAMP early repressor (ICER) via a cAMP/PKA-mediated increase of ICER protein stability; ICER in turn suppressed expression of the anti-apoptotic molecule Bcl2 and triggered cardiomyocyte apoptosis [46,47]. In accordance with these *in vitro* findings, myocardial overexpression of PDE3A1 in transgenic mice reduces ICER levels, increases Bcl2 expression, reduces cardiomyocyte apoptosis and prevents ischemia/reperfusion induced myocardial infarction [201]. Interestingly, PDE3A downregulation has been found in human failing hearts as well as in hearts from animals with cardiac hypertrophy and/or dysfunction [45,48–51]. Restoring cardiac function in experimental models of heart failure, including treatment with angiotensin receptor antagonists or overexpression of constitutively active mitogen-activated protein/extracellular signal-regulated protein kinase (CA-MEK5 α), prevented PDE3A downregulation and ICER induction [50]. These results strongly suggest that chronically blocking PDE3A function promotes cardiomyocyte apoptosis and accelerates development of cardiac dysfunction. A topic of interest for future study is whether pathological cardiac remodeling and dysfunction is accelerated in *PDE3A* knockout mice. In addition, PDE3B appears to be an important mediator for PI3K γ in regulating cardiac cAMP signaling [35–37]. Despite this, no change in cardiac function, such as heart rate and contractility, has been observed in PDE3B knockout mice [31,33,52]. Future studies investigating the specific contribution of PDE3B to cardiac physiology and pathology would be desirable. As PDE3A and PDE3B have very divergent roles in the myocardium, as well as in other tissues, development of isoform-specific PDE3 inhibitors is extremely desirable. Although there is a high degree of homology between the two isoforms [6], it has been reported that certain inhibitors may already have different affinities for PDE3A and 3B variants [53].

PDE4

The PDE4 family is highly specific for cAMP, and contains four different genes, *PDE4A–4D*. Among them, *PDE4A*, *4B* and *4D* have been reported in the heart [54,55]. The relative contribution of PDE4 activity in the myocardium seems to vary significantly with species. *In vitro* PDE activity assays revealed that PDE4 is responsible for 50–60% of total cAMP hydrolytic activity in rat, approximately 30% in mouse, and only about 10% in human [56]. Physiologically, PDE4 isoforms associate with β 1 and β 2 adrenergic receptors, and are involved in termination of signaling from these receptors [57–59]; tethering of PDE4D5 to the β 2-AR has also been reported to block hypertrophic signals produced in response to chronic β 2 stimulation [60]. Genetic models have further underscored the unique roles of various PDE4 isoforms in the heart. Studies in isolated murine cardiomyocytes with genetic depletion of PDE4B and PDE4D have found that both these PDE4 isoforms regulate cardiac

contractility and Ca^{2+} cycling [55]. Furthermore, the PDE4D3 isoform was reported to interact with the Ryr2 complex and act as a key regulator of PKA-mediated RyR2 phosphorylation [61]. Blocking PDE4D function in the complex resulted in RyR2 hyperphosphorylation and SR Ca^{2+} leakage [61]. PDE4D3-mediated RyR2 hyperphosphorylation was believed to be responsible for the adverse effects of PDE4 inhibitors on exercise- or catecholamine-induced arrhythmogenesis [61,62]. *PDE4D* global knockout mice also developed age-dependent cardiomyopathy associated with RyR2 hyperphosphorylation, increased RyR2 leakage, and severe contractile dysfunction [61]. Worsened cardiac dysfunction post myocardial infarction in *PDE4D* knockout mice was significantly attenuated in phospho-RyR2 null mice, supporting a critical role for PDE4D in regulating RyR2 phosphorylation status. Interestingly, a decrease in PDE4D in the RyR2 complex and an increase in RyR2 phosphorylation were also detected in human failing hearts [61]. However, in a different study, PDE4D was found to associate exclusively with SERCA2, but not RyR2 [63]. These different results may suggest that PDE4D associates with various sets of proteins in discrete complexes in the cell, which are detected under different experimental conditions. Mice with genetic ablation of *PDE4B* are predisposed to developing tachycardia and burst-pacing induced ventricular arrhythmias in the presence of catecholamines, probably via enhanced ICa,L [55]. Interestingly, it was also recently reported that PDE4A and 4B are both tethered to $\text{PI3K}\gamma$, and that loss of this tethering (by $\text{PI3K}\gamma$ depletion) causes increased phospholamban and LTCC phosphorylation, Ca^{2+} leak, and arrhythmias [40]. Together, these findings indicate that PDE4 isoforms, and their proper localization in the cell, play a critical role in protecting against cardiac dysfunction in mice. Given its multiple reported interactions with PDE3 and PDE4 isoforms, $\text{PI3K}\gamma$ appears to be emerging as a central regulator of cardiomyocyte cAMP levels and localization [35,37,40]. Despite the dramatic detrimental cardiac effects seen with chronic PDE4 inhibition or PDE4B/4D depletion in mice, similar effects associated with PDE4 inhibition in humans have not been well characterized. Because PDE4 represents a much smaller portion of total cAMP-hydrolyzing activity in human ventricular tissue compared with rodents [56], the cardiac effects elicited by PDE4 inhibition may be more significant in rodents than in humans. The relative contributions of PDE4 may also be different between ventricle and atrium. A recent study reported that PDE4, particularly PDE4D, represents a larger portion of cAMP hydrolyzing activity in human atrial tissue and that pharmacological inhibition of PDE4 in human atrial trabeculae increased incidence of arrhythmias in response to β_1 and β_2 stimulation [64]. Cardiac PDE4D expression was also reduced in humans with atrial fibrillation, and a one-year clinical trial investigating the effects of the PDE4 inhibitor rofluminate in humans found that the rofluminate-treated group had a slight increase in incidence of atrial arrhythmias [65]. Therefore, PDE4D may play an important role in human atrial function and susceptibility to arrhythmias. Further studies to this effect, particularly utilizing *in vivo* approaches, would be highly desirable. As with PDE3, development of isoform-specific PDE4 inhibitors could be extremely beneficial, and could help reduce side effects associated with inhibitor treatment. Numerous crystal structures for PDE4 isoforms are already available, and some progress has already been made in deriving isoform-selective PDE4 inhibitors [66].

PDE5

PDE5 specifically hydrolyzes cGMP with a high affinity, and is also known as the cGMP-specific PDE. It contains cGMP-binding GAF domains and a PKG phosphorylation site in the N-terminal regulatory region. Increasing cGMP levels activates PDE5 through cGMP binding to GAFs and phosphorylation by PKG, which is a critical mechanism by which PDE5 regulates cGMP signaling in a negative feedback loop. *PDE5A* is the only gene thus far cloned in PDE5 family [67]. In the healthy myocardium, *PDE5* appears to be expressed at very low levels in human [13,68] and in mouse [69,70]. In normal hearts, administration of the PDE5 inhibitor sildenafil attenuated isoproterenol-stimulated contractility in both human [71] and mouse [72], suggesting that PDE5-cGMP signaling negatively regulates catecholamine-induced contractile function. The negative effect of PDE5 inhibition on contractility depends on both PKG and NOS-mediated signaling [73]. Via fluorescent and electron microscopy, PDE5 has been shown to be concentrated in the Z-lines of murine cardiomyocytes [74]. The localization of PDE5 to Z-bands is dependent on eNOS and is essential for its regulation of cardiac contractility [73], implying that PDE5 regulates highly localized cGMP pools in normal hearts [73].

Under pathological conditions, PDE5 expression in the heart can be highly upregulated. For example, in human diseased hearts, increased cardiac PDE5 expression was reported in hypertrophied, dilated and ischemic cardiomyopathies [13,69,75], as well as in congestive heart failure [76]. Cardiac PDE5 expression also appears to correlate well with severity of heart failure in human patients [77]. In mouse, PDE5 expression is upregulated in experimental heart failure induced by myocardial infarction [13] and transverse aortic constriction (TAC) [76]. However, one study has reported that *PDE5A* is present only in cardiac fibroblasts, not myocytes, in the hypertrophied mouse heart [11]. This discrepancy might be due to differences in *PDE5A* antibody specific and/or the type and severity of cardiac dysfunction in animal models. PDE5 targeting and coupling with other signaling molecules may be also subjected to changes in diseased states: a recent study showed that pathological cardiac hypertrophy induced by TAC alter PDE5 targeting from eNOS-mediated cGMP to natriuretic peptide-mediated cGMP, which could block the antihypertrophic effects of ANP/BNP signaling [78].

PDE5 inhibitors such as sildenafil have been widely used to explore the role of PDE5 inhibition in experimental animal disease models. For example, administration of sildenafil dramatically reduced or reversed pre-existing TAC-induced development of cardiac dysfunction, fibrosis, and hypertrophy in mouse [79]. Sildenafil treatment has also been shown to improve cardiac function and attenuate apoptosis in other models of cardiac dysfunction, including ischemia-reperfusion injury in mouse [80] and rabbit [81], Ang II-infusion in mouse [82], and doxorubicin-induced cardiotoxicity in mouse [82,83]. Sildenafil can also block phenylephrine or Ang II-stimulated hypertrophy in neonatal rat cardiomyocytes [84], as well as apoptosis in adult mouse cardiomyocytes induced by ischemia-reperfusion [85]. The downstream signaling mediating sildenafil's protective effects appear to include activation of RGS2 [86,87] and mitoKATP channels [81], suppression of TRPC channel activation and calcineurin/NFAT signaling [84,88], as well as inhibition of Rho kinase activity [89] and ERK activation [79]. Many of these signaling

molecules are directly or indirectly modulated by PKG [79,88,89]. The role of PKG in pathological cardiac remodeling remains controversial. One study reported that cardiomyocyte-specific deletion of PKG 1 does not alter isoproterenol-infusion or TAC-induced cardiac hypertrophy [11]. However, another study using a different strain of mice found that PKG deletion did worsen cardiac remodeling induced by chronic Ang II-infusion [90], so it appears that cGMP/PKG signaling may be more important in some types of heart failure than in others, and it has also been argued that the magnitude of cardiac stress (particularly in TAC) contributes to the relevance of this signaling [91]. Interestingly, the effects of sildenafil on cardiac contractility in diseased heart may be different from that in normal heart. For example, in the hypertrophied right rat ventricle, sildenafil treatment has actually been reported to increase cardiac contractility, likely via cGMP-mediated PDE3 inhibition, and in turn, cAMP elevation [26]. Such a difference could conceivably result from an alteration in PDE5A subcellular targeting between normal and diseased hearts [78].

A number of studies have called into question whether sildenafil's antihypertrophic effects might actually be through off-target inhibition of PDE1. For example, Lukowski *et al.* reported that in both normal and hypertrophic mouse myocardium, the majority of sildenafil-inhibited PDE activity is responsive to Ca^{2+} /CaM stimulation, suggesting that it is PDE1, not PDE5, being inhibited [11]. *In vitro*, sildenafil inhibits PDE1 with a reported IC_{50} of 300–400 nM [9,11], and in healthy adult humans, a 100 mg dose of sildenafil can result in plasma drug levels above 600 nM [202]. So, it is possible that sildenafil also inhibits PDE1 in some studies in which high concentrations of sildenafil were used. However, considerable effort has been made to demonstrate the specific role of PDE5 in cardiac biology and disease. For example, in a mouse study in which sildenafil dramatically reduced TAC-induced cardiac dysfunction, blood drug levels were assessed and found to be around 10 nM, too low to likely inhibit PDE1 [79]. Additionally, a different PDE5 inhibitor, tadalafil, which is more specific for PDE5, has also been used to protect against ischemia-reperfusion in mouse, again supporting the cardioprotective role of PDE5 inhibition [92]. Additionally, genetically modified PDE5A mice have been created and examined. Cardiomyocyte-specific overexpression of PDE5A in transgenic mice worsened TAC-induced cardiac remodeling and cardio-myocyte hypertrophy, which is diminished by PDE5 inhibitor treatment [93]. Another line of PDE5 overexpressing mice also displayed a worsened response to myocardial infarction [69]. Because PDE5A overexpression could potentially produce non-physiological effects, development of a *PDE5* knockout mouse would be the best method for confirming the role of PDE5 in the myocardium; to date, however, multiple attempts to generate such a mouse have been unsuccessful [94]. In addition to *in vivo* approaches, specific PDE5A depletion with siRNA in isolated cardiomyocytes further demonstrated an important role of PDE5A in cardiomyocyte hypertrophy [70].

Based on the cardioprotective effects observed in animals, there is now significant interest in using PDE5 inhibitors to treat heart failure in humans. To date, two NIH sponsored clinical trials investigating the effects of PDE5 inhibitors on heart failure have been initiated. The RELAX trial aimed to investigate the effect of 24 weeks of sildenafil treatment in more than 200 elderly patients with diastolic heart failure [95], while the PITCH-HF trial, also NIH sponsored, will investigate the effects of tadalafil treatment on more than 2000 patients with

heart failure with reduced ejection fraction [94]. Additionally, several smaller clinical trials have indicated that PDE5 inhibitor treatment does appear to have beneficial effects on several forms of heart failure. Two studies, designed to investigate the effects of either 1 year of sildenafil treatment on patients with ischemic, idiopathic or hypertensive heart failure (Class II–III), or 3 months sildenafil treatment on patients with nonischemic, diabetic cardiomyopathy, found that sildenafil treatment appeared to reduce cardiac hypertrophy and improve function, and reduce levels of inflammatory markers [2,96]. Additionally, a retrospective study of effects of long-term sildenafil in patients with mean New York Heart Association class III heart failure and pulmonary hypertension, awaiting transplant, found that sildenafil treated patients experienced an improvement in cardiac function, and significant reduction in New York Heart Association heart failure class after 3 months treatment, and improved survival pre- and acutely post-transplant [97]. Sildenafil treatment also shows potential for improving cardiac performance in children given the Fontan procedure [98]. Despite the promising findings of these smaller studies, however, the RELAX Trial recently reported that sildenafil treatment was not associated with improved outcomes in any endpoint in patients with heart failure with preserved ejection fraction, and in fact, sildenafil treatment was associated with slightly increased, though non-statistically significant, increases in mortality and frequency of adverse events [99]. Additionally, sildenafil treatment was associated with a mild but significant worsening of renal function. The authors of the study speculated that the lack of efficacy of sildenafil treatment relative to other studies was due to the relatively mild degree of left ventricular hypertrophy present in these patients relative to those in other studies. It is also possible that sildenafil is more effective at treating heart failure with reduced ejection fraction. Certainly, the potential benefit of sildenafil treatment in patients with various types of heart failure, and the prospect that sildenafil treatment worsens renal function, deserve further investigation.

PDE8

PDE8 is highly specific for cAMP, and is unique amongst the PDEs for its insensitivity to the pan- PDE inhibitor IBMX. Two PDE8 genes, *PDE-8A* and *-8B*, have been characterized. *PDE8A* has been reported to be expressed in the heart [100]. The role of cardiac *PDE8A* was recently characterized in a study using *PDE8A* knockout mice. Ventricular myocytes isolated from *PDE8A* knockout mice displayed heightened isoproterenol-induced increases in Ca^{2+} transients, probably due to increased ICa_L [101]. Basal, but not isoproterenol-induced Ca^{2+} spark frequency was also increased in *PDE8A* knockout myocytes. SR Ca^{2+} load was similar between WT and knockout myocytes, yet the rate of SR Ca^{2+} refilling appeared to be increased in *PDE8A* knockout cells [101]. Together, these data indicate that PDE8A may be important in regulating LTCC Ca^{2+} under conditions of catecholamine stimulation, but SR Ca^{2+} load under basal conditions. A specific role for PDE8A regulation in cardiac pathology has not yet been reported, but given its role in regulating Ca^{2+} signaling, it seems conceivable that PDE8 could play a role in protecting against cardiac arrhythmias or other forms of cardiovascular disease, and this warrants further study. Such a role may provide future rationale for the development of PDE8-modulating drugs.

Conclusion & future perspective

In the cardiac myocyte, by controlling cAMP and cGMP, PDEs are crucial in regulation of a variety of biological functions, including pacemaking, contractility, cell growth and survival. With regards to some functions, the roles of cAMP and cGMP are divergent or occasionally even antagonistic: PDE3/cAMP-mediated signaling potentiates contractility, while PDE5/cGMP-mediated signaling may reduce it. Different PDE isoforms appear to regulate unique pools of cyclic nucleotides in distinct regions of the cell. For example, PDE2 appears to primarily control cGMP produced by membrane-localized pGC [19], while PDE5 plays a bigger role in regulating cGMP produced by cytosolic sGC [78]. Pathologically, dysregulation of PDE expression/activity as well as cyclic nucleotide signaling have been found in a variety of types of human and animal heart disease, which appears to be important for disease development and progression. For instance, PDE3A and PDE4D levels are significantly reduced, either globally or locally, in various human and animal failing hearts [45,61]. PDE3A and PDE4D downregulation have been shown to promote myocyte apoptosis [45,47] and cardiac arrhythmias [61] respectively. However, PDE1A and PDE5A expression are significantly increased in human and animal disease hearts, which may play causative roles in development of cardiac hypertrophy and heart failure [12,69].

In recent years, specific inhibitors to a number of PDE proteins have been developed. Not only have these inhibitors allowed more detailed characterization of roles of PDEs in cells or animals, but may represent a novel treatment strategy for cardiovascular disease. PDE5 inhibitors, may show translational potential, but further research and clinical trials should determine the efficacy of these agents in treating human cardiovascular disease. Equally important, research into the cardiovascular roles of the PDEs may also serve to uncover hazards of inhibiting PDEs to treat diseases in other tissues, due to detrimental cardiac side effects. The deleterious effects observed in patients with chronic PDE3 inhibitor treatment provide a good example, and it is also conceivable that PDE4 inhibitors may be dangerous due to potential arrhythmogenic effects, and this possibility certainly warrants future study. Furthermore, given the unique roles of different PDE isozymes, such as PDE3A versus 3B and PDE4B versus PDE4D, in regulating cardiac function, development of isoform-specific PDE inhibitors could prove to be a powerful tool, allowing targeting of very specific signaling in cells with relatively few side effects. Development of novel strategies to restore the activity or localization of PDEs such as PDE3A or PDE4D, or PI3K γ -PDE complexes, in heart failure could also be extremely beneficial, although this would likely necessitate gene therapy, or development of PDE activators.

Animal models with tissue-specific depletion or overexpression of various PDEs have also been powerful tools for studying cardiac cyclic nucleotide signaling. Compared to inhibition strategies, genetic knockout models have several advantages. Studies comparing PDE3A and 3B depletion, and depletion of various PDE4 isoforms [55,61], have uncovered unique roles for these PDEs isoforms in myocytes [31], which could not have been discovered through use of pan-PDE3 or -PDE4 inhibitors. Increased expression of PDEs is a hallmark in many types of disease, and genetic overexpression strategies allow this phenomenon to be modeled, an approach which currently cannot be achieved pharmacologically. For example,

multiple studies have used mice with cardiomyocyte PDE5 [69,77,93] or PDE3A overexpression [100]. The use of tissue-specific depletion strategies, such as cardiomyocyte-specific or fibroblast-specific PDE depletion, can also demonstrate cardiomyocyte, or other tissue-specific roles of PDEs. While relatively few studies to date have utilized this approach, it remains a potentially powerful approach to studying the cardiac role of PDEs.

While the findings summarized in this article are of great interest, the physiological role and clinical significance of some of the PDEs in the cardiovascular system deserve further investigation. For instance, large numbers of *in vitro* studies have indicated that PDE1 [10], PDE2 [20], and PDE8 [101] may play important roles in development of cardiovascular pathology. The precise roles and underlying mechanisms of action of these PDEs have yet to be well characterized in intact animal models. At the same time, caution must be used in interpreting findings from animal models, as the expression levels of several PDEs vary between human and rodents. Thus, while inhibiting a particular PDE may have dramatic effects in one animal, it may have little to no effect in another – therefore, verifying animal model-derived findings in human cardiac tissue or cardiac IPS cells is quite important. Finally, the role of PDEs in regulating fibroblast activation and function in the heart also merits further research, and could have considerable therapeutic potential.

Key Terms

cAMP	Acts as a second messenger important for a variety of biological functions. In the cardiomyocyte, it is notably produced in response to catecholamines binding to β -adrenergic receptors, which activates adenylyl cyclase proteins. This results in activation of PKA and an increase in myocyte contractile force and pacemaking rate. In addition, chronic activation of cAMP/PKA signaling has pro-hypertrophic and pro-apoptotic effects
cGMP	Acts as a second messenger. In the cardiac myocyte, it is produced via two separate pathways; pGC that is membrane bound and activated in response to natriuretic peptides (ANP, BNP, CNP), and soluble guanylylcyclase that is activated in response to nitric oxide. The primary effector for cGMP is PKG. In the cardiac myocyte, cGMP-signaling is associated with anti-contractile, anti-apoptotic, and anti-hypertrophic effects
Heart failure	Pathological condition characterized by the inability of the heart to provide sufficient blood to the body. Common causes include genetic abnormalities, hypertension, or myocardial infarction. Characterized by an increase in heart size, cardiomyocyte hypertrophy, and cardiac fibrosis
Cardiac fibroblasts	Type of connective tissue cells, which represents the major non-myocyte population (approximately 70% of cells) in the heart. Involved in deposition and degradation of extracellular matrix in the heart. Pathological insults such as myocardial infarction can cause

these to become activated into myofibroblasts, which have increased extracellular matrix-depositing, proliferative, and migratory properties, and contribute to cardiac fibrosis and pathological cardiac remodeling

Cardiomyocytes

Contractile cells of the heart. Multinucleate, striated muscle cells. Coordinated contraction of these cells provides the pumping action of the heart. Significant morphological and biochemical changes occur in cardiac myocytes in a diseased state, such as cellular hypertrophy, fetal gene reactivation, and sarcomere disorganization

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Executive summary

Overview of cardiac PDEs

- cAMP and cGMP play important roles in regulating cardiac functions from acute myocyte contraction/relaxation to chronic cell growth/survival.
- Alteration in the behavior of cyclic nucleotide-mediated signaling plays important roles in pathological cardiac remodeling and dysfunction. Much of this altered cyclic nucleotide signaling can be attributed to changes in expression, activation, or localization of PDEs.
- Alterations in PDE expression/activity are found in various types of human and animal diseased hearts, which may play causative roles in pathological cardiac remodeling and/or cardiac dysfunction.

PDE1

- Increased expression of PDE1 isoforms may worsen development of heart failure and cardiac fibrosis, and pharmacological inhibition of PDE1 has the potential to ameliorate these effects – further research is needed. High levels of PDE1 activity in human hearts could possibly make it a promising therapeutic target.

PDE2

- PDE2 plays important roles in regulating natriuretic cGMP levels and cardiomyocyte contractility, but its behavior varies significantly in a species-dependent manner. A role for PDE2 in cardiovascular pathology has not yet been reported.

PDE3

- PDE3A plays important roles in regulating cardiac contractility. Acute PDE3 inhibition can increase cardiac contractility and output and enhance Ca^{2+} signaling by activating phospholamban and RyR2 in a cAMP/PKA-dependent fashion.
- Chronic PDE3 inhibition worsens mortality in heart failure, which may be through potentiation of ICER expression, decreased Bcl-2 expression, and increased apoptosis. PDE3A expression is also reduced in heart failure, and restoration of PDE3A expression could potentially improve cardiac function.

PDE4

- Several PDE4 isoforms, including PDE-4A, -4B and -4D, are expressed in heart, and PDE4B and 4D play important roles in controlling Ca^{2+} signaling and protecting against arrhythmias in mouse, via regulation of LTCCs, RyR2, or possibly SERCA2. PDE4D3 also protects against myocardial infarction and age-dependent cardiac remodeling.

- PDE4 isoforms may play a less dramatic role in regulating human myocardial function than in mouse. However, atrial PDE4 does appear to be important in humans, and protects against arrhythmias.
- PI3K γ , by regulating localization and activity of various PDE3 and PDE4 isoforms, appears to be a key regulator of cardiovascular PDE signaling. Maintenance of certain PI3K γ /PDE complexes protects against arrhythmias or pathological cardiac remodeling, and could represent a potential therapeutic target.

PDE5

- Physiologically, PDE5 is expressed at low levels in the heart and regulates catecholamine-induced cardiac contractility in an eNOS-dependent manner.
- PDE5 expression is strongly increased in heart failure and appears to worsen disease etiology. Pharmacological inhibition or reduction in expression of PDE5 are able to ameliorate cardiac dysfunction in a number of animal models.
- While clinical trials using PDE5 inhibitors in humans with heart failure demonstrate some promising results, the negative results of the RELAX Trial indicate that the further study of the potential for PDE5 inhibitors in treating human heart failure is needed.

PDE8

- Cardiac PDE8 is involved in regulating RyR and L-type Ca²⁺ channel Ca²⁺ signaling and Ca²⁺ leak.

Conclusion & future perspective

- Inhibition of certain PDEs (PDE1, PDE5) represent an intriguing therapeutic approach for treating heart failure.
- Caution must be used in inhibiting certain PDEs (PDE3, PDE4) to treat non-cardiovascular diseases due to the hazards of cardiovascular side effects.
- Development of isoform specific PDE inhibitors or PDE activators in the future could prove useful for treating heart failure, and perhaps other diseases as well.

Table 1

Overview of cardiomyocyte PDE function and the tools available for PDE characterization.

PDE family	Expression	Physiological and/or pathological roles	Transgenic mice	Inhibitors used
PDE1	PDE1A expression is upregulated in hypertrophic and failing hearts [12] PDE1C is highly expressed in normal human hearts and is located along the Z-lines and M-lines of human cardiac myocytes [8] PDE1C expression is reported to be unchanged in a mouse TAC model [11]	Inhibiting PDE1 activity reduces myocardial hypertrophy in a chronic ISO-infusion mouse model [10] PDE1A regulates cardiomyocyte hypertrophy [10] PDE1A regulates cardiac fibroblast activation and cardiac fibrosis [12]	Global <i>PDE1C</i> knockout mice [102]	IC86340 Vinpocetine 8-MM-IBMX
PDE2	PDE2 expression is increased in experimental heart failure in rat [20]	Mediating cGMP effects on blunting β -AR-induced cAMP production, contractility [14] and LTCC activity [15]		EHNA
PDE3	PDE3A is found in the SERCA2/ PLB complex in SR [33] PDE3B is found in a complex with PI3K γ , PDE4A and PDE4B [40] in the cardiomyocyte PDE3A expression is reduced in rodent and human failing hearts [45]	PDE3 regulates β -adrenergic signaling, cardiac contractility, pacemaking, and output [31] PDE3A represents the primary isoform responsible for the inotropic and chronotropic effects of PDE3 inhibitors [31] PDE3A downregulation or chronic PDE3 inhibition promotes cardiomyocyte apoptosis [45,46] Myocardial overexpression of PDE3A1 in transgenic mice reduces cardiomyocyte apoptosis and prevents ischemia/reperfusion induced myocardial infarction [100] PDE3A regulates cardiac contractility, LTCC activity, and arrhythmias via PI3K γ [40]	Global <i>PDE3A</i> knockout mice [31] Global <i>PDE3B</i> knockout mice [31] Cardiomyocyte <i>PDE3A</i> overexpressing mice [100]	Milrinone Amrinone Enoximone
PDE4	Reduced PDE4A and PDE4D expression in failing human heart [56] PDE4D depletion in the Ryr2 complex is reported in human failing hearts [61] PDE4D downregulation reported in patients with atrial fibrillation [64]	PDE4A and 4B regulate cardiac contractility, LTCC activity, and arrhythmias via PI3K γ [40] Depletion of PDE4B causes arrhythmogenesis in mouse [55] PDE4D regulates β -adrenergic cAMP [59] PDE4D regulates cardiac contractility via regulation of Ryr2 phosphorylation status [61] and Ca ²⁺ signaling; and/or via regulation of SERCA2 activity [63] Depletion of PDE4D causes RyR2 hyperphosphorylation, arrhythmia, and age-dependent cardiomyopathy [61]	Global <i>PDE4A</i> knockout mice [55] Global <i>PDE4B</i> knockout mice [55] Global <i>PDE4D</i> knockout mice [55]	Rolipram Rofluminalast
PDE5	PDE5 expression upregulated in heart failure in mouse and human [69,77]	PDE5 inhibition negatively regulates Isomediated contractility [71] Inhibiting PDE5 reduces adverse cardiac remodeling, cardiomyocyte hypertrophy and apoptosis in multiple models of heart failure in mouse [79,80,82,83] Mice with cardiomyocyte-specific PDE5 overexpression display a greater degree of maladaptive	Cardiomyocyte <i>PDE5A</i> overexpressing mice [69,77] Cardiomyocyte <i>PDE5A</i> overexpressing mice with doxycycline suppression [93]	Sildenafil Tadalafil Vardenafil

PDE family	Expression	Physiological and/or pathological roles	Transgenic mice	Inhibitors used
		remodeling in response to TAC or MI [69,77,93] Sildenafil demonstrates beneficial effects on some human subjects [2,96] but also no benefit in a large clinical trial [97]		
PDE8	PDE8A has been detected in cardiomyocytes in mouse [101]	Regulation of LTCC Ca^{2+} signaling, Ryr2 Ca^{2+} load [101]	Global <i>PDE8A</i> knockout mice [101]	PF-04957325

ISO: Isoporterenol; LTCC: L-type Ca^{2+} channel; MI: Myocardial infarction; SR: Sarcoplasmic reticulum; TAC: Transverse aortic constriction.