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Cyclic nucleotide phosphodiesterases as therapeutic targets in cardiac hypertrophy and heart failure

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Abstract | Cyclic nucleotide phosphodiesterases (PDEs) modulate the neuro-hormonal regulation of cardiac function by degrading cAMP and cGMP. In cardiomyocytes, multiple PDE isozymes with different enzymatic properties and subcellular localization regulate local pools of cyclic nucleotides and specific functions. This organization is heavily perturbed during cardiac hypertrophy and heart failure (HF), which can contribute to disease progression. Clinically, PDE inhibition was once considered a promising approach to compensate for the catecholamine desensitization that accompanies HF. Although PDE3 inhibitors, such as milrinone or enoximone, have been used clinically to improve systolic function and alleviate the symptoms of acute HF, their chronic use has proven to be detrimental. Other PDEs, such as PDE1, PDE12, PDE14, PDE15, PDE19 and PDE110, have emerged as new potential targets to treat HF, each having a unique role in local cyclic nucleotide signalling pathways. In this Review, we describe cAMP and cGMP signalling in cardiomyocytes, and present the various PDE families expressed in the heart as well as their modifications in pathological cardiac hypertrophy and HF. We also appraise the evidence from pre-clinical models as well as clinical data pointing to the use of inhibitors or activators of specific PDEs that could have future therapeutic potential in HF.

[H1] Introduction

Heart failure (HF) is a chronic clinical syndrome characterized by a progressive loss of cardiac function. Ultimately, cardiac output becomes insufficient to meet the body's metabolic demand for oxygen, resulting in organ failure and death. HF has a variety of causes, including arrhythmias, cardiomyopathies, coronary artery disease, congenital heart diseases, infections, hypertension, valve diseases and the cardiotoxicity of anticancer drugs¹. Regardless of the cause, decreased cardiac function triggers the activation of neurohormonal systems and the development of cardiac hypertrophy to normalize ventricular wall stress². However, this compensated state is usually transient and gradually evolves toward chamber dilatation and, ultimately, HF.

In physiological conditions, cardiac function is controlled by two intracellular cyclic nucleotides with opposing effects — cAMP and cGMP. Cardiac output is increased by cAMP—cAMP-dependent protein kinase catalytic subunit α (PKA) signalling upon β -adrenergic stimulation in response to exercise, whereas cGMP stimulated by the production of nitric oxide (NO) and by natriuretic peptides (NPs) can exert both synergistic and opposite effects to cAMP on heart function. In HF, a decline in cardiac function causes over-activation of the sympathetic nervous system and chronic catecholamine elevation³. A pathophysiological 'vicious circle' is initiated in which excessive β -adrenergic stimulation has a central role, as attested by the beneficial effects of β -blockers in HF⁴. Continuous cAMP—PKA signalling elicits maladaptive remodelling, comprising pathological cardiac hypertrophy, cardiomyocyte death and cardiac fibrosis and precipitates HF. By contrast, NO and NPs exert anti-hypertrophic and anti-fibrotic effects. Accordingly, drugs that elevate NPs or synergize with NO to stimulate cGMP synthesis improve classical treatments in patients with heart failure with reduced ejection fraction (HFrEF)⁵⁻⁷.

Cyclic nucleotide levels are tightly regulated through their hydrolysis by cyclic nucleotide phosphodiesterases (PDEs), which break their phosphodiester bond to produce 5'-

single-phosphate nucleotides. The PDE superfamily comprises several distinct isoforms that regulate the intensity and duration of cAMP and cGMP signals in discrete compartments of the cardiomyocyte. Data published in the past 5 years underline the localized nature of cAMP degradation by PDEs in nanometre-size domains⁸⁻¹⁰. Together with strategic PKA localization by A-kinase anchoring proteins (AKAPs), PDEs ensure the specificity of cAMP signalling in physiological conditions. This organization is strongly remodelled in pathological hypertrophy and HF, and this is thought to be involved in deterioration of heart function. Indeed, in HF, β -adrenergic signalling undergoes profound remodelling^{11,12} and NO bioavailability and NP signalling are impaired^{13,14}. Unsurprisingly, changes in PDE expression, activity and subcellular localization lead to alterations in cAMP and cGMP signalling in HF. To date, inhibitors of PDE3 and PDE5 have proven to be either detrimental in patients with HFrEF¹⁵, or inefficient in patients with heart failure with preserved ejection fraction (HFpEF), respectively¹⁶. However, evidence continues to accumulate in the literature pointing to specific PDEs as potential therapeutic targets to prevent cardiac remodelling and HF^{17, 18}.

In this Review, we discuss the role of cardiac PDEs in the regulation of compartmentalized cAMP and cGMP signalling and their alteration in cardiac hypertrophy and HF. After a brief overview of cAMP and cGMP signalling in cardiomyocytes, we describe the structure, regulation and enzymatic properties of the various PDEs (**FIG. 1**) and their subcellular localization within the cardiomyocyte (**FIG. 2**). A summary of PDE expression in the normal human myocardium and their modification in pathological hypertrophy and HF is provided in **TABLES 1** and **2**, respectively. **TABLE 3** lists the main clinical trials of PDE inhibitors in chronic HF. Finally, we discuss PDE-mediated crosstalk between cAMP and cGMP signalling and its deregulation in HF.

[H1] cAMP and cGMP in cardiomyocytes

Various G-protein-coupled receptors (GPCRs) control cAMP levels in cardiomyocytes.

Among the stimulatory G protein (G_s)-coupled receptors, the most functionally important are undoubtedly the β_1 -adrenergic receptors (β_1 -ARs), but cardiomyocytes also express other G_s -coupled receptors, including β_2 -ARs, prostaglandin EP₄ receptors and the adenosine receptor A_{2a} (A₂R) that elicit distinct functional responses despite increasing levels of the same second messenger (cAMP). The actions of these receptors are opposed by inhibitory G protein (G_i)-coupled receptors, among which the muscarinic acetylcholine receptor M₂ and the adenosine receptor A₁ have a prominent role. In the classical view, GPCRs act from the plasma membrane, although evidence is emerging for intracellular β -adrenergic receptors (β -ARs) localized at the nuclear envelope¹⁹ to control gene transcription, at the Golgi and the sarcoplasmic reticulum that regulate cardiac hypertrophy and contractility, respectively^{20,21}. G_s -coupled receptors combine with transmembrane adenylyl cyclases (tmACs), with tmAC5 and tmAC6 being the predominant isoforms expressed in ventricular cardiomyocytes²². Different G_s -coupled receptors localize with tmAC5 or tmAC6 in distinct subdomains, such as the caveolae and T-tubules^{12, 23-25}, contributing to individual regulation of distal effectors, such as the L-type Ca^{2+} channel (LTCC) current, $I_{Ca,L}$ ²⁶. In addition to tmAC, the bicarbonate-sensitive and Ca^{2+} -sensitive soluble adenylyl cyclase (sAC) has been proposed as a local source of cAMP regulating mitochondrial function in cardiomyocytes^{27,28}. In pathological hypertrophy and HF, chronic elevation of norepinephrine and epinephrine causes β -adrenergic desensitization for several reasons — down-regulation of β_1 -ARs, uncoupling of β_2 -ARs from G_s , increased expression of G_i , a decrease in tmAC (particularly in dog models of HF) and increased expression and activity of the G-protein-coupled receptor kinases, GRK2 (also known as β -adrenergic receptor kinase 1) and GRK5¹¹. Although these

changes dampen cardiac β -adrenergic reserve, they also limit the cardiotoxicity of chronically elevated catecholamines and, on the whole, should be regarded as protective⁴.

Once synthesized, cAMP exerts its effects through four major effectors in the heart. The first is PKA, which has an essential role during the fight-or-flight response by regulating cardiac excitation–contraction coupling (ECC)²⁹. PKA increases $I_{Ca,L}$ by phosphorylating the regulatory protein Rad³⁰ (replacing the old tenet of direct PKA phosphorylation and regulation of Cav1.2³¹ (also known as voltage-dependent L-type calcium channel subunit alpha-1C)), increases Ca^{2+} release from the sarcoplasmic reticulum by phosphorylating ryanodine receptor 2 (RYR2) and accelerates Ca^{2+} re-uptake by sarcoplasmic/endoplasmic reticulum calcium ATPase 2 (SERCA2) through phospholamban phosphorylation. PKA also controls myofilament function by phosphorylating troponin I and myosin-binding protein C³². Acutely, PKA also activates glycogenolytic enzymes³³ and favours the nuclear retention of class II histone deacetylases 4 and 5, thereby repressing myocyte-specific enhancer factor 2 activity and the transcription of genes involved in pathological hypertrophy³⁴⁻³⁷. PKA is widely recognized as being involved in the acute regulation of cardiac function; in addition, transgenic mouse models have shown that chronic PKA activation induces HF³⁸, whereas chronic PKA inhibition protects against experimental HF³⁹. Although many of the detrimental effects of chronic PKA activation are likely to be the consequence of Ca^{2+} overload and Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) activation³⁹, PKA could also exert pro-apoptotic effects through upregulation of cAMP-responsive element (CRE) modulator (also known as inducible cAMP early repressor, ICER), an endogenous inhibitor of CRE-mediated transcription^{40,41}. The second type of cAMP effector are the hyperpolarization-activated cation non-selective channels (HCN), which are cyclic nucleotide-gated channels expressed in cardiac automatic tissue where they control diastolic potential⁴². The third type cAMP effector are the Popeye domain containing proteins (also

known as the blood vessel epicardial substance proteins), which are highly expressed in the conduction system and contribute to cardiac automatism⁴³. The fourth type of cAMP effectors are the exchange proteins directly activated by cAMP (EPAC) — guanine exchange factors of the small G proteins, Rap1 GTPase-activating protein 1 and Ras-related protein Rap2, which are directly activated by cAMP⁴⁴. EPAC proteins are involved in the regulation of cardiac hypertrophy and ECC in a complex, and controversial, manner^{45,46}. EPAC proteins have a role in cardiomyocyte hypertrophy triggered by β -ARs via a CaMKII and calcineurin signalling pathway^{47,48}. CaMKII activation, which can also result from PKA-dependent increases in Ca^{2+} , also leads to RYR2 phosphorylation, thereby promoting sarcoplasmic reticulum Ca^{2+} leak and arrhythmias⁴⁹. In cardiomyocytes, EPAC proteins also localize at the perinuclear envelope together with muscle-specific A-kinase anchoring protein β (mAKAP β), mitogen-activated protein kinase 7 (MAPK7; also known as extracellular signal-related kinase 5; ERK5) and 1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase β (also known as phospholipase C β , PLC β) to regulate cardiomyocyte hypertrophy⁵⁰⁻⁵². In addition, EPAC proteins have been shown to localize to mitochondria where they regulate cardiomyocyte death, although whether mitochondrial EPAC exert protective or apoptotic effects remains controversial^{27,53}.

Levels of cGMP in the myocardium can be increased via the stimulation of soluble guanylyl cyclases (GC-1 and GC-2) by NO and particulate guanylyl cyclases (GC-A and GC-B) by NPs⁵⁴. These guanylyl cyclases define two cGMP microdomains, at the sarcolemma and in the cytosol, distinctly regulated by PDEs¹⁰. GC-A localizes to the T-tubules, whereas GC-B is uniformly distributed at the plasma membrane, which further defines two subsarcolemmal cGMP pools with different spatial distributions and functional outcomes⁵⁵⁻⁵⁷.

In cardiomyocytes, the protective effects of cGMP are mainly attributed to cGMP-dependent protein kinase (PKG). PKG can phosphorylate many PKA targets — including

LTCC⁵⁸, phospholamban⁵⁹, troponin I⁶⁰, myosin binding protein-C, cardiac type⁶¹ and titin⁶² — although often at different residues. However, because PKG inhibits $I_{Ca,L}$ augmented by cAMP–PKA⁵⁸, it opposes adrenergic effects. The same mechanism might explain PKG-mediated inhibition of calcineurin–nuclear factor of activated T cells (NFAT) signalling and the anti-hypertrophic effects of PKG⁶³. However, PKG has also been shown to exert anti-hypertrophic effects by targeting regulator of G-protein signalling 2 (RGS2)⁶⁴, RGS4⁶⁵ and short transient receptor potential channel 6 (TRPC6)⁶⁶. In 2019, PKG was shown to phosphorylate tuberin (also known as tuberous sclerosis 2 protein), a negative regulator of mammalian target of rapamycin complex 1 (mTORC1) to provide cardioprotection against pressure overload⁶⁷. Another favourable effect of PKG is activation of proteasome function and thus degradation of misfolded proteins⁶⁸, which confers protection against myocardial infarction in mice⁶⁹. Although these and other findings strongly support cardiomyocyte PKG as a potent anti-hypertrophic and cardioprotective enzyme⁷⁰, in mice with PKG expressed only in smooth muscle cells, chronic isoprenaline⁷¹ or angiotensin II (Ang II) infusion did not provoke an exacerbated hypertrophic response⁷². Therefore, the relevance of cardiomyocyte PKG in maladaptive remodelling of the heart remains controversial.

The pleiotropic effects of cyclic nucleotides suggest that inhibition or activation of PDE isoforms, by modulating specific intracellular pools of cAMP or cGMP, could improve heart function, convey cardioprotection or both. Therefore, extensive work has been carried out to decipher the role of cyclic nucleotides PDEs in cardiomyocytes.

[H1] Properties of cardiac PDEs

Mammalian PDEs are a diverse superfamily of enzymes comprising eleven families encoded by 21 genes, which generate over 80 different isoforms due to alternative splicing and the existence of different translation initiation sites⁷³. With the exception of PDE6, which is

selective of photoreceptors, all the other families have been found to be expressed in the heart. Nothing is known on the potential functional roles of PDE7⁷⁴⁻⁷⁶ and PDE11⁷⁷; therefore, only eight PDE families have been characterized in the heart, in terms of gene and protein expression, function and biochemical properties — PDE1, PDE2, PDE3, PDE4, PDE5, PDE8, PDE9 and PDE10. These PDEs have a fairly conserved C-terminal catalytic domain and a more variable N-terminal regulatory domain (**FIG. 1**). On the basis of their substrate specificity and selectivity, cardiac PDE families can be grouped into three broad categories — PDE1, PDE2, PDE3 and PDE10 hydrolyse both cAMP and cGMP, whereas PDE4 and PDE8 are selective for cAMP and PDE5 and PDE9 are selective for cGMP. The N-terminal domains contain diverse elements involved in enzyme dimerization, binding of small molecules, phosphorylation, protein–protein interactions and localization. PDE2, PDE5 and PDE10 contain GAF domains (named after mammalian cGMP-dependent phosphodiesterase, *Anabaena* adenylyl cyclase, *E. Coli* FhlA)⁷⁸ for allosteric regulation by cGMP (PDE2⁷⁹ and PDE5⁸⁰) or cAMP (PDE10)⁸¹. Other families present distinctive modulatory domains, such as calmodulin-binding domains in PDE1, upstream conserved regions (UCRs) in PDE4, response regulator receiver (REC) and Per-Arnt-Sim (PAS) domains in PDE8, and a pat7 nuclear localization motif in PDE9 (**FIG. 1**)⁸². Detailed descriptions of the PDEs, including their structure, regulation, physiological roles and pharmacology have been published elsewhere⁸³⁻⁸⁶. **TABLE 1** summarizes the available information on the expression of the various PDE isoforms in the human heart, together with the detection method employed. When available, the cell type as well as the PDE splice variant and subcellular localization are indicated. Similar information in animal models is provided in **SUPPLEMENTARY TABLE 1**.

[H1] PDEs in cAMP and cGMP compartmentation

A single cardiomyocyte expresses a large repertoire of PDE variants with distinct enzymatic properties, distributed in multiple subcellular locations (**FIG. 2**). At the plasma membrane, various PDE isoforms were shown to couple with hormone receptors (β -AR⁸⁷⁻⁹⁴, prostaglandin EP₄ receptors^{92, 95}, A₂R⁹⁶, and NP receptors^{56, 97-99}) and also to associate with ion channels^{100, 101} and the Na⁺/K⁺-transporting ATPase¹⁰². Isoforms of PDE3 and PDE4 were found to control Ca²⁺ release and reuptake in the sarcoplasmic reticulum by RYR2 and SERCA2, respectively¹⁰³⁻¹⁰⁶, or are localized to the myofilaments^{107, 108} or the nuclear envelope^{52, 106, 109}. By contrast, PDE2 isoforms are distributed in the cytosol, at the plasma membrane and in the mitochondria^{110, 111}, and PDE5 is localized at the Z-band¹¹². Thus, the general concept is that strategically located PDEs dictate the spatiotemporal pattern of cAMP and cGMP in cells, and allow local regulation of a limited subset of effectors¹¹³. Many studies using cyclic nucleotide-gated channels and fluorescent resonance energy transfer (FRET)-based biosensors to measure cyclic nucleotide dynamics generally support this concept^{54, 114-116}.

[H1] PDEs in cardiac hypertrophy and HF

In addition to altered cyclic nucleotide synthesis, cardiac hypertrophy and HF are accompanied by complex remodelling of PDEs. The modifications in PDE expression reported in human and animal models of HF are listed in **TABLE 2 and SUPPLEMENTARY TABLE 2**, respectively. Although exceptions have been reported, the general consensus from published studies is that levels of PDE1, PDE2, PDE5, PDE9 and PDE10 are increased in pathological hypertrophy and HF. For PDE1, PDE5 and PDE9, these changes seem to be maladaptive, as pharmacological inhibition or genetic suppression alleviate pathological remodelling in experimental models of HF. Whether an increase in PDE2 is beneficial or detrimental is controversial, and the data on modifications of PDE3 and

PDE4 expression and activity in cardiac hypertrophy and HF are variable. These divergent results could be explained differences between species, HF aetiology or disease stage, or by technical issues. Whereas a global increase in these two main cAMP-PDEs might counter the detrimental effects of β -AR overstimulation at the expenses of β -AR reserve, a decrease is likely to amplify the cardiotoxicity of catecholamines and, therefore, to be maladaptive in HF. However, the existence PDEs that hydrolyse both cAMP and cGMP, compartmentalized isozymes and crosstalk between cyclic nucleotides might complicate this general view, as discussed in the following sections.

[H3] PDE1. The PDE1 family is encoded by three distinct genes (*PDE1A*, *PDE1B*, *PDE1C*) that are each expressed as multiple protein variants. *PDE1B* expression has not been found in the heart. The PDE1 family is unique in being activated by the Ca^{2+} -calmodulin complex and might, therefore, have an important role in crosstalk between Ca^{2+} and the cyclic nucleotides. The PDE1 enzymes bind and hydrolyse cAMP and cGMP in a mutually competitive manner. Each PDE1 isoform catalyses the hydrolysis of both cAMP and cGMP, but with different substrate affinities. In vitro, PDE1A and PDE1B hydrolyse cGMP with greater affinity than cAMP. However, PDE1C hydrolyses cAMP and cGMP with a similarly high affinity¹¹⁷.

Most of the cAMP and cGMP hydrolytic activity in the human myocardium involves PDE1. These enzymes are mostly found in the soluble fractions¹¹⁸⁻¹²⁰, but are also associated with the nucleus¹⁰⁶. PDE1 was initially thought to be expressed in non-myocyte cardiac cells¹²¹, and PDE1A is the main cAMP-hydrolysing enzyme in rat cardiac fibroblasts¹²². However, in human myocardium, PDE1C was found to be localized at the cardiomyocyte Z-lines and M-lines¹²⁰, and *PDE1C* transcripts were detected only in isolated murine cardiac myocytes¹²³. *PDE1A* and *PDE1C* are expressed in rabbit, dog and human hearts (**TABLE 1**

and **SUPPLEMENTARY TABLE 1**). In these species, PDE1C is predominant whereas, in rat and mouse hearts, the dominant isoform is PDE1A.

PDE1A expression is increased in human HF and in several animal models of HF. Upregulation of PDE1A was replicated in isolated rat cardiomyocytes stimulated with the α_1 -adrenergic agonist phenylephrine. PDE1 inhibition or *Pde1a* gene silencing blocked the hypertrophic effect of the α_1 -AR agonist and prevented a phenylephrine-induced decrease in cGMP levels and PKG activity¹²⁶. These findings suggest that PDE1A induction by phenylephrine promotes cardiac hypertrophy by suppressing cGMP–PKG signalling¹²⁶. PDE1A is also upregulated in cardiac fibroblasts activated by pro-fibrotic stimuli, and PDE1A inhibition or silencing were shown to limit myofibroblast transformation and extracellular matrix synthesis¹²². Mechanistic studies revealed that PDE1A modulates unique pools of cAMP and cGMP, predominantly in perinuclear and nuclear regions of cardiac fibroblasts. Both cAMP–EPAC1–Rap1 and cGMP–PKG signalling were involved in PDE1A-mediated regulation of collagen synthesis¹²². Together, the findings from these studies suggest that PDE1A is a potential therapeutic target, and its inhibition could counteract cardiac hypertrophy and fibrosis.

Expression of the PDE1C isoform is also increased in human and mouse failing heart¹²³. *Pde1c* knockout (-/-) mice showed remarkable protection against cardiac contractile dysfunction and remodelling induced by transverse aortic constriction (TAC). In isolated cardiac myocytes, the lack of PDE1C prevented hypertrophy and apoptosis induced by isoprenaline and Ang II, which was largely dependent on cAMP–PKA and PI3K–AKT-signalling pathways¹²³. Although expression of PDE1C in cardiac fibroblasts is absent or negligible, fibrosis was also attenuated in *Pde1c*^(-/-) mice subjected to TAC, and conditioned medium from *Pde1c*^(-/-) cardiomyocytes prevented fibroblast activation, suggesting that paracrine factors regulated by PDE1C in cardiomyocytes control fibroblast activation¹²³.

Consistent with a detrimental role for PDE1 upregulation, the pan-PDE1 inhibitor vinpocetine prevented cardiomyocyte hypertrophy and fibroblast activation, hence blunting pathological remodelling induced by Ang II¹²⁸. Furthermore, the anti-apoptotic effect of PDE1C inhibition or deletion was prevented by pharmacological blockade or knockdown of A₂Rs, suggesting that the cAMP-hydrolysing activity of PDE1C selectively modulates A₂R–cAMP signalling in cardiomyocytes⁹⁶. The activation of PDE1C by Ang II required a plasma membrane-localized macromolecular complex, including the short transient receptor potential channel 3 (TRPC3), A₂R and PDE1C⁹⁶. In this scheme, Ca²⁺ entering via TRPC3 activates Ca²⁺/CaM-dependent PDE1C, which in turn hydrolyses the cAMP signals emanating from A₂R (**FIG. 2**) and exerting cardioprotective effects against Ang II, isoprenaline, hydrogen peroxide or doxorubicin⁹⁶. Additionally, mice with the R120G mutation in the gene for alpha-crystallin B chain (*Cryab*), a small heat shock protein, have been reported to develop HFpEF accompanied by an increase in PDE1A levels¹²⁹. In these mice, the PDE1-selective inhibitor IC86430 protected against HF by promoting PKA phosphorylation of proteasome proteins, which facilitates the degradation of misfolded proteins¹²⁹.

Interestingly, in healthy dogs and rabbits and in those with HF, a newly developed PDE1 inhibitor, ITI-214, produces acute inotropic and lusitropic effects as well as arterial vasodilation by promoting a cAMP pool independent of β -AR signalling, specifically emanating from A₂R¹³⁰. In guinea pig cardiomyocytes, ITI-214 (with a low dose of the adenylate cyclase stimulator, forskolin) stimulated contractility, mainly by enhancing $I_{Ca,L}$, with little effect on Ca²⁺ transients and myofilament protein phosphorylation, in contrast with β -AR stimulation or PDE3 inhibition¹³¹. These findings prompted the first clinical trial of ITI-214 in humans with HF¹³². In this small, phase I/II randomized, double-blind, placebo-controlled trial, a single-dose of ITI-214 was tested in patients with HFpEF. ITI-214 was generally well-tolerated and acutely reduced arterial vascular resistance while augmenting

cardiac inotropy, output and heart rate¹³². Together, the results of these studies suggest that PDE1 inhibition could exert beneficial effects in acute and, potentially, chronic HF. PDE1 seems to be a critical modulator of cyclic nucleotide levels at the crossroads of the cAMP, cGMP and Ca²⁺ signalling pathways, by limiting hypertrophy and adverse remodelling and producing vasorelaxant effects, as well as improving cardiac output.

[H3] PDE2. PDE2 was originally isolated from bovine heart and adrenal tissues and is a cGMP-stimulated enzyme that hydrolyses both cAMP and cGMP¹³³. PDE2 has a relatively low affinity for cAMP and cGMP, but a high hydrolytic capacity. The binding of cGMP to the GAFB domain of PDE2 (**FIG. 1**) increases cAMP hydrolysis by a factor 5–30, a mechanism that accounts for local cAMP depletion in nearby LTCC and *I*_{Ca,L} inhibition by cGMP in frog^{135,136} and human myocytes¹³⁷. The *PDE2A* gene encodes three splice variants, giving rise to three isoforms — PDE2A1 is a soluble form, whereas PDE2A2 and PDE2A3 are membrane-associated^{111,138-141}. PDE2 is enriched in cardiac mitochondria¹¹⁰ and PDE2A2 was shown to localize to mitochondria in neonatal cardiomyocytes¹¹¹. PDE2A is also present in neonatal fibroblasts¹⁴² and in endothelial cells¹⁴³⁻¹⁴⁵. The presence of PDE2 in various species, cardiac cell types and cellular compartments is summarized in **TABLE 1**, **SUPPLEMENTARY TABLE 1** and **FIG. 2**. In addition to regulating ECC¹⁴⁶, PDE2 also regulates mitochondrial function, dynamics and morphology^{110,111}.

In rat and dog models of HF and in human failing hearts, increased PDE2 expression and cAMP-hydrolytic activity was correlated with decreased β -AR responsiveness¹⁴⁷⁻¹⁴⁹, suggesting that increased PDE2 expression either contributes to HF progression or exerts antiadrenergic, cardioprotective effects¹⁴⁸. PDE2 contribution to cAMP hydrolysis was also increased locally in the vicinity of SERCA2a in hypertrophied cardiomyocytes¹⁵⁰. Zoccarato et al. described an anti-hypertrophic effect of PDE2 inhibition in isolated myocytes and in

mice subjected to TAC¹⁵¹. This effect was attributed to enhanced PKA phosphorylation of NFAT, and its consequent retention in the cytosol, to curtail expression of hypertrophic genes¹⁵¹. In another study, Baliga et al. showed that PDE2 inhibition counteracts hypertrophy and pathological remodelling, notably fibrosis, in response to abdominal aortic stenosis and chronic catecholamine infusion¹⁵². Although PDE2 was shown to regulate cGMP generated by NP signalling, the cardioprotective effects of PDE2 inhibition were attributed to mobilization of the NO–GC-1–cGMP signalling pathway¹⁵². In this study, PDE2 inhibition induced modest coronary vasodilatation, raising the possibility that this contributes to its beneficial effects. Moreover, Liu et al. showed that PDE2 inhibition, or overexpression of catalytically inactive PDE2A, re-establishes modulation of cardiac norepinephrine release by brain NP (BNP) in stellate ganglia neurons from rats and humans with sympathetic hyperactivity¹⁵⁵ — another potentially protective effect in HF. By contrast, studies by our group¹⁵⁶ and by Mehel et al.¹⁴⁸ have shown that PDE2 overexpression in cardiomyocytes confers cardioprotection. PDE2 overexpression blunts the response to acute β -AR stimulation and protects from norepinephrine-induced hypertrophy¹⁴⁸. Moreover, transgenic mice overexpressing PDE2 specifically in cardiomyocytes showed no significant cardiac hypertrophy and were less susceptible to arrhythmias induced by β -adrenergic stimulation, a phenotype associated with decreased phosphorylation of RYR2, sarcoplasmic reticulum Ca²⁺ leaks and sparks¹⁵⁶. PDE2A transgenic mice also showed attenuated cardiac dysfunction and reduced mortality associated to ventricular tachycardia in the setting of myocardial infarction^{156,157}. The discrepancy in the literature concerning the regulation of cardiac hypertrophy by PDE2 could be attributed to differences in the PDE2 isoforms expressed, the constructs used or the cellular localization of the heterologous PDE2. Generally, whether inhibition or activation of PDE2 is therapeutic in HF might depend on the aetiology. Indeed, the currently available evidence suggests that inhibition of endogenous PDE2 in

cardiomyocytes (and potentially in cardiac endothelial cells, fibroblasts and neurons) could be beneficial to counteract pathological remodelling in HF induced by pressure-overload^{151,152,155,156}, whereas PDE2 activation in cardiomyocytes might be beneficial in ischaemic heart failure to improve Ca²⁺ homeostasis, limit contractile dysfunction and prevent cardiac arrhythmias^{156,157}. Further studies are needed to clarify whether inhibition or activation of PDE2 provides therapeutic effects in HF of various aetiologies.

[H3] PDE3. PDE3 is a dual cyclic nucleotide hydrolysing enzyme with submicromolar affinities for cAMP and cGMP, but a higher turnover rate for cAMP than for cGMP. Thus, cGMP behaves as a competitive inhibitor of cAMP hydrolysis by PDE3, which is sometimes referred to as the cGMP-inhibited, cAMP-PDE. PDE3 is expressed in the myocardium of many species (**TABLE 1** and **SUPPLEMENTARY TABLE 1**) and it is particularly abundant in large mammals, including humans. In human myocardium, PDE3 activity is present in the cytosolic and microsomal fractions and constitutes the majority of cAMP-hydrolysing activity in the latter.

PDE3 is encoded by two genes, *PDE3A* and *PDE3B*. Both are expressed in the heart, but *PDE3A* is predominant^{118,164}. Knock out of these genes in mice shows that *PDE3A* accounts for the cardiotoxic effects of PDE3 inhibitors^{103,165}. Three isoforms of *PDE3A* (*PDE3A1–3*), differing in the length of their N-terminal domain, localize in distinct subcellular compartments. The longest form, *PDE3A1*, contains both hydrophobic domains NHR1 and NHR2 and is found in the particulate fraction. *PDE3A2* contains only NHR2 and is detected in cytosolic and particulate fractions. The shortest form, *PDE3A3*, lacks both domains and is cytoplasmic¹⁶⁴. Thus far, only one isoform of *PDE3B* has been described¹⁶⁶. *PDE3B* is localized in the T-tubules in proximity to mitochondria, whereas *PDE3A* is associated with the sarcoplasmic reticulum¹⁶⁷. *PDE3A1*, *PDE3A2* and *PDE3B* contain

phosphorylation sites for various kinases, including PKA and RAC-alpha serine/threonine-protein kinase (AKT1, also known as PKB) (**FIG. 1**) that increase catalytic activity and regulate protein–protein interactions¹⁶⁶.

PDE3 controls cardiac ECC by modulating cAMP–PKA regulation of $I_{Ca,L}$ ^{137,168-171} and Ca^{2+} uptake in the sarcoplasmic reticulum^{172,173}. PDE3 activity has long been known to be associated with the human cardiac sarcoplasmic reticulum¹⁷⁴, and PDE3A1 was shown to be part of a multiprotein signalling complex controlling phospholamban phosphorylation and SERCA2 activity in mice and human hearts^{103,175}. PDE3 was also shown to control cAMP in a microdomain around the $Na^+–K^+–ATPase$ –phospholemman complex¹⁷⁶. Studies with a phospholemman-targeted cAMP biosensor in rat cardiomyocytes revealed preferential regulation of this cAMP pool by β_2 -Ars, and tight control by PDE3¹⁰². In failing myocytes, cAMP generated by β_2 -ARs is substantially reduced and is accompanied by a switch from PDE3 to PDE2 regulation, illustrating local PDE remodelling in HF¹⁰². However, the literature on modifications of PDE3 expression and activity in humans with HF and in experimental models is contradictory (**TABLE 2, SUPPLEMENTARY TABLE 2**). In some studies of human failing hearts, no change in PDE3 activity was reported^{162,177}, whereas decreased PDE3 activity was found in others¹⁷⁸. Similarly, in animal models of cardiac hypertrophy or HF, decreased PDE3 activity or PDE3A expression was reported in some studies¹⁷⁸⁻¹⁸³ and increased activity or expression in others¹⁸⁴⁻¹⁸⁷.

PDE3 is the target of potent cardiotoxic drugs, such as milrinone, which causes vasodilation and inhibits platelet aggregation¹⁶⁰. However, PDE3 inhibition increases arrhythmias in patients with HFrEF^{188,189}, and the short-term benefit of these drugs is outweighed by an increase in mortality from sudden cardiac death of ~3% per year when administered chronically¹⁶⁶. In 2019, a polymorphism in the human PDE3A promoter was reported to regulate *PDE3A* gene transcription by cAMP–PKA, and might explain the

variable tolerance to milrinone in patients¹⁹⁰. A non-exhaustive list of PDE3 inhibitors used in the clinical arena is given in **TABLE 3**, and these drugs have been reviewed elsewhere¹⁹¹.

Despite the adverse effects observed in humans, PDE3 inhibition was shown to decrease infarct size in canine hearts after myocardial ischaemia–reperfusion (IR) via a mechanism dependent on the cAMP–PKA–MAPK11 (also known as p38 MAPK) pathway¹⁹². Moreover, in an acute mouse IR model, *Pde3b*^(-/-), but not *Pde3a*^(-/-), mice had reduced infarct size¹⁶⁷. Herein, the cardioprotective effects of PDE3B were dependent on cAMP–PKA signalling, which potentiated the opening of Ca²⁺-activated potassium channels, decreasing reactive oxygen species formation by mitochondrial fractions that were resistant to calcium-induced mitochondrial permeability transition pore opening¹⁶⁷. In a TAC mouse model, milrinone was shown to attenuate ventricular remodelling, decrease fibrosis and macrophage infiltration and de-activate the p38 MAPK pathway¹⁸⁵. Global knock out of *Pde3a* provided protection against ventricular remodelling similar to that induced by TAC, and milrinone had no additional effect in these mice¹⁸⁵.

In contrast to these positive outcomes reported for PDE3 inhibition or genetic ablation, Nakata et al. found that PDE3 inhibition worsened chamber stiffness and increased collagen type I deposition induced by chronic β -AR stimulation in rats¹⁹³. Furthermore, a reduction in PDE3A has been shown to upregulate ICER, a negative regulator of Bcl2-associated agonist of cell death (Bcl2) and potent pro-apoptotic factor in cardiomyocytes¹⁹⁴. In addition, Nash et al. showed that PDE3 negatively regulates EPAC-mediated activation of PLC β and the downstream hypertrophic response in neonatal cardiomyocytes¹⁹⁵. These mechanisms could contribute to the detrimental effects of long-term PDE3 inhibition, and suggest that increasing PDE3A activity would be beneficial to prevent HF. Accordingly, mice with cardiac-specific overexpression of PDE3A1 show protection against myocardial IR, presenting ameliorated infarct size associated with decreased expression of ICER, increased

expression of Bcl2 and cardiomyocyte resistance to apoptosis¹⁹⁶. The reason for the discrepancies in the literature is not clear, and further investigations on the role of PDE3 variants are required to better understand their respective roles in hypertrophy and HF.

[H3] PDE4. PDE4 hydrolyses cAMP specifically with micromolar affinity¹⁹⁷. Four genes (*PDE4A–D*) encode multiple isoforms that can be long, short or super-short, based on the presence or absence of upstream conserved region (UCR) domains separated by linker regions⁸² (**FIG. 1**). Both UCR1 and UCR2 domains are required for dimerization^{198, 199}, meaning that short and super-short forms lacking UCR1 remain as monomers. PDE4 activity can be regulated by PKA phosphorylation in the UCR1 domain or by MAPK1 (also known as ERK2) in the C-terminal region^{201, 202} (**FIG. 1**). This provides an important feedback mechanism to control cAMP levels in various cell types, including cardiomyocytes^{168,203,204}. In PDE4D, CaMKII phosphorylation has been shown to increase catalytic activity and control cAMP levels in cardiomyocytes, suggesting that PDE4D underlies crosstalk between Ca²⁺ and cAMP signaling²⁰⁵.

PDE4 expression and function are conserved between human and rodent hearts, but the contribution of this enzyme to total cellular cAMP-PDE activity is much smaller in humans than in rodents. Accordingly, PDE4 is a major negative regulator of β -AR responses in healthy rat cardiomyocytes^{146,168}. However, during cardiac hypertrophy and HF, the balance between PDE3 and PDE4 in the regulation of β -AR responses is shifted toward PDE3^{180,209,210}. Approximately equal proportions of PDE4A, PDE4B and PDE4D are found in the mouse heart¹⁰⁰, and these isoforms regulate cAMP levels in multiple subcellular compartments²¹¹ (**FIG. 2**). At the plasma membrane, distinct PDE4D variants interact with β_1 -AR and β_2 -AR subtypes, either directly or through β -arrestin, thereby modulating both autologous and heterologous receptor desensitization and alternative signalling by β_1 -AR and

β_2 -AR^{87, 90, 91, 93}. In particular, PDE4D5 recruitment to β_2 -AR by β -arrestin has been shown to be critical to prevent activation of EPAC1 and CaMKII and the pro-hypertrophic effects of β_2 -AR activation⁸⁹. A different isoform, PDE4B, was shown to associate with LTCC and to negatively modulate β -AR stimulation of $I_{Ca,L}$ ¹⁰⁰. In mice deficient for the *Pde4b* gene, enhanced β -AR stimulation of $I_{Ca,L}$ is associated with increased susceptibility to cardiac arrhythmias¹⁰⁰.

At the level of the sarcoplasmic reticulum, PDE4D3 localizes within the RYR2 macromolecular complex, but this association is decreased in failing human hearts leading to hyperphosphorylated ‘leaky’ channels¹⁰⁵. A pathogenic role for depressed PDE4D3 in the RYR2 complex is suggested by the phenotype of *Pde4d*-deficient mice, in which PKA hyperphosphorylation of RYR2 is associated with increased sensitivity to exercise-induced arrhythmias and the development of a late-onset dilated cardiomyopathy¹⁰⁵. In 2021, decreased RYR2-associated PDE4 in hypertrophy was confirmed by Berisha et al. in mice expressing a RYR2-targeted cAMP biosensor, enhancing RYR2 phosphorylation upon β_2 -AR stimulation²¹². In addition to interacting with RYR2, PDE4D also associates with the SERCA2–phospholamban complex¹⁰³. In this microdomain, local cAMP measurements also suggest an altered contribution of PDE4 in hypertrophied cardiomyocytes¹⁵⁰. In contrast to decreased PDE4 activity at RYR2, decreasing PDE4 activity near SERCA2 is expected to enhance Ca^{2+} re-uptake in the sarcoplasmic reticulum through increased phospholamban phosphorylation. This effect could be beneficial in HF where phospholamban phosphorylation is decreased and SERCA2 function depressed²¹³⁻²¹⁷.

PDE4 activity (as for PDE3) is predominant at the nuclear envelope¹⁰⁶. At this location, PDE4D3 was shown to be part of a mAKAP β -based macromolecular complex, including PKA and EPAC1, that regulates cardiomyocyte hypertrophy^{52, 109}. This localized pool of PDE4 also controls the integration of β -AR–cAMP–PKA signalling in the nucleus

and, consequently, PKA regulation of gene expression with potentially important consequences for cardiac hypertrophy and apoptosis^{41,218,219}. In particular, PDE4 bound to mAKAP β was found to curtail nuclear PKA activation after β_2 -AR stimulation, and to limit induction of the pro-apoptotic factor, ICER⁴¹. Moreover, overexpression of the C-terminal portion of the UCR domain (UCR1C), which was shown to activate PDE4, inhibits nuclear PKA activity, decreases phosphorylation of the transcription factor cAMP responsive element-binding protein and attenuates cardiomyocyte hypertrophy²²⁰. PDE4D also regulates a specific cAMP pool that controls PKA phosphorylation of the heat shock protein Hsp20, which when phosphorylated protects against hypertrophic response²²¹. Interestingly, PDE4B expression is decreased in a model of cardiac hypertrophy in rats¹⁸⁰ and in human HF²²², suggesting that decreased PDE4B expression could contribute to cardiac pathological remodelling. Moreover, our group showed that moderate, cardiac-specific overexpression of PDE4B in transgenic mice or by gene transfer with an adeno-associated virus (AAV9) counteracts the pathological remodelling induced by chronic isoproterenol infusion or TAC²²². Small-molecule, pharmacological activators of PDE4, which mimic the effect of PKA phosphorylation on PDE4 long forms, have been reported to reduce abnormally elevated cAMP levels in animal and human cell models of autosomal dominant polycystic kidney disease²²³. Such compounds could provide an interesting alternative to gene therapy in HF.

[H3] PDE5. PDE5 is cGMP-activated and specifically hydrolyses cGMP. This enzyme is encoded by a single gene, *PDE5A*, generating three splice variants (PDE5A1–A3). At the N-terminal domain, PDE5 harbours two GAF domains (GAFA and GAFB). GAFA is the cGMP-binding domain that mediates allosteric activation of PDE5^{225,226}, whereas GAFB contributes to dimerization²²⁷ (**FIG. 1**). PKA and PKG can phosphorylate PDE5 in the N-

terminal region resulting in activation of the enzyme²³¹. Although the expression and function of PDE5 in the heart were initially a matter of debate^{71,186,232-234}, several studies have now indicated that PDE5 is expressed in the myocardium of many species (**TABLE 1**, **SUPPLEMENTARY TABLE 1**). In cardiomyocytes, PDE5 localizes to both the cytosol and the Z-lines¹¹², and controls a specific pool of cGMP produced by soluble (but not particulate) GC, unlike PDE2 and PDE9⁹⁹ (**FIG. 2**). PDE5 inhibition was shown to oppose β -AR stimulation of cardiac contractility in several species, including humans²³⁵. Studies in mice showed that this effect is associated with cGMP elevation and PKG activation and depends on the presence of functional soluble GC and NO-synthase 3 (NOS3)¹¹².

PDE5 expression was found to be increased in HF in mice, rats and humans^{127, 237-240}, but decreased in dogs²⁴¹. PDE5 inhibitors were initially developed to treat coronary artery disease, but were then used clinically to treat erectile dysfunction. With time, the PDE5 inhibitors sildenafil and tadalafil were re-investigated to examine their potential efficacy in HF²⁴² (**TABLE 3**). Effects varied from improving microvascular and endothelial dysfunction and ameliorating various types of cardiovascular impairment, to providing no benefit or harm in patients with HF²⁴³. In mice subjected to aortic constriction, PDE5 inhibition provided cardioprotection by promoting cGMP–PKG signalling to prevent cardiac myocyte hypertrophy and cardiac dysfunction²⁴⁴. Subsequently, PDE5 inhibition was shown to attenuate diastolic dysfunction, and to decrease fibrosis and collagen type I deposition in a rat model of catecholamine-induced myocardial injury¹⁹³. In vitro, sildenafil was found to produce anti-hypertrophic effects²⁴⁵ independently of PKA phosphorylation, but involving PKG, which phosphorylates NFAT to inhibit its nuclear translocation¹⁵¹. Additionally, PKG was proposed to phosphorylate TRPC6 leading to its inhibition and, therefore, decreasing pro-fibrotic and pro-hypertrophic effects coupled to calcineurin–NFAT signalling^{246, 247}.

Conversely, PDE5 upregulation has proven deleterious. Transgenic mice overexpressing PDE5 subjected to myocardial stress exhibited worsened cardiac function and amplified fibrosis via an decrease in PKG activity in models of either pressure overload²⁴⁸ or myocardial infarction²³⁷. However, the contribution of PKG to cardiac anti-hypertrophic effects has been contested. Deletion of the *Prkg1* gene (encoding PKG) in the myocardium did not alter the progression of cardiac hypertrophy in pathological models, such as isoprenaline-induced dysfunction⁷⁰, and the lack of cardiomyocyte PKG activity did not worsen Ang II-induced cardiac hypertrophy⁷². By contrast, decreased levels of PKG exacerbated dilation and systolic dysfunction in mice chronically treated with Ang II or subjected to aortic constriction⁷⁰. PKG downregulation also exacerbated pressure-overload-induced HF and prevented the anti-remodelling effects of sildenafil²⁴⁹. These findings support a cardioprotective role for PKG. Furthermore, the anti-fibrotic effects of PDE5 inhibition require PKG expression in one or more cell types other than smooth muscle cells⁷², suggesting a role for the cGMP–PKG pathway under PDE5 control to counteract adverse cardiac remodelling.

Other targets of PKG have been described, suggesting alternative mechanisms for some of the positive outcomes obtained with PDE5 inhibition. Among them, phosphorylation of tuberin with PDE5 inhibition suppresses mTORC1, which stimulates macro-autophagy⁶⁷. Pleiotropic cardioprotective effects of PDE5 inhibition have been linked not only to NOS3 signalling^{112, 250}, but also to the opening of mitochondrial ATP-sensitive K⁺ (K⁺_{ATP}) channels²⁵⁰⁻²⁵² and the RGS2–PKG–G protein α q subunit signalling pathway⁶⁴. Moreover, PDE5 inhibition has been shown to enhance phosphorylation of MAPK2/3 (also known as ERK1/2) and glycogen synthase kinase-3 β ²⁵³, restore transverse tubule and catecholamine sensitivity²⁵⁴, bolster proteasome-dependent degradation of damaged proteins⁶⁸ and prevent Ca²⁺ triggered arrhythmias in a model of long QT syndrome²⁵⁵, which could confer additional

protection in HF. Interestingly, the sildenafil-induced cardioprotective effects of PKG are lost in female mice after ovary removal, suggesting that PDE5 inhibition efficacy is dependent on oestrogen levels²⁵⁶. In support of this theory, the oestrogen receptor- α non-nuclear signalling pathway was linked to PKG activity in female mouse hearts and was critical to the therapeutic efficacy of PDE5 inhibition²⁵⁷. These studies suggest sex differences in the efficacy of PDE5 inhibitors for treating heart disease and the critical role of oestrogen status when these agents are used in female patients.

Despite the few suggested adverse effects of sildenafil, such as the prolonged repolarization of cardiomyocytes (by blocking the rapid component of the delayed rectifier K^+ current) that favours ventricular arrhythmias^{193, 258}, pre-clinical studies have generated legitimate enthusiasm for therapies targeting PDE5 in HF. However, patients with HFpEF, sildenafil treatment did not improve physical capacity or clinical status¹⁶ and did not decrease filling pressure at rest or during exercise in in patients with diastolic dysfunction after myocardial infarction²⁵⁹. These null findings could be due to downregulation of the NOS–cGMP pathway in the disease state, low cardiac expression of PDE5 or sex differences in the participants. However, further testing of PDE5 inhibitors in other HF aetiologies seems to be worth pursuing. Beneficial effects of PDE5 inhibition have been reported from several clinical trials in patients with HF_rEF (**TABLE 3**), although all are small, single-centre, studies. In a meta-analysis, therapy with PDE5 inhibitors led to a statistically significant improvement of clinical outcomes, exercise capacity and pulmonary haemodynamics in patients with HF_rEF²⁶⁰. Randomized, multicentre, large-scale clinical trials are required to determine whether DE5 inhibition is of therapeutic interest in other HF aetiologies.

[H3] PDE8. PDE8 is a high-affinity, cAMP-specific enzyme^{261, 262} encoded by two genes — — *PDE8A* and *PDE8B* — resulting in at least nine splice variants. In contrast to most PDEs,

PDE8 (like PDE9) is insensitive to 3-isobutyl-1-methylxanthine (IBMX)²⁶¹. PDE8 contains REC and PAS domains at the N-terminus that are involved in protein–protein interactions (**FIG. 1**). PDE8A is activated by PKA phosphorylation²⁶³, and PDE8A transcript and protein are expressed in mouse^{261, 264} and human heart^{265, 266}. In mouse ventricular cells, PDE8A was found to regulate ECC by controlling a specific pool of cAMP involved in β -AR regulation of Ca²⁺ homeostasis²⁶⁴. Although PDE8A expression has been shown to be increased in the human atrium in atrial fibrillation²⁶⁵, no data are currently available on the expression level and putative role of PDE8 in HF.

[H3] PDE9. PDE9 is highly specific for cGMP hydrolysis. Although the presence of this enzyme in human heart tissue was first observed in 1998²⁶⁷, it has been contested¹⁸⁶ and its role in the heart was not reported until 2015⁹⁷, and little is known about the specific role of PDE9 in the myocardium. PDE9 is encoded by the single gene, *PDE9A*, producing more than 20 splice variants in human tissues²⁶⁸. In cardiac cells, PDE9A5 and PDE9A1 were found to be localized exclusively in the cytoplasm and in the nucleus, respectively²⁶⁹. In contrast to most PDEs, PDE9 (like PDE8) is insensitive to IBMX. Unlike PDE5A, PDE9A controls a cGMP pool coupled to NP signalling⁹⁷ (**FIG. 2**). Both PDE9A and GC-A are localized to the T-tubules^{56, 97}. PDE9 was found to be upregulated in a rat model of ischaemic preconditioning²⁷⁰, in mice subjected to TAC and in tissues from patients with dilated cardiomyopathy or HFpEF⁹⁷. In mice, PDE9 inhibition produced anti-hypertrophic and anti-fibrotic effects requiring PKG activation via a NP–cGMP pathway (but not a NO/cGMP pool, which is under PDE5 control)⁹⁷. PDE9 hydrolysis of this specific cGMP pool at the membrane that transduces NP-coupled signalling might explain why PDE9 inhibitors, but not PDE5 inhibitors, are efficient in models where NO generation is disrupted⁹⁷. However, PDE9 inhibition, like PDE5 inhibition, induces PKG activation of, which in turn inhibits the

NFAT–calcineurin pathway, even though PDE9A or PDE5A inhibition lead to differential phosphorylation of various proteins⁹⁷. Interestingly, in a model of pressure overload, PDE5 inhibition broadly reduced the expression of >120 microRNAs, whereas PDE9 inhibition barely had an effect on any microRNAs²⁷¹, suggesting differential effects of PDE5 and PDE9 inhibition. In a sheep model, with pacing-induced HF, PDE9 inhibition increased plasma cGMP:NP ratio and urinary cGMP concentration, and led to improved cardiac output and arterial vasodilatation, suggesting that the benefits of PDE9 inhibition come from effects on renal, as well as cardiac, function²⁷². However, in two mouse models of HFpEF, although PDE9 inhibition improved cardiomyocyte passive stiffness in a cGMP–PKG-dependent manner, this treatment had no therapeutic effects on end-diastolic function or pressure, relaxation, fibrosis or hypertrophy of the left ventricle²⁷³. In fact, PDE9 inhibition led to impaired systolic function, thus mitigating any beneficial effects²⁷³. Nonetheless, the therapeutic potential of chronic PDE9 inhibition in HFrEF were confirmed in a mouse model with the use of CRD-733²⁷⁴, a specific inhibitor of the enzyme that is currently being advanced in human clinical studies. The compound improved key features of HF after pressure overload induced by transverse aortic constriction²⁷⁴. Therefore, PDE9 inhibitors alone or combined with cGMP-generating drugs might constitute new therapeutic approaches in HFrEF and could be soon translated to patients²⁷⁴.

[H3] PDE10. PDE10, like PDE1–3, hydrolyses both cAMP and cGMP; it contains two N-terminal GAF domains and a PKA phosphorylation site²⁷⁶ (**FIG. 1**). cAMP binding to the catalytic domain inhibits cGMP hydrolysis^{277, 278}. However, cAMP binding to the GAF domain activates PDE10 and, depending on cAMP concentration, cGMP hydrolysis can be increased (low levels) or decreased (high levels)²⁷⁹. PDE10 expression is low in the healthy heart, but is upregulated in mouse and human failing hearts²⁸⁰. Inhibition or deficiency of

PDE10 was shown to attenuate cardiomyocyte hypertrophy induced by Ang II, phenylephrine or isoprenaline in vitro, and was also efficient in vivo in reducing myocardial hypertrophy, cardiac fibrosis and dysfunction induced by TAC or chronic Ang II infusion²⁸⁰. However, further work is needed to better understand the role of PDE10 in the regulation of cardiac function and its involvement in HF.

[H1] Altered PDE-mediated crosstalk in HF

The existence of microdomain compartments, to which PDEs are recruited to hydrolyse cAMP, cGMP or both with various affinities and kinetics, creates functionally relevant crosstalk between cGMP and cAMP signalling pathways. PDE2, PDE3 and PDE5 are important mediators of this crosstalk²⁸¹ (**FIG. 3**). In healthy cardiomyocytes, NO donors or cGMP can modulate cAMP levels and PKA activity near LTCC through inhibition of PDE3 or stimulation of PDE2, hence increasing or decreasing $I_{Ca,L}$ ^{136,137,282-284}. In neonatal rat cardiomyocytes, PDE2 is activated by cGMP generated upon β_3 -AR stimulation, thereby counteracting cAMP and functional responses produced by β_1/β_2 -ARs stimulation²⁸⁵. In 2020, Schobesberger et al. showed that β_3 -ARs are redistributed from the T-tubules to the surface sarcolemma in HF, leading to impaired PDE2-dependent negative regulation of cAMP signalling²⁸⁶. In addition to β_3 -ARs, paracrine NO signalling and NP-cGMP signalling also modulates β_1/β_2 -AR-cAMP responses in healthy cardiomyocytes. PDE5 inhibition with sildenafil was shown to attenuate β -AR contractile responses in a NOS3-dependent manner, whereas atrial NP (ANP) had no effect^{112,287}. In a study that used targeted FRET biosensors in neonatal rat cardiomyocytes, cGMP elicited by the NO donor S-nitroso-N-acetylpenicillamine was shown to increase cAMP levels in the PKA type I compartment due to inhibition of PDE3, whereas it decreased cAMP in the PKA type II compartment due to stimulation of PDE2. By contrast, activation of GC-A with ANP specifically decreased

cAMP in the PKA type II compartment²⁸⁸. In both failing and non-failing rat hearts, activation of GC-B by C-type NP potentiates β_1/β_2 -AR contractile responses through cGMP-mediated inhibition of PDE3, whereas this effect was not observed with stimulation of GC-A with BNP^{210,289}. However, a positive inotropic effect of ANP was reported in early hypertrophy induced by TAC²⁹⁰. This effect of ANP was associated with the redistribution of PDE2 from the β_1 -AR to the β_2 -AR at the plasma membrane in early hypertrophy, leading to an increase in global β_1 -AR signalling and a more pronounced contractile effect²⁹⁰. In contrast to the overall protective effect of NPs in HF, these mechanisms could help to sustain cardiac contractility in the early stages of disease, but are likely to be detrimental in the long term. Therefore, these studies might have uncovered a negative side to NP signalling in HF, which could be prevented by augmenting or restoring PDE2 compartmentation.

A 2021 study by Manfra et al. showed that cGMP signals generated by GC-A or GC-B in cardiomyocytes are differentially regulated by PDE2 and PDE3 in the vicinity of phospholamban and troponin I⁵⁵. This finding illustrates that cGMP-degrading PDEs, like cAMP-degrading PDEs, are precisely localized in the vicinity of kinase substrates and might be remodelled in HF. The intricate synergism between cardiac PDEs in the control of β -AR–cAMP signalling has also been demonstrated by computational modelling in healthy myocytes²⁹¹, but these interactions remain largely unresolved in HF. Further studies are needed to fully understand how modifications in expression and spatial localization of PDEs impact crosstalk between cAMP and cGMP in HF to improve treatments for HF that target cyclic nucleotide signalling.

[H1] Conclusions

Our understanding of the pathophysiological mechanisms of HF and associated arrhythmias has come a long way. Studies in animal models of HF have demonstrated that PDEs are

plausible therapeutic targets. However, the cellular mechanisms controlled by each PDE family or subtype are complex and point to the need to target specific PDE isoforms in circumscribed microdomains of myocardial cells, rather than using a pan-PDE approach to produce global changes in cyclic nucleotide concentrations. Accordingly, beneficial effects might stem from isoform-specific PDE inhibition or activation (for example, by modifying expression level, targeting GAF domains, targeting regulatory domains or by post-translational modifications) as well as alteration of PDE location⁸³.

Several PDE inhibitors have been tested in clinical trials for potential cardioprotective effects in patients with HF but, unfortunately, despite encouraging preliminary observations, no clear beneficial effects have been finally reported (**TABLE 3**). Several factors might explain the unsuccessful translation of promising findings from pre-clinical studies into the clinical arena. First, the different patterns of expression of PDE families and isoforms between species. Second, in most preclinical studies, PDE modulation occurs before (transgenic or knockout models) or concurrent with (pharmacological treatment) the onset of HF, favouring the prevention of disease onset. In clinical studies, however, patients with established HF are treated, a scenario that might require different PDE modulations. Third, the even PDE-isoform-selective modulators can be ineffective when the isoform is expressed in different compartments, particularly when those compartments serve opposing functions. The development of compartment-specific, PDE-isozyme-selective modulators (for example, using cell-permeable peptide disrupters to displace the PDE isozyme from its signalling complex)²⁹² could help to solve this problem. Finally, the complex crosstalk between cAMP and cGMP intracellular signalling, and the presence of various PDE isoforms in different compartments, could require multi-drug therapy to fine-tune the cyclic nucleotide levels in specific subcellular compartments. For example, activating PDE4D near RYR2 or PDE4B or PDE2A near LTCCs while, at the same time, inhibiting PDE3A or PDE5 near

phospholamban, could restore normal compartmentalization of cAMP and cGMP during hypertrophy and prevent the transition towards HF. Future studies addressing these questions will determine whether PDE modulators can be successfully translated to the clinical arena for the treatment of patients with cardiac hypertrophy and HF.

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Competing interests

The authors declare no competing interests.

Key points

- Cyclic nucleotide phosphodiesterases (PDEs) hydrolyse cAMP and cGMP to modulate the neuro-hormonal regulation of cardiac function.
- Multiple PDE isozymes with various enzymatic properties and subcellular localizations finely tune local pools of cyclic nucleotides and control specific functions.
- Changes in PDE expression, activity and subcellular localization during cardiac hypertrophy and heart failure can contribute to disease progression.

- Despite limited success in the clinical arena, evidence in the literature continues to point to inhibitors or activators of specific PDEs as promising future therapies for heart failure.

Table 1 | **Phosphodiesterase (PDE) isoform expression in the human heart**

PDE family	PDE gene	Tissue/cell type	Subcellular compartment	Detection methods	Splice variants
PDE1	<i>PDE1A</i>	Heart tissue ¹²² ; Heart ¹²⁶ ; LV ¹³⁰ ;	NA	RT-qPCR, WB ^{126,130} ; IH ¹²²	NA
	<i>PDE1B</i>	Heart ¹²⁶	NA	Low mRNA levels ¹²⁶	NA
	<i>PDE1C</i>	LV ^{130,186} ; Myocardium in the Z and M lines (cardiomyocytes) ¹²⁰	Microsomal and soluble fraction ¹²⁰	WB, IF and cAMP, cGMP hydrolytic activities ¹²⁰ ; RT-qPCR and WB ¹³⁰ ; WB ¹⁸⁶	PDE1C1 (microsomal and soluble fraction) ¹²⁰ , PDE1C3 ¹²⁰
PDE2	<i>PDE2A</i>	Isolated LV or RV cardiomyocyte homogenates from failing hearts ²⁹³	NA	PCR product on gel and cGMP hydrolytic activity ²⁹³	NA
		LV ¹⁴⁸	NA	WB ¹⁴⁸	NA
PDE3	<i>PDE3A</i>	Myocardium ^{118,164} ; LV ^{175,186}	Cytosolic and/or microsomal ^{118,164} ; Sarcomere Z band ¹⁷⁵	cAMP and cGMP hydrolytic activity ¹¹⁸ ; WB, NB and SB ¹⁶⁴ ; IF, WB and cAMP hydrolytic activity ¹⁷⁵ ; WB ¹⁸⁶	PDE3A-94 and PDE3A-118 (derived from PDE3A2 mRNA, microsomal and cytosolic), PDE3A-136 (derived from PDE3A1 mRNA, microsomal) ^{118,164} ; PDE3A1 (membrane associated, SR with SERCA2– phospholamban– AKAP18 after phosphorylation by PKA), PDE3A2 (cytosolic fraction) and PDE3A3 (cytosolic fraction) ¹⁷⁵
PDE4	<i>PDE4A</i>	Myocardium ²⁰⁸	NA	WB and cAMP hydrolysing activity ²⁰⁸	NA
	<i>PDE4B</i>	LV from patients with HF and NF controls ²²²	NA	WB ²²²	NA
	<i>PDE4D</i>	Myocardium ^{105,208}	Colocalized with RYR2 ¹⁰⁵	IP, WB and cAMP hydrolysing activity in RYR2 complex ¹⁰⁵ ; WB and cAMP hydrolysing activity ²⁰⁸	PDE4D3 (RYR2 colocalization) ¹⁰⁵ ; PDE4D3, 5, 8, 9 ²⁰⁸
PDE5	<i>PDE5A</i>	Isolated LV or RV cardiomyocyte homogenates from failing hearts ²⁹³ ; heart tissue ²⁹⁴ ; adult and foetal heart tissue ²⁹⁵	NA	PCR product on gel and cGMP hydrolytic activity ²⁹³ ; PCR product on gel ^{294, 295}	PDE5A1, 2, 3 ^{294,295}
PDE7	<i>PDE7A</i>	Foetal human heart ⁷⁵ ; human heart cDNA library ⁷⁶	Both soluble and particulate fractions ⁷⁵	PCR ^{75,76} ; WB ⁷⁵	PDE7A1 and PDE7A2 ⁷⁵

	<i>PDE7B</i>	Human heart RNA ⁷⁴	NA	RNA dot blot analysis ⁷⁴	NA
PDE8	<i>PDE8A</i>	Human atrium ²⁶⁵ ; whole heart cDNA ²⁶⁶	NA	RT-qPCR, WB ²⁶⁵ ; PCR ²⁶⁶	PDE8A1 and PDE8A2 ²⁶⁶
PDE9	<i>PDE9A</i>	Heart tissue ^{97,267}	NA	WB, immunostaining, in situ hybridization and cGMP hydrolysing activity ⁹⁷ ; northern blot ²⁶⁷	NA
PDE10	<i>PDE10A</i>	Heart tissue ²⁸⁰	NA	RT-qPCR, WB and cAMP hydrolysing activity ²⁸⁰	PDE10A2 ²⁸⁰

AKAP18, A-kinase anchor protein 7 isoform; HF, heart failure; IF, immunofluorescence; IH, immunohistochemistry; IP, immunoprecipitation; LV, left ventricle; NA, not available; NB, northern blot; NF, non-failing; PCR, polymerase chain reaction; PKA, cAMP-dependent protein kinase catalytic subunit α ; RT-qPCR, quantitative reverse transcription polymerase chain reaction; RV, right ventricle; RYR2, ryanodine receptor 2; SB, Southern blot; SERCA2, sarcoplasmic/endoplasmic reticulum calcium ATPase 2; SR, sarcoplasmic reticulum; WB, western blot.

Table 2 | **Phosphodiesterase (PDE) remodelling in human cardiac hypertrophy and heart failure**

PDE family	PDE gene(s)	Pathology	Regulation and method of detection	Functional consequences	Ref.
PDE1	<i>PDE1A</i>	MI	↑ Immunostaining	Fibrosis (collagen deposition and increase in α -SMA expression)	122
	<i>PDE1A, PDE1C</i>	DCM	↑ PDE1A (WB), PDE1C (RT-qPCR)	NA	130
	<i>PDE1C</i>	HF	= (WB)	NA	186
PDE2	<i>PDE2A</i>	DCM, ICM, AS	↑ (RT-qPCR, WB, cAMP and cGMP hydrolysing activity) in DCM, ICM. = In human AS	NA	148
PDE3	<i>PDE3A</i>	DCM and IHD	↓ (WB, cAMP hydrolysing activity)	NA	178
		HFrEF	↑ (WB)	NA	186
		IDCM	= (cAMP hydrolysing activity)	NA	162
		IDCM (mainly)	= (cAMP hydrolysing activity)	NA	177
PDE4	<i>PDE4A, PDE4D</i>	IDCM	↓ (WB and activity)	NA	208
	<i>PDE4D3</i>	HF	↓ (WB and activity in RYR2 complex)	Increase in RYR2 phosphorylation levels and decrease in cAMP-hydrolysing activity of RYR2-bound PDE4D3	105
	<i>PDE4B</i>	ICM, DCM	↓ (WB)	NA	222
PDE5	<i>PDE5A</i>	DCM or ICM	↑ (WB and IH)	NA	237
		RV hypertrophy (various pathologies; e.g. hypoplastic left heart, rheumatic valve disease, Tetralogy of Fallot)	↑ (RT-qPCR, WB, IH and activity)	NA	238
		Transplant recipients with end-stage ICM or IDCM	↑ (WB, IH)	PDE5 inhibition increased contractility in isolated RV trabeculae	239
		Transplant recipients with single ventricle congenital heart disease of RV (secondary to systolic HF)	↑ (WB and activity)	NA	240

PDE9	<i>PDE9A</i>	LV hypertrophy from AS (pressure overload); HFpEF or DCM	↑ (WB, immunostaining and cGMP esterase activity assay)	NA	97
PDE10	<i>PDE10A</i>	End-stage HF	↑ (RT-qPCR, WB and cAMP hydrolysing activity assay)	NA	280

α -SMA, α smooth muscle actin; AS, aortic stenosis; DCM, dilated cardiomyopathy; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; IHD, ischaemic heart disease; ICM, ischaemic cardiomyopathy; IDCM, idiopathic dilated cardiomyopathy; LV, left ventricle/ventricular; MI, myocardial infarction; NA, not available; RT-qPCR, quantitative reverse transcription polymerase chain reaction; RV, right ventricle/ventricular; WB, western blot.

Table 3 | **Phosphodiesterases (PDE) as therapeutic targets in heart failure**

Drug tested (PDE modulation)	Cardiovascular disease	Clinical outcome
ITI-214 (PDE1 inhibitor)	HFrEF ¹³²	A single dose was well-tolerated and induced inodilator effects
Milrinone (PDE3 inhibitor)	NYHA class III–IV congestive HF ²⁹⁶	Acutely: positive inotropic effect and ↑ in stroke work or positive chronotropic plus vasodilator effect depending on dosage
	Moderately severe HF in sinus rhythm ¹⁸⁹	↑ Ventricular arrhythmias
	Severe chronic HF or advanced LV dysfunction ¹⁵	↑ Hospitalization and all-cause mortality
	Worsening systolic HF not requiring intravenous inotropic support ²⁹⁷	Sustained hypotension, increase in atrial arrhythmias
	HFpEF ²⁹⁸	↓ PCWP with exercise, resting RAP and mean PAP; ↑ Resting HR and cardiac index
Extended release milrinone formulation (PDE3 inhibitor)	Stage D HF ²⁹⁹ (no treatment options)	Improvements in NYHA class, MLFHQ score and 6-min walk distance
	Symptomatic HFpEF ³⁰⁰	Improvements in KCCQ score and 6-min walk distance
Enoximone (PDE3 inhibitor)	NYHA class III–IV HF and LVEF ≤30% with one hospitalization or two ambulatory visits for worsening HF in the previous year ³⁰¹	↑ 6-min walk distance
Sildenafil (PDE5 inhibitor)	Diabetic cardiomyopathy ³⁰²	Improved LV torsion and strain, mass:volume ratio and circulating markers (monocyte chemotactic protein-1 and transforming growth factor)
	HFpEF ¹⁶	= Exercise capacity or clinical status
	Chronic HF ³⁰³	= LVEF; ↓ Mean PAP, arteriolar resistance and oxygen debt and improved exercise parameters
	Stable chronic HF with LVEF <45% ³⁰⁴	↓ Systolic PAP, improved exercise ventilation; ↑ flow-mediated dilatation
	Chronic NYHA class II–III HF with LVEF <40% ³⁰⁵	↑ Flow-mediated dilatation of the brachial artery (an additive effect when administered concomitantly with ramipril)
	NYHA class III HF ³⁰⁶	Selective pulmonary vasodilation during rest and exercise by ↓ PAP, systemic vascular resistance and pulmonary vascular resistance; ↑ Resting and exercise cardiac index
	NYHA class II–IV HF with LVEF <40% and mean PAP >25 mmHg ³⁰⁷	↓ Pulmonary vascular resistance; ↑ CO with exercise, 6-min walk distance and MLFHQ score; ↓ Hospitalizations
	Chronic HF with LVEF <28% ³⁰⁸	Improved maximal oxygen uptake, ventilatory efficiency and oxygen uptake kinetics; ↓ PASP

	NYHA class II–III HF ³⁰⁹	↑ LVEF and improved exercise performance, ventilation efficiency and quality of life
	NYHA class I–III HF with LVEF <35% ³¹⁰	= LV parameters
	HFpEF with LVEF >50% and PASP >40 mm Hg ³¹¹	Improvements in mean pulmonary artery pressure and vasomotility, RV function; ↑ left-sided cardiac function
	AMI with diastolic dysfunction and near normal LVEF ²⁵⁹	= PCWP; ↑ Cardiac index, systemic vascular resistance index and DBP
	HFpEF with mean PAP >25 mmHg and PAWP >15 mmHg ³¹²	= PAP or CO
	Chronic HF and LVEF <40% ³¹³	↓ Cardiac norepinephrine spill-over, mean PAP and mean arterial pressure
	HFpEF with mean PAP >25 mmHg and PAWP >15 mmHg ³¹⁴	= Cardiac structure and function and quality of life
Udenafil (PDE5 inhibitor)	Chronic HFrEF, optimal medical therapy for >30 days before enrollment ³¹⁵	Improvement in parameters of LV systolic and diastolic function and exercise capacity vs conventional pharmacotherapy
Tadalafil (PDE5 inhibitor)	LV hypertrophy, diastolic dysfunction and resistant hypertension ³¹⁶	Improvement in LV relaxation; ↓ BNP-32 levels

AMI, acute myocardial infarction; CO, cardiac output; DBP, diastolic blood pressure; HF, heart failure; HFrEF, heart failure with reduced ejection fraction; HFpEF, heart failure with preserved ejection fraction; HR, heart rate; KCCQ, Kansas City Cardiomyopathy Questionnaire; LV, left ventricular; LVEF, left ventricular ejection fraction; MLHFQ, Minnesota Living With Heart Failure Questionnaire; NYHA, New York Heart Association; PAP: pulmonary artery pressure; PAWP: pulmonary artery wedge pressure; PASP, pulmonary arterial systolic pressure; PCWP, pulmonary capillary wedge pressure; RAP, right atrial pressure; RV, right ventricular; ↑, increase; ↓, decrease; =, no change.

Fig. 1 | Structure and enzymatic properties of cardiac PDEs. The conserved catalytic domains in the C-terminal halves of the phosphodiesterases (PDEs) are shown as purple cylinders for enzymes degrading both cAMP and cGMP and are depicted as red or blue cylinders when they specifically hydrolyse cAMP or cGMP, respectively. These proteins differ in their biochemical properties. For each PDE family, the K_m (Michaelis constant) and V_{max} (maximal rate) values are reported in the tables. Specific targeting domains and numerous types of protein domain provide regulatory control of PDE catalytic activity and allow subcellular localization of the enzymes. Domains such as linker region (LR), N-terminal hydrophobic region (NHR), response regulator receiver (REC), Per-Arnt-Sim (PAS), binding domains for the Ca^{2+} -calmodulin complex (Ca^{2+} -CaM) are located in the N-terminal regions. Some of these domains harbour phosphorylation sites for various kinases such as cAMP-dependent protein kinase catalytic subunit α (PKA), mitogen-activated protein kinases (MAPK, also known as ERK) or Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) to modulate their catalytic activities. PDE2 and PDE5 can undergo dimerization via their GAF domains (cGMP-dependent phosphodiesterase, *Anabaena* Adenylyl cyclase, *E. Coli* FhlA), as indicated. Long isoforms of PDE4 can also dimerize, because they include two required upstream conserved regions (UCR1 and UCR2) whereas short forms lacking UCR1 remain as monomers. PDE2, PDE5 and PDE10A are among a group of PDEs containing GAF domains, which allow their regulation by cyclic nucleotides — PDE2 and PDE5 being activated by cGMP and PDE10A inhibited by cAMP.^{317,318} AKT1, RAC-alpha serine/threonine-protein kinase (also known as PKB); P, phosphorylation; PKC, protein kinase C; PKG, cGMP-dependent protein kinase; SERCA2, sarcoplasmic/endoplasmic reticulum calcium ATPase 2.

Fig. 2 | Subcellular localization and activities of PDE isoforms within macromolecular complexes in cardiac myocytes. Various signalosomes in the ventricular cardiomyocyte,

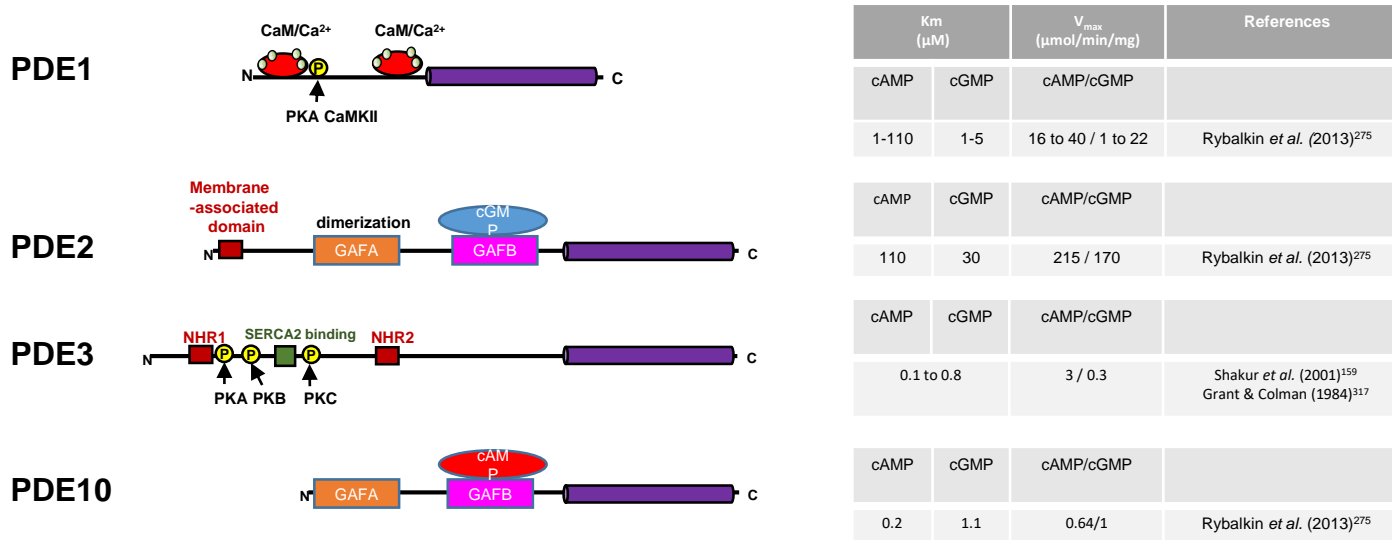
including phosphodiesterases (PDEs), control differential cAMP pools (red) synthesized by adenylyl cyclase (AC) at the plasma membrane when activated by heterotrimeric G-protein stimulating adenylyl cyclase (Gs), or produced by soluble ACs (sAC) within the mitochondria. cGMP signals (blue) catalysed by particulate and soluble guanylyl cyclases (pGC and sGC) are also shown. Various PDE isoforms are associated with or control cyclic nucleotide concentrations emanating from β -adrenergic receptors (β -AR), adenosine 2A receptors (A_2R) and GCs activated by natriuretic peptides or nitric oxide (NO). Some of these enzymes interact with β -ARs directly or via β -arrestin (β -Arr), others are associated to A-kinase anchoring protein (AKAP), phosphoinositide 3-kinases (Pi3K), heat shock protein (Hsp20), myomegalin or membranes. These interactions define their subcellular localization to control cyclic nucleotides levels within specific nanodomains to control cAMP effectors, such as the cAMP-dependent protein kinase catalytic subunit α (PKA) or the exchange protein directly activated by cAMP (EPAC), and cGMP effectors, such as the cGMP-dependent protein kinase (PKG). By degrading cAMP and cGMP, PDEs control the phosphorylation, and thus the activity, of numerous channels present at the sarcolemma, such as the L-type Ca^{2+} channels (LTCC), the potassium voltage-gated channel subfamily KQT member 1 (KCNQ1) and the short transient receptor potential channel 3 (TRPC3). Other PDEs are found at the sarcoplasmic reticulum (SR) and modulate phosphorylation of the ryanodine receptor (RYR) as well as proteins physically and functionally coupled to pumps, such as phospholemman (PLM) or phospholamban (PLB) associated to Na^+/K^+ -ATPase or sarcoplasmic/endoplasmic reticulum calcium ATPase 2 (SERCA2), respectively. Thus, these enzymes are crucial modulators of numerous cellular responses to various external stimuli, fine-tuning cAMP and cGMP levels in subcellular domains to modulate excitation–contraction coupling, but also control apoptosis and expression of hypertrophic genes by

limiting the phosphorylation of transcriptional factors such as cAMP-responsive element-binding protein (CREB) or nuclear factor of activated T-cells (NFAT).

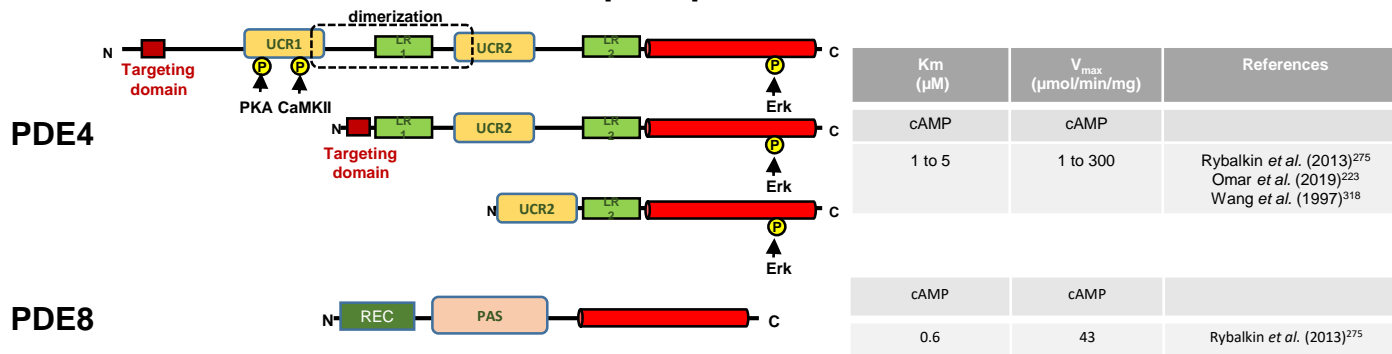
Fig. 3 | Enzymes involved in cAMP and cGMP synthesis and degradation in

cardiomyocytes. The adenylyl cyclases (AC) and the guanylyl cyclases (GC) are involved in cAMP and cGMP synthesis, respectively, and various phosphodiesterases (PDEs) degrade both cyclic nucleotides in cardiomyocytes. PDEs are at the interplay between the two signalling pathways in cardiac myocytes (red for cAMP and blue for cGMP). PDE1, PDE2, PDE3 and PDE10 hydrolyse both cAMP and cGMP, whereas PDE4 and PDE5 are specific for cAMP or cGMP, respectively. The involvement of PDEs in the intricate interactions between these signalling pathways is promoted by their different enzymatic properties and their direct regulation by the cyclic nucleotides. For example, the activation of PDE2 and PDE5 by cGMP, the limited degradation of cAMP by PDE3 when cGMP binds to its catalytic region and the inhibition of PDE10 by cAMP. Therefore, depending on their subcellular localization, PDEs can modify the enzymatic activities of other PDEs by degrading either of the cyclic nucleotides. Furthermore, some PDEs are phosphorylated by kinases, such as cAMP-dependent protein kinase catalytic subunit α (PKA), which activates PDE1, PDE3, PDE4 and PDE8 or by cGMP-dependent protein kinase (PKG), which increases PDE5 hydrolytic activity, providing negative feedback loops to shape cAMP and cGMP signals within the cardiomyocyte. P, phosphorylation.

cGMP/cAMP phosphodiesterases



cAMP phosphodiesterases



cGMP phosphodiesterases

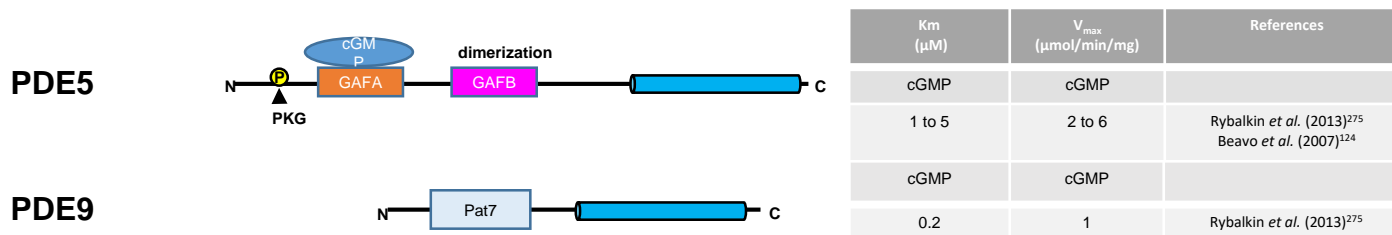


Figure 1

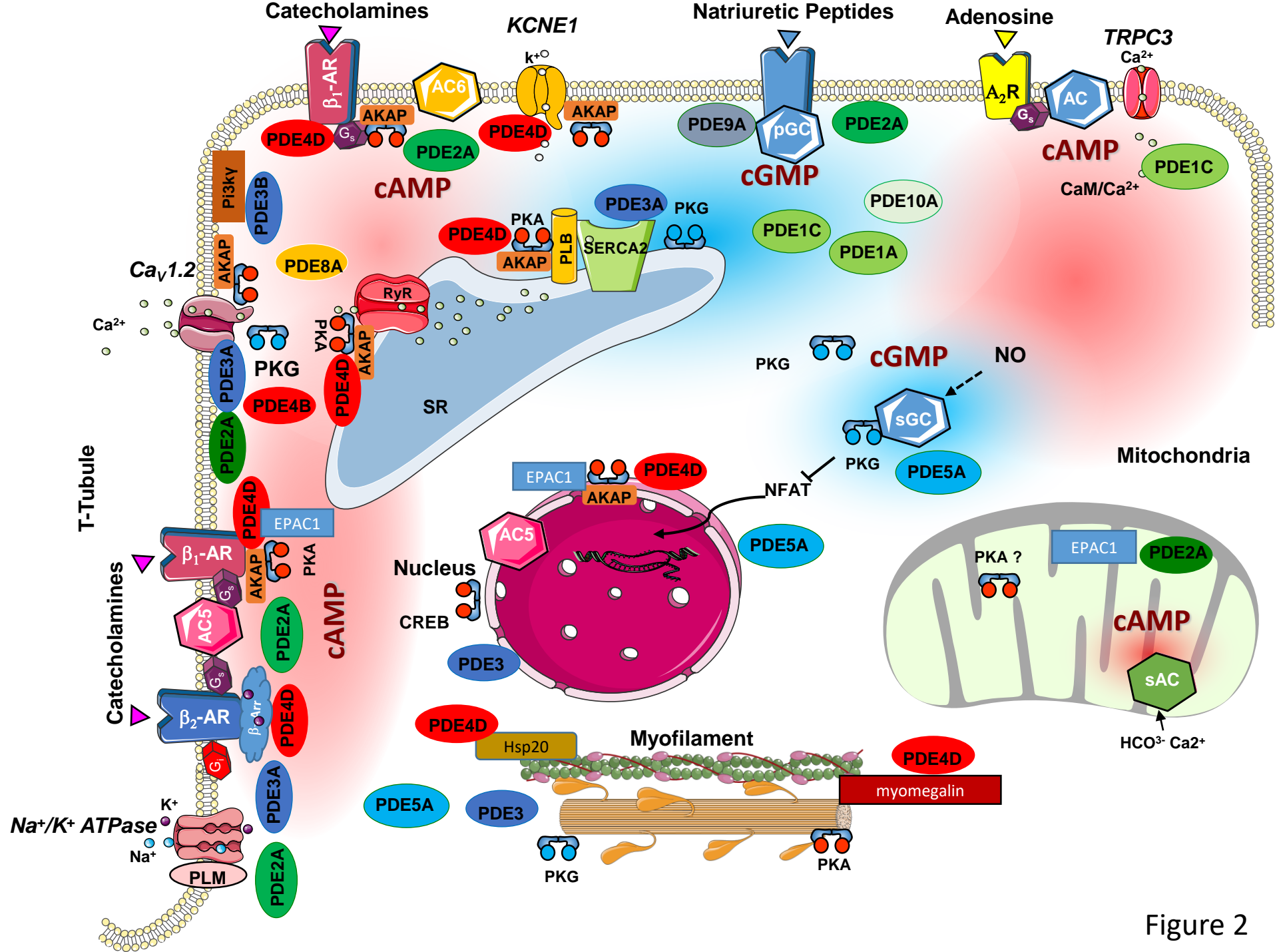


Figure 2

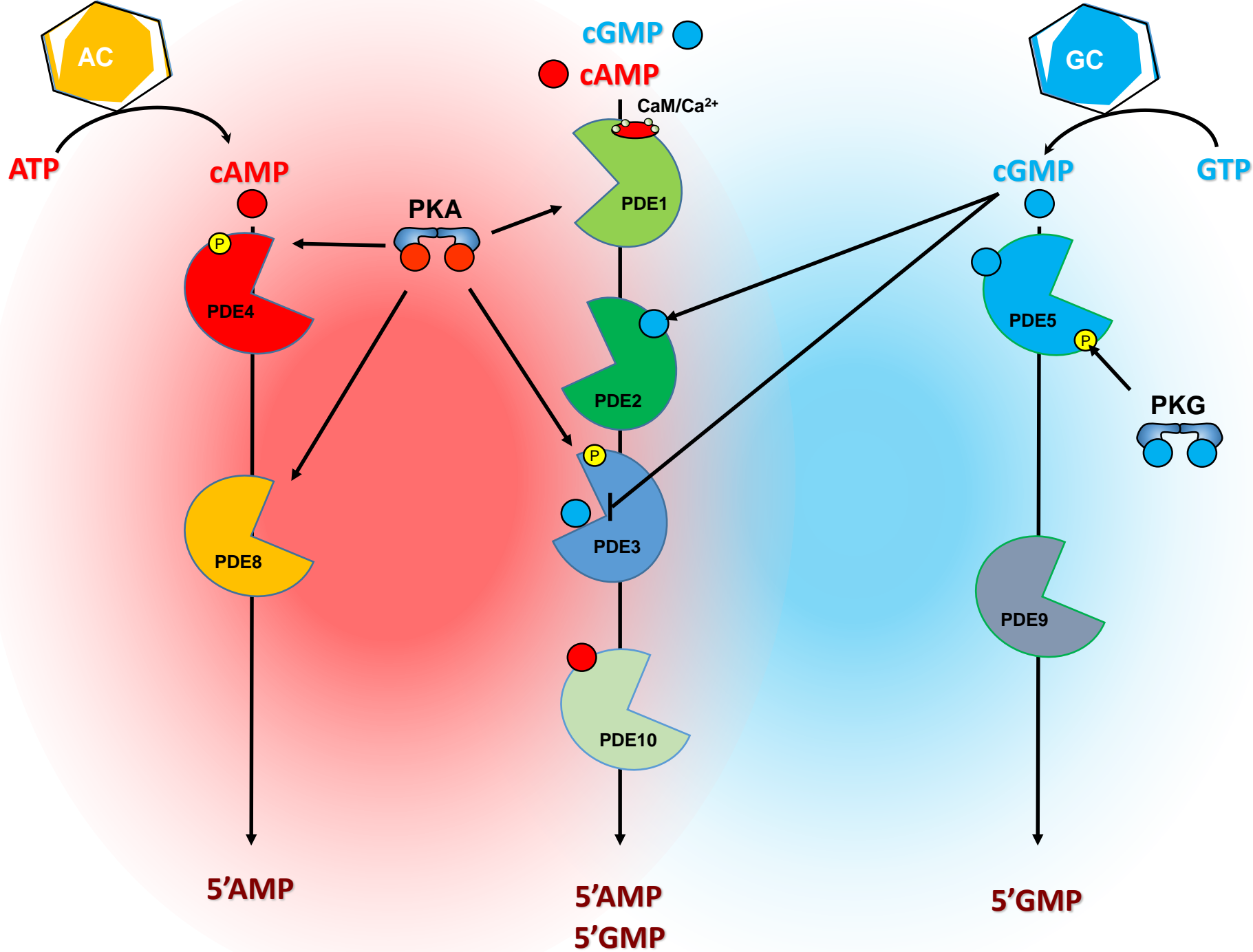


Figure 3

SUPPLEMENTARY MATERIAL

Cyclic nucleotide phosphodiesterases: Therapeutic targets in cardiac hypertrophy and heart failure

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Supplementary Table 1 | **Expression of PDE isoforms in the heart from different animal species.** AC: atrial cells; AM: adult myocytes; AT: atrial tissue; AVM: adult ventricular myocytes; IF: immunofluorescence; IH: immunohistochemistry; IP: immunoprecipitation; LA: left atria; LV: left ventricle; Mito: mitochondria; N/A: not available; NM: neonatal myocytes; NVM: neonatal ventricular myocytes; RT-qPCR: quantitative reverse transcription polymerase chain reaction; RV: right ventricle; RA: right atria; SAN: sinoatrial node; VC: ventricular cells; VT: ventricular tissue; WB: western blot.

PDE Family	PDE Gene	Species	Tissue/cell type	Subcellular compartment	Detection methods	Splice variants
PDE1	PDE1A	Mouse ^{1,3}	Heart ¹ ; LV ² ; Heart tissue ³	N/A	RT-qPCR, WB ^{1,2} ; IH ³	N/A
		Rat ^{1,5}	Heart, NM and AM ¹ ; LV ² ; AT, VT, AC, VC ⁵ ; cardiac fibroblasts and heart tissue ³ , heart ⁴	Nuclear and perinuclear regions ³	RT-qPCR, WB ^{1,5} ; PDE1 cGMP hydrolyzing activity and IH ³	N/A
		Rabbit ^{2,6,7}	Myocardium ² ; LV, RV, RA, LA, septum and SAN cells ⁶ ; SAN cells ⁷	subsarcolemmal region of SAN cells ⁶	RT-qPCR, WB ^{2,6} ; RT-qPCR ⁷	N/A
		Dog ²	Myocardium ²	N/A	RT-qPCR, WB ²	N/A
	PDE1B	Mouse ¹	Heart ¹	N/A	Low mRNA levels ¹	N/A
		Rat ^{1,5}	Heart and AVM ¹ ; AT, VT, VC, VT ⁵	N/A	Low mRNA levels ¹ RT-qPCR, WB (higher in AC) ⁵	N/A
		Rabbit ^{2,6,7}	Myocardium ² ; LV, RA and SAN cells ⁶ ; SAN cells and ventricular myocardium ⁷	N/A	Low mRNA and protein expression ² ; mRNA levels ^{6,7}	N/A
		Guinea Pig ⁸	Isolated ventricular cardiomyocyte homogenates ⁸	N/A	PCR product on gel ⁸	N/A
	PDE1C	Dog ^{2,9}	Myocardium ² ; LV, RV ⁹	N/A	RT-qPCR and WB ² ; WB ⁹	PDE1C1 (microsomal and soluble fraction) ¹⁰ , PDE1C3 ¹⁰
		Rabbit ^{2,6,7}	Myocardium ² ; LV, RA and SAN cells ⁶ , SAN cells and ventricular myocardium ⁷	N/A	RT-qPCR and WB ² ; RT-qPCR ^{6,7}	N/A
		Mouse ^{1,11}	Heart ¹ AVM ¹¹	N/A	RT-qPCR and a Low protein expression ¹ , WB ¹¹	N/A

		Rat ^{1, 5, 12}	Heart ¹² , AVM ¹ ; AT, VT, AC, VC ⁵	N/A	RT-qPCR ¹² , Low mRNA signal in AVM ¹ ; WB ⁵	N/A
		Guinea Pig ⁸	Ventricular cardiomyocyte homogenates ⁸	N/A	PCR product on gel ⁸	N/A
PDE2	PDE2A	Sheep	Ventricular tissue ¹⁴ ; Posterior LV myocardium ¹³	Nucleus ¹⁴	WB; cAMP and cGMP hydrolytic activity ¹⁴	N/A
		Rat ^{4, 5, 8, 15-18}	Myocardium tissue and isolated cardiomyocytes ¹⁸ ; Heart Homogenate and total isolated mitochondria ¹⁵ ; AT, VT, AC, VC ⁵ ; Ventricular cardiomyocyte homogenates ⁸ ; Isolated NM ¹⁷ ; primary rat NVM ¹⁶	Subsarcolemmal and interfibrillar mitochondria, more specifically mitoplast (in the inner membrane and the matrix) ¹⁵ ; membrane associated fraction (plasma membrane in the cell-to-cell junction and at the Z line) ¹⁷ ; mitochondria, plasma membrane and cytosol ¹⁶	RT-qPCR ¹⁸ ; WB and cAMP hydrolytic activity ¹⁵ ; RT-qPCR and WB ^{4, 5} ; PCR product on gel and cGMP hydrolytic activity ⁸ ; IF ¹⁷ ; Fluorescent labeling, protein expression, immunogold labeling ¹⁶	N/A
		Mouse ^{15, 19, 20}	Myocardial subsarcolemmal mitochondria ¹⁵ ; Whole heart homogenates ²¹ ; Ventricular myocardium ²⁰	Plasma membrane, subsarcolemmal mitochondria ¹⁵	WB ^{15, 19} ; RT-qPCR and WB ²⁰	PDE2A2 ¹⁸ (membrane associated fraction ¹⁷); PDE2A1 (cytosol), PDE2A2 (outer and inner mitochondrial membranes), PDE2A3 (plasma membrane) ¹⁶
		Guinea Pig ⁸	Isolated ventricular cardiomyocyte homogenates ⁸	N/A	PCR product on gel and cGMP hydrolytic activity ⁸	PDE2A3 (plasma membrane, subsarcolemmal mitochondria) ¹⁵
		Frog ²²	Atrial fibers ²² ; ventricles ²²	Cytosol ²²	DEA chromatography, cAMP and cGMP hydrolytic activity ²²	N/A

PDE3	PDE3A	Mouse ²³⁻²⁵	Cardiac tissue ²³⁻²⁵	Sarcoplasmic reticulum with SERCA2/PLB/AKAP18 ²⁴ ; Sarcoplasmic reticulum ²⁵	WB ²³ ; WB, cAMP hydrolyzing activity and IF ²⁴ ; Immunogold labeling ²⁵	PDE3A-94 and PDE3A-118 (derived from PDE3A2 mRNA, microsomal and cytosolic), PDE3A-136 (derived from PDE3A1 mRNA, microsomal) ^{26, 27} ; PDE3A1 (membrane associated, SR with SERCA2/PLB/AKAP18 after phosphorylation by PKA), PDE3A2 (cytosolic fraction) and PDE3A3 (cytosolic fraction) ²⁸
		Rat ^{4, 5, 15, 29, 30}	Rat AVM ³⁰ ; Heart ⁴ ; Heart and Mito ¹⁵ ; AT, VT, AC, VC ⁵ ; Heart tissue ²⁹	Mitochondria ¹⁵ ; trabecular staining suggesting internal membrane localization ³¹	Activity (after IP) ³⁰ ; WB and cAMP hydrolytic activity ¹⁵ ; RT-qPCR and WB ^{4, 5} ; RT-qPCR, WB and cAMP hydrolyzing activity ²⁹	N/A
		Guinea-pig ³²	LV and RV ³²	Cytosolic and nuclear membrane ³²	cAMP hydrolyzing activity ³²	N/A
		Frog ²²	Atrial fibers ²² ; ventricles ²²	Cytosol ²²	DEA chromatography, cAMP hydrolytic activity ²²	N/A
		Rabbit ^{7, 33}	LV ³³ ; SAN cells and ventricular myocardium ⁷	Microsomal and cytosolic fraction ³³ ; In the SAN, colocalization at the Z line with SERCA2, PLB, α -actinin and PDE4D and colocalization under the sarcolemma with PDE4B ⁷	Northern blot (mRNA) and cAMP hydrolyzing activity ³³ ; RT-qPCR, WB and immunostaining ⁷	N/A
		Pig ³⁴	RV and adult pig RV myocytes ³⁴	N/A	WB and cAMP hydrolyzing activity ³⁴	N/A
		Sheep ^{13, 14}	Posterior LV myocardium ¹³ ; Ventricles ¹⁴	Nuclear envelope ¹⁴	WB ¹³ ; cAMP hydrolyzing activity ¹⁴	N/A
		Canine ^{9, 33}	LV ³³ and RV ⁹	Microsomal and cytosolic fraction ³³	Northern blot (mRNA) and cAMP hydrolyzing activity ³³ ; WB ⁹	N/A

	PDE3B	Mouse ^{25, 35}	Heart tissue ^{25, 35}	Associated to PI3K ³⁵ ; Co-localization with caveolin-3 on T-tubule membranes, along the Z-line and within the I-band (corbular sarcoplasmic reticulum of the I-band) ²⁵	WB and cAMP-dependent PDE activity ³⁵ ; Immunogold labeling ²⁵	PDE3B1 ¹⁸
		Rat ^{5, 18, 29}	Myocardium tissue and isolated cardiomyocytes ¹⁸ ; AT, VT, AC, VC ⁵ ; Heart tissue ²⁹	N/A	RT-qPCR ¹⁸ ; RT-qPCR and WB ⁵ ; RT-qPCR ²⁹	PDE3B1 ³⁵
PDE4	PDE4A	Mouse ^{36, 37}	Myocardium ³⁶ ; Isolated NM ³⁷	N/A	WB and cAMP hydrolyzing activity ³⁶ ; cAMP hydrolyzing activity ³⁷	N/A
		Rat ^{4, 5, 15, 18, 30, 31, 36}	Myocardium tissue and isolated cardiomyocytes ¹⁸ ; Adult RV cardiomyocytes ³⁰ ; Heart Homogenate and total isolated mitochondria ¹⁵ ; AT, VT, AC, VC ⁵ ; Heart ⁴ ; NM ³¹ ; Myocardium ³⁶	Mitochondria ¹⁵	mRNA levels ¹⁸ ; Activity (after IP) ³⁰ ; WB and cAMP hydrolyzing activity ¹⁵ ; RT-qPCR and WB ^{4, 5} ; cAMP hydrolyzing activity ³¹ ; WB and cAMP hydrolyzing activity ³⁶	N/A
		Rabbit ⁷	SAN cells and ventricular myocardium ⁷	N/A	RT-qPCR, WB ⁷	N/A
		Pig ³⁴	RV and adult pig RV myocytes ³⁴	N/A	WB and cAMP hydrolyzing activity ³⁴	PDE4A5 ¹⁸
	PDE4B	Mouse ³⁶⁻³⁸	Myocardium ³⁶ ; isolated NM ³⁷ ; Adult mouse hearts, AVM ³⁸	Colocalization with CaV1.2 along the T-tubules ³⁸	WB and cAMP hydrolyzing activity ^{36, 38} ; cAMP hydrolyzing activity ³⁷	N/A
		Rat ^{4, 5, 12, 15, 18, 30, 31, 36}	Heart ^{4, 12} ; Myocardium tissue and isolated cardiomyocytes ¹⁸ ; Rat AVM ³⁰ ; Heart Homogenate and total isolated mitochondria ¹⁵ ; AT, VT, AC, VC ⁵ ; NM ³¹ ; Myocardium ³⁶	Mitochondria ¹⁵ ; Cytosolic granular pattern and localization to the sarcomere M line ³¹	RT-qPCR ^{12, 18} ; Activity (after IP) ³⁰ ; WB ¹⁵ ; RT-qPCR and WB ^{4, 5} ; WB and cAMP hydrolyzing activity ³¹ ; WB, cAMP hydrolyzing activity and IF ³⁶	N/A
		Rabbit ⁷	SAN cells and ventricular myocardium ⁷	In the sinoatrial cell, colocalization under the sarcolemma with PDE3A ⁷	RT-qPCR and WB and immunostaining ⁷	PDE4B1, PDE4B2, PDE4B3 ^{12, 18, 31}
		Pig ³⁴	RV and adult pig RV myocytes ³⁴	N/A	WB and cAMP hydrolyzing activity ³⁴	N/A

	PDE4D	Mouse ³⁶⁻⁴⁰	Myocardium ³⁶ ; isolated NM ³⁷ ; heart tissue ⁴⁰ ; mouse heart homogenates ^{38, 39}	$\beta_{1/2}$ -AR compartment ³⁷ ; localization at Z lines along with RyR2 channels ⁴⁰ ; immunoprecipitated with SERCA2a ³⁹	WB and cAMP hydrolyzing activity ^{36, 37} ; WB, cAMP hydrolyzing activity in RyR2 complexes and FRET-PKA ⁴⁰ ; IP followed by WB ³⁹	PDE4D3, 5, 8, 9 ³⁶ ; PDE4D3 (RyR2 colocalization) ⁴⁰
		Guinea Pig ⁸	Isolated ventricular cardiomyocyte homogenates ⁸	N/A	PCR product on gel ⁸	N/A
		Rat ^{4, 5, 12, 15, 18, 31, 36, 41}	Heart ^{4, 12, 41} ; Myocardium tissue and isolated cardiomyocytes ¹⁸ ; AT, VT, AC, VC ⁵ ; NM ³¹ ; Myocardium ³⁶	Mitochondria ¹⁵ ; Cytosolic granular pattern and localization to the sarcomere Z line ³¹	RT-qPCR, WB and enzyme activity ^{12, 41} ; WB ¹⁵ ; WB ⁵ ; WB and cAMP hydrolyzing activity ³⁶ ; RT-qPCR and WB ⁴	N/A
		Rabbit ⁷	SAN cells and ventricular myocardium ⁷	In the sinoatrial cell, colocalization at the Z line with SERCA2, PLB, α -actinin and PDE3A ⁷	RT-qPCR, WB and immunostaining ⁷	PDE4D1, PDE4D2, PDE4D3, PDE4D5, PDE4D7, PDE4D8, PDE4D9 ^{12, 18, 31, 41} ; PDE4D3, 5, 8, 9 ³⁶ ; PDE4D3 is more prominently expressed than PDE4D5 ³¹
		Pig ³⁴	RV and adult pig RV myocytes ³⁴	N/A	WB and cAMP hydrolyzing activity ³⁴	PDE4D3, 5, 8, 9 ³⁶ ; PDE4D3, 4, 5, 8, 9 (PDE4D8 localized at the β_1 -AR and PDE4D5 at β_2 -AR) ³⁷ ; PDE4D3 (associated to RyR2) ⁴⁰
PDE5	PDE5A	Mouse ^{11, 42-45}	Heart and cardiac myofibroblasts ¹¹ ; Adult heart tissue ⁴² and isolated cardiomyocytes ⁴³⁻⁴⁵	Z bands, cytosol ⁴⁴ and nucleus ⁴³	WB ^{11, 42} ; RT-qPCR and WB, IF staining and cGMP hydrolyzing activity ⁴³⁻⁴⁵	PDE5A1, 2, 3 ^{46, 47}
		Guinea Pig ⁸	Isolated ventricular cardiomyocyte homogenates ⁸	N/A	PCR product on gel and cGMP hydrolytic activity ⁸	N/A
		Rat ^{4, 5, 8, 48, 49}	AT, VT, AC, VC ⁵ ; Isolated ventricular cardiomyocyte homogenates ⁸ ; LV ⁴⁸ ; NVM and AVM ⁴⁹ ; Heart ⁴	Z bands ⁴⁸	RT-qPCR and WB ^{4, 5} ; PCR product on gel ⁸ ; WB, cGMP hydrolyzing activity and immunostaining ^{48, 49}	N/A
		Sheep ¹³	Posterior LV myocardium ¹³	N/A	RT-qPCR ¹³	N/A
		Dog ⁵⁰	LV myocardium and isolated cardiomyocytes ⁵⁰	Z bands ⁵⁰	WB, cGMP-hydrolyzing activity, IH ⁵⁰	PDE5A1, PDE5A2 (present from the embryonic state until adult) whereas PDE5A3 is present in embryos but absent in adult mice ⁴³

PDE7	PDE7B	Mouse ^{51, 52}	Whole heart tissue ⁵³	N/A	Northern hybridisation ^{51, 52}	N/A
PDE8	PDE8A	Mouse ⁵⁴	Ventricles and isolated cardiomyocytes ⁵⁴	N/A	RT-qPCR, WB and cAMP-dependent PDE activity ⁵⁴	N/A
	PDE8B	Mouse ⁵⁴	Ventricles and isolated cardiomyocytes ⁵⁴	N/A	RT-qPCR ⁵⁴	N/A
PDE9	PDE9A	Mouse ⁵⁵	Heart tissue and isolated cardiomyocytes ⁵⁵	N/A	PCR product on gel and immunostaining ⁵⁵	N/A
		Rat ⁵⁵	Isolated NM ⁵⁵	Co-localization at the T-tubules with SERCA2a ⁵⁵	WB and immunostaining ⁵⁵	N/A
		Rabbit ⁵⁶	LV ⁵⁶	N/A	WB ⁵⁶	N/A
PDE10	PDE10A	Mouse ⁵⁷	Heart tissue, cardiomyocyte, cardiac fibroblast ⁵⁷	N/A	RT-qPCR, WB and cAMP hydrolyzing activity ⁵⁷	PDE10A2 ⁵⁷

Supplementary Table 2 | **Phosphodiesterase remodeling in animal models of cardiac hypertrophy and HF.** AAC: abdominal aortic constriction; AF: atrial fibrillation; Adr: adrenaline; Ang II: angiotensin II; AS: aortic stenosis; d: days; DCM: dilated cardiomyopathy; HFpEF: Heart failure with a preserved ejection fraction; HFrEF: Heart failure with a reduced ejection fraction; Iso: isoprenaline; MI: myocardial infarction; Nor: noradrenaline; rTOF: repaired tetralogy of Fallot; TAC: transverse aortic constriction.

PDE Family	PDE Gene(s)	Species	Pathology/model	Regulation and method of detection	Functional consequences	References
PDE1	PDE1A,C	Mouse	Ang II infusion	↑ (RT-qPCR and WB)	Cardiac hypertrophy and fibrosis with an increased expression of pro-fibrotic markers	58
	PDE1A	Mouse, rat	Mouse: Iso infusion (1-2 weeks) post-MI Rat: Ang II infusion (2 weeks)	↑ (RT-qPCR, WB, cGMP hydrolyzing activity and immunostaining)	Fibrosis (collagen deposition and increase in α -SMA expression), PKG and PKA signaling pathway implication	3
	PDE1A	Rat	AAC (1-14 d)	↑PDE1(cAMP and cGMP hydrolyzing activity)	↑ in cGMP and cAMP hydrolyzing activity	59
	PDE1A	Mouse and rat	<i>In vivo</i> : Mouse Iso infusion (7 d), and TAC (3 weeks). Rats: Ang II infusion <i>In vitro</i> : Iso or Phe-treated NRVM; Iso or Ang II treated ARVM	↑ (RT-qPCR and WB)	↓ in cGMP levels, decrease in PKG activity, cardiomyocyte hypertrophy	1
	PDE1A	Rat	Diabetic cardiomyopathy	↑ (RT-qPCR)	N/A	4
	PDE1A	Rat	Ang II infusion (2 weeks)	↑ (cGMP hydrolyzing activity)	N/A	48
	PDE1A, C	Dog	Dog: DCM induced by tachypacing	↓ PDE1C (RT-qPCR)	N/A	2

PDE Family	PDE Gene(s)	Species	Pathology/model	Regulation and method of detection	Functional consequences	References
	PDE1C	Dog	HFpEF induced by bilateral renal wrapping and administration of desoxycorticosterone pivalate	↓ PDE1C in RV not in LV (WB)	N/A	9
PDE2	PDE2A	Mouse	Iso infusion, AAC (6 weeks)	↑ (WB)	PDE2 inhibition increases cGMP and counteracts pathological remodeling induced by Iso infusion and AAC	19
		Rat	AAC (1-14 d)	↑ (cAMP and cGMP hydrolyzing activity)	Increase in cGMP and cAMP hydrolyzing activity	59
		Rat	Rat: Iso infusion	↑ (RT-qPCR, WB, cAMP and cGMP hydrolyzing activity)	Increase in cAMP and cGMP hydrolyzing activity, decreased β-AR responsiveness, calcium transients and sarcomere shortening	60
		Rat	Iso infusion	↑ (cAMP hydrolyzing activity)	↓ chronotropic, inotropic and potency of Nor (+) and Adr (+++), β-AR desensitization	61
		Rat	Diabetic cardiomyopathy	↑ (RT-qPCR)	N/A	4
		Rat	Ang II infusion (2 weeks)	↑ (cGMP hydrolyzing activity)	N/A	48
PDE3	PDE3A	Mouse	TAC (6 weeks)	↑ (WB and cAMP hydrolyzing activity)	Increase in cAMP hydrolyzing activity, in p38 phosphorylation levels and in macrophage/monocyte densities	62
		Mouse, rat	Mouse: TAC Rat: Iso and Ang II treated NRVM	↓ (WB, cAMP hydrolyzing activity)	Cardiomyocyte apoptosis, ↑ CREB phosphorylation, ICER induction, decrease of Bcl-2	23
		Rat	Rat: Ang II infusion (7d) Ang II treated NRVM (12 h)	↓ (WB)	↑ ICER and activation of cAMP/PKA signaling	63

PDE Family	PDE Gene(s)	Species	Pathology/model	Regulation and method of detection	Functional consequences	References
		Rat	AS (5 weeks)	↓ (WB and cAMP hydrolyzing activity)	↓ in subsarcolemmal cAMP response to cilostamide	64
		Rat	Diabetic cardiomyopathy	↑ (RT-qPCR, WB)	N/A	4
		Rat	Dahl	↑ (cAMP-hydrolyzing activity)	N/A	65
		Dog	4/5-week Pacing-induced congestive HF	↓ (mRNA (Northern blot) and cAMP activity)	Decrease in microsomal cAMP PDE3 activity	66
		Dog	3 week and 4/5 weeks pacing-induced HF	↓ (mRNA (Northern blot) and immunoblot)	RV: ↓ PDE3A mRNA and protein; LV: Only at 4/5 week after pacing, ↓ in PDE3A mRNA and protein in the cytosolic fraction	9
		Dog	Dog: HFpEF induced in elderly mongrel dogs via bilateral renal wrapping and administration of deoxycorticosterone pivalate	↑ (WB)	N/A	9
		Pig	rTOF	↓ (WB)	N/A	34
PDE4	PDE4A, PDE4B	Rat	TAC (5 weeks)	↓ (WB and activity)	↓ in β-AR response and subsarcolemmal cAMP levels and a decrease in total cAMP hydrolyzing activity	64
	PDE4A, PDE4B, PDE4D	Rat	Diabetic cardiomyopathy	↑ (RT-qPCR, WB)	N/A	4
	PDE4	Rat	MI	↓ (activity)	N/A	67
	PDE4, PDE4B	Rat	Dahl	↑ (activity, RT-qPCR)	N/A	65
	PDE4, PDE4A, PDE4D	Rat	Ang II infusion (2 weeks)	↑ PDE4 (activity) ↑ PDE4A (WB) ↓ PDE4D (WB, RT-qPCR)	N/A	48

PDE Family	PDE Gene(s)	Species	Pathology/model	Regulation and method of detection	Functional consequences	References
PDE5	PDE5A	Mouse	10-week myocardial infarction mouse model	↑ (WB and IH)	N/A	68
		Rat	Pulmonary arterial hypertensive rat model via intraperitoneal injection of monocrotaline; Ang II infusion (2 weeks)	↑ (RT-qPCR, WB, IH and activity)	PKG decreased activity, increase in cGMP-PDE activity	
		Rat	Diabetic cardiomyopathy	↑ (RT-qPCR)	N/A	4
		Rat	Ang II infusion (2wks)	↑ (activity, WB, Immunostaining)	N/A	48
		Dog	HF induced by 4 week left ventricular-tachycardia pacing	↓ (WB and activity)	Dampening of the β -AR pathway and disruption of localization to the Z bands)	50
PDE9	PDE9A	Mouse	TAC Mouse model	↑ (WB)	Anti-hypertrophic and fibrotic effects	55
		Rabbit	Rabbit model showing cardiac dysfunction as a result of an atherogenic diet	↑ (WB)	No change in cGMP levels but an increase in PKG1 protein levels	56
PDE10	PDE10A	Mouse	8-week TAC mouse model (cardiac tissue as well as isolated myocytes and fibroblasts)	↑ (RT-qPCR, WB and cAMP hydrolyzing activity assay)	N/A	57

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