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Cyclic nucleotide phosphodiesterases and compartmentation in normal and diseased heart

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Abstract

Cyclic nucleotide phosphodiesterases (PDEs) degrade the second messengers cAMP and cGMP, thereby regulating multiple aspects of cardiac function. This highly diverse class of enzymes encoded by twenty-one genes encompasses eleven families which are not only responsible for the termination of cyclic nucleotide signalling, but are also involved in the generation of dynamic microdomains of cAMP and cGMP controlling specific cell functions in response to various neurohormonal stimuli. In myocardium, the PDE3 and PDE4 families are predominant to degrade cAMP and thereby regulate cardiac excitation-contraction coupling. PDE3 inhibitors are positive inotropes and vasodilators in human, but their use is limited to acute heart failure and intermittent claudication. PDE5 is particularly important to degrade cGMP in vascular smooth muscle, and PDE5 inhibitors are used to treat erectile dysfunction and pulmonary hypertension. However, these drugs do not seem efficient in heart failure with preserved ejection fraction. There is experimental evidence that these PDEs as well as other PDE families including PDE1, PDE2 and PDE9 may play important roles in cardiac diseases such as hypertrophy and heart failure. After a brief presentation of the cyclic nucleotide pathways in cardiac cells and the major characteristics of the PDE superfamily, this chapter will present their role in cyclic nucleotide compartmentation and the current use of PDE inhibitors in cardiac diseases together with the recent research progresses that could lead to a better exploitation of the therapeutic potential of these enzymes in the future.

Keywords: cAMP, cGMP, cyclic nucleotide phosphodiesterases, heart failure

Abbreviations: AC, adenylyl cyclases; AKAP, A-kinase anchoring protein; ANP, atrial natriuretic peptide; β -ARs, β -adrenergic receptors; BNP, brain natriuretic peptide; CaM, calmodulin; CaMKII, Ca²⁺/Calmodulin-dependent kinase II; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; CN, cyclic nucleotides; CNP, C-type natriuretic peptide; ECC, excitation-contraction coupling; Epac, exchange protein directly activated by cAMP; ERK, extracellular signal-regulated kinase; FRET, Förster resonance energy transfer; GAF, cGMP-stimulated phosphodiesterases, *Anabaena* adenylyl cyclases, *Fhla* transcription factor; GC, guanylyl cyclase; HF, heart failure; ICER, inducible-cAMP early repressor; IR, ischemia-reperfusion; KO, knockout; LTCC, L-type Ca²⁺ channels; mAKAP, muscle AKAP; NO, nitric oxide; NOS, NO synthase; PDE, cyclic nucleotide phosphodiesterase; pGC, particulate guanylyl cyclase; PLB, phospholamban; PKA, cAMP-

dependent protein kinase; PKG, cGMP-dependent protein kinase; PGE, prostaglandin; PI3K γ , phosphoinositide 3-kinase, γ isoform; RyR2, ryanodine receptor type 2; SERCA, sarcoplasmic reticulum Ca²⁺-ATPase; sGC, soluble guanylyl cyclase; SR, sarcoplasmic reticulum; TnI, troponin I.

Introduction

The cyclic nucleotides (CN) cAMP and cGMP participate in the main regulations of cardiac function. They act as second messengers for sympathetic and parasympathetic systems, nitric oxide (NO) and natriuretic peptides. CN may exert beneficial or deleterious effects on the heart, depending on the strength and duration of the stimulation. Acute elevation of CN regulates cardiac excitation-contraction coupling (ECC). However, chronic elevation of cAMP contributes to the development of cardiac hypertrophy and progression to heart failure (HF), while cGMP possesses anti-hypertrophic properties. The amplitude, duration and localization of CN responses are determined by the balance between synthesis of cAMP and cGMP by adenylyl and guanylyl cyclases, respectively, and degradation by cyclic nucleotide phosphodiesterases (PDEs). PDEs represent the main route to rapidly lower CN levels inside the cells, and constitute a highly diverse superfamily of enzymes. Different enzymatic properties and localization of multiple PDE isoforms within the cell participate in CN compartmentation, which is critical to determine specific physiological responses (Conti et al. 2014; Steinberg and Brunton 2001). In addition, modification in the expression and activity of specific PDEs are observed in several cardiovascular diseases. Thus, the members of the PDE superfamily are well placed to be the targets for pharmacological interventions in cardiovascular diseases. This is actually the case for a few of them, with PDE inhibitors being approved for the treatment of acute heart failure, erectile dysfunction, pulmonary hypertension, and intermittent claudication. In the following, we will present an overview of the roles of PDEs in cardiac muscle, the current indication of PDE inhibitors in heart diseases and the recent research advances holding promises for future therapeutic developments in cardiovascular diseases.

CN signaling in cardiac myocytes

In response to activation of G_s -coupled receptors, cAMP is produced by transmembrane adenylyl cyclases, which constitute the main source of cAMP in cardiac cells. Two types of guanylyl cyclases (GC) produce cGMP, the soluble GC (sGC) which is activated by NO, and particulate GCs (pGC) which constitute the receptors for natriuretic peptides (ANP, BNP and CNP). Once synthesized, CN exert their effects by acting through a limited number of cellular effectors: for cAMP, these include the cAMP-dependent protein kinase (PKA), cyclic nucleotide-gated ion channels (CNG), the exchange proteins directly activated by cAMP

(Epac), and the recently discovered popeye-domain containing proteins (Schindler and Brand 2016). Similarly to cAMP, cGMP may exert its effects through activation of cGMP-dependent protein kinase (PKG) or CNG channels. Both nucleotides also bind directly to PDEs, not only at the catalytic site but also at allosteric sites, thereby modulating their activity.

During the fight or flight response, epinephrine and norepinephrine bind to β -adrenoceptors (β -ARs) in cardiomyocytes, leading to cAMP elevation and PKA activation. PKA phosphorylation of sarcolemmal L-type Ca^{2+} channels (LTCC), ryanodine receptors type 2 (RyR2), phospholamban (PLB, which controls the activity of the Ca^{2+} -ATPase from the sarcoplasmic reticulum, SERCA2) and troponin I (TnI) enhances the amplitude and kinetics of Ca^{2+} transients in cardiomyocytes (Figure 1), underlying the classical positive inotropic and lusitropic effects of acute sympathetic stimulation. However, sustained stimulation of β -ARs, as occurs during hypertension or chronic heart diseases, is detrimental to the heart as it favors maladaptive hypertrophic remodeling, apoptosis and arrhythmias. Along with PKA, Epac is activated by cAMP and may play an important role in this context. Epac activation triggers a signaling pathway involving the phosphatase calcineurin and Ca^{2+} /Calmodulin-dependent kinase II (CaMKII) to stimulate hypertrophic growth (Lezoualc'h et al. 2016). CaMKII activation, which can also result from PKA-dependent increases in Ca^{2+} , also phosphorylates RyR2 and promotes a pro-arrhythmogenic sarcoplasmic reticulum (SR) Ca^{2+} leak which may ultimately lead to chamber dilatation and HF (Ruiz-Hurtado et al. 2012) (Figure 1).

In the heart, cGMP is often viewed as the mirror of cAMP, opposing its effects on cardiac function. Indeed, cGMP can exert negative inotropic effects via PKG-mediated inhibition of the L-type Ca^{2+} current (Méry et al. 1991; Yang et al. 2007) and phosphorylation of TnI to decrease myofilament sensitivity to Ca^{2+} (Layland et al. 2005). In addition, cGMP can modulate cAMP levels through regulation of distinct PDEs (see below). One proposed mechanism by which cGMP-PKG signaling exerts its antihypertrophic action is by inhibiting the calcineurin pathway (Tsai and Kass 2009) (Figure 1).

Overview of the PDE superfamily

Eleven PDE families that differ in their primary structure, catalytic properties, affinities for cAMP and/or cGMP, as well as in their mechanisms of regulation are known in mammals (Figure 2). Most PDE families are encoded by several genes, which together generate close to 100 different PDE isoforms by the use of different translation initiation sites and alternative

mRNA splicing. Specific isoforms are designated according to a common nomenclature: PDE is followed by a family number (1-11), a capital letter indicating the gene (A, B, C or D) and a final number corresponding to the splice variant. Some PDE families selectively hydrolyze cAMP (PDE4, 7, 8), while other are specific for cGMP (PDE5, 6, 9). A third category, so called dual PDEs, hydrolyzes both cAMP and cGMP (PDE1, 2, 3, 10, 11).

PDEs share a conserved catalytic domain (C-domain) showing approximately 25–52% AA sequence identity, but differ markedly in their regulatory N-domain (Figure 2). N-domains contain diverse elements involved in enzyme dimerization, binding of regulatory small molecules, phosphorylation and localization. They are characteristic for each family and their variants. For instance, the unique distinguishing feature of the PDE1 family is the existence of two binding sites for the Ca^{2+} -binding protein calmodulin (CaM) in the N-domain which are responsible for enzyme stimulation by Ca^{2+} . Other important domains found in several PDE families (PDE2, PDE5, PDE6, PDE10 and PDE11) are the so-called GAF domains (this acronym is based on the first letters of the three proteins in which they were first identified: G: cGMP-stimulated phosphodiesterases; A: Anabaena adenylyl cyclase; F: FhlA transcription factor) which are involved in enzyme dimerization and allosteric regulation by cyclic nucleotides. In particular, in PDE2 and PDE5, cGMP-binding to their respective GAF domain stimulates enzymatic activity (Martins et al. 1982; Rybalkin et al. 2003). In contrast to PDE2, PDE3 is inhibited by cGMP, by direct competition at the catalytic site. The N-domains of various PDEs contain phosphorylation sites for distinct kinases which modulate enzymatic activity. For instance, long isoforms of PDE4 are phosphorylated by PKA, leading to an increase in cAMP-hydrolytic activity (Sette and Conti 1996), whereas PKG phosphorylation of PDE5 increases cGMP-hydrolytic activity (Francis et al. 2011). Long isoforms of PDE4D have been largely studied in that respect and were shown to be phosphorylated by multiple other kinases, including in particular ERK2, ERK5 and CaMKII (Mika et al. 2015). N-domains are also important for intracellular localization, through specific regions that provide membrane association or protein-protein interaction. PDEs can associate with multiple protein partners including scaffold proteins such as A-kinase anchoring proteins (AKAPs) or β -arrestin strategically located within the cells. Recently, an interaction between PDE8 and the regulatory subunit $\text{RI}\alpha$ of PKA was described which brings together regions spanning the phosphodiesterase active site and cAMP-binding sites of $\text{RI}\alpha$ to facilitate cAMP hydrolysis (Krishnamurthy et al. 2014). More detailed presentations of PDEs including their structure,

regulation, physiological roles and pharmacology are available in several recent reviews (Conti and Beavo 2007; Francis et al. 2011; Keravis and Lugnier 2012; Maurice et al. 2014).

Role of PDEs in cyclic nucleotide compartmentation

The notion of compartmentation arose from studies of cAMP signalling in the heart. Almost 40 years ago, Corbin and co-workers provided evidence that the two subtypes of PKA, designated type I and type II, are differentially distributed in the heart: while PKA type I was found mostly in the soluble fraction, PKA type II was predominant in the particulate fraction (Corbin et al. 1977). It was shown subsequently by Brunton and colleagues that the classical positive inotropic effect of β -AR stimulation was accompanied by an increase in cAMP and PKA in both fractions, whereas prostaglandin E₁ (PGE₁) stimulation increased cAMP and activated PKA only in the soluble fraction and was devoid of a positive inotropic effect (Buxton and Brunton 1983; Hayes et al. 1980). Two main conclusions were drawn from these experiments; the first was that cAMP generated by a given receptor does not activate all possible PKA molecules in the cell, and the second that active PKA cannot phosphorylate all possible substrates (Steinberg and Brunton 2001). The discovery of AKAPs as scaffold proteins able to direct PKA to specific subcellular compartments provided a mean to spatially restrict PKA activity (Dodge-Kafka et al. 2006; Wong and Scott 2004). A large number of PKA type II-specific AKAPs were shown to be associated with critical components of β -AR signalling, ECC or hypertrophic signalling in heart (for review see (Diviani et al. 2011)). The role of AKAPs in defining the range of PKA action is further supported by recent provocative experiments in HEK293 cells suggesting that when PKA is anchored to an AKAP, the catalytic subunits of the kinase may not dissociate from the regulatory subunits upon hormonal stimulation (Smith et al. 2013). Yet, these sophisticated assemblies of cAMP signalling components would not be sufficient to ensure specificity if cAMP can diffuse uniformly and indistinctly activate all PKAs present in a given cell. The observation that a local β -AR stimulation is much more efficient at stimulating local than remote Ca²⁺ channels in frog cardiomyocytes provided evidence that cAMP diffusion must be hindered. Importantly, global PDE inhibition with IBMX increased β -AR stimulation of the remote Ca²⁺ channels, implying that PDEs can act as a diffusion barrier preventing cAMP diffusion (Jurevicius and Fischmeister 1996). More direct evidence of the existence of cAMP gradients and extended cAMP spreading upon PDE inhibition were subsequently obtained by expressing cyclic nucleotide gated channels or Förster resonance energy transfer (FRET)

biosensors to measure cAMP in real time in mammalian cardiomyocytes (Leroy et al. 2008; Molina et al. 2014; Mongillo et al. 2004; Nikolaev et al. 2006; Nikolaev et al. 2010; Zaccolo and Pozzan 2002). Based on experiments performed in HEK293 cells, it was proposed that differentially localized PDEs may function as local sinks, that drain cAMP concentration in defined domains by locally degrading the second messenger (Terrin et al. 2006). The interaction of PDE4D isoforms with several AKAPs facilitating PKA-mediated activation of the PDE by PKA also suggest that cAMP can be controlled locally, at the level of one macromolecular complex (Dodge et al. 2001; Terrenoire et al. 2009). Additional evidence that PDEs control local cAMP signals in cardiomyocytes come from studies showing that the contribution of the different PDEs to cAMP degradation depends on the type of G_s-coupled receptors generating the signal (Nikolaev et al. 2006; Rochais et al. 2006; Xiang et al. 2005). These studies highlighted the predominant role of the PDE4 family in this control, and in neonatal cardiac myocytes, distinct PDE4 variants were shown to associate with and regulate the cAMP signals generated by β_1 -ARs *versus* β_2 -ARs (Baillie et al. 2003; De Arcangelis et al. 2009; Mika et al. 2014; Richter et al. 2008; Richter et al. 2013). The use of cAMP biosensors specifically targeted to distinct subcellular compartments also suggests local cAMP degradation by distinct PDEs. For instance, targeting of FRET-based cAMP biosensors to the plasma membrane and the cytosol indicates a spatially restricted function of a specific PDE4 variant (PDE4B) to regulation of subsarcolemmal cAMP generated by β_1 -ARs in neonatal cardiomyocytes (Mika et al. 2014). Moreover, cAMP biosensors targeted to PKA type I and II compartments are differentially activated by distinct receptors and PDE inhibitors: while cell stimulation with PGE₁ or PDE2 inhibition preferentially increase cAMP in the PKA type I compartment, stimulation with Iso or PDE4 inhibition preferentially increase cAMP in the PKA type II compartment (Di Benedetto et al. 2008). Activation of PKA type II correlates with phosphorylation of SR proteins such as PLB by Iso, whereas PGE₁/E₂ fails to phosphorylate PLB (Di Benedetto et al. 2008; Liu et al. 2012). The use of a FRET-based PKA biosensor (AKAR) localized to the SR confirmed a lack of PKA activation in the SR upon PGE₂ stimulation, but also showed that PGE₂ is able to blunt β -AR inotropic response by activating PDE4D and preventing cAMP generated by β -ARs at the membrane to diffuse and activate PKA at the SR (Liu et al. 2012). Inhibition of PDE4 or ablation of the PDE4D gene were also shown to enhance PKA activation in the nucleus following a short stimulation of β -ARs, suggesting that PDE4D also impedes the diffusion of cAMP from the plasma membrane to the nucleus (Haj Slimane et al. 2014).

While the works related above argue for a critical role of PDEs in cAMP compartmentation, a number of modelling studies suggest that PDEs are not sufficient to establish cAMP gradients if cAMP diffusion in the cell cytoplasm is as fast as in water (Feinstein et al. 2012; Iancu et al. 2007; Rich et al. 2001; Saucerman et al. 2014; Saucerman et al. 2006). Indeed, given their *in vitro* kinetics and estimated concentration in cells, PDEs may not be able to metabolize cAMP fast enough to avoid its replacement by diffusion from surrounding areas. Yet, at least for PDE2, there is convincing evidence that upon activation of the enzyme by cGMP, hydrolysis of cAMP is fast enough to balance the hormonal stimulation of cAMP synthesis (Fischmeister and Hartzell 1987; Hartzell and Fischmeister 1986; Nikolaev et al. 2005). Also, it should be kept in mind that PDE activity can be enhanced during hormonal stimulation (Conti et al. 2014). Whereas several studies indicated fast diffusion rates for cAMP in neurons (Bacskai et al. 1993; Chen et al. 1999; Nikolaev et al. 2004), recent investigations suggest that cAMP diffusion is markedly slower in adult cardiomyocytes (Agarwal et al. 2016; Richards et al. 2016). The reasons for this are not entirely clear, but the above mentioned recent studies propose an important role of the mitochondrial network, acting either as a physical barrier (Richards et al. 2016) or as a support for anchored PKA acting as a cAMP buffer system (Agarwal et al. 2016).

PDEs and cyclic nucleotide compartmentation in heart failure

In the 1970s and 1980s, PDE3 inhibitors were discovered to exhibit cardiotoxic, inotropic, bronchodilatory and vasodilatory activities in several species, and were initially developed as cardiotoxic agents to replace or add to cardiac glycosides in the treatment of HF (Movsesian et al. 2011). However, despite beneficial hemodynamic effects on the short term, chronic use of PDE3 inhibitors were associated with increased cardiac arrhythmias and sudden death (Packer et al. 1991). Thus, the use of PDE3 inhibitors is now limited to acute decompensated HF. Nevertheless, PDE3 inhibitors are targeting several functionally distinct isoforms which are co-expressed in the heart, raising the hope that more selective targeting might provide some benefits. PDE3 is encoded by two genes, PDE3A and PDE3B. Evidence from global PDE3A and PDE3B knockout (KO) mice indicate that PDE3A but not PDE3B is responsible for the inotropic and chronotropic effects of PDE3 inhibitors (Sun et al. 2007). Three isoforms of PDE3A are expressed in cardiomyocytes, which differ only in their N-terminal domain, giving rise to different intracellular localization (Wechsler et al. 2002). In mice and humans, PDE3A1 controls PLB-SERCA2 activity and Ca^{2+} re-uptake in the SR (Ahmad et al. 2015; Beca et al. 2013) (Figure 1). Because dephosphorylated PLB and depressed SERCA2 activity

are a hallmark of HF, PDE3 inhibitors targeting specifically the PDE3A1 associated with PLB-SERCA2 may improve contractile performance and provide therapy for HF (Movsesian 2015). However, currently available PDE3 inhibitors have little selectivity for PDE3A *versus* PDE3B isoforms, whose catalytic domains are similar, and no selectivity for individual PDE3A isoforms, whose catalytic domains are identical. Phosphorylation of PDE3A1 was recently shown to regulate its interaction with SERCA2 (Ahmad et al. 2015). Targeting this mechanism may offer an alternative to selectively enhance contractility without the harmful effects of global inhibition of PDE3 activity.

The second major PDE involved in cAMP hydrolysis in the heart is the cAMP-specific PDE4. The PDE4 family is encoded by 4 genes (PDE4A-D). Most of our knowledge on the role of individual PDE4 subtypes in the heart is limited to PDE4D. Deletion of this gene in mice leads to PKA hyperphosphorylation of RyR2, increased sensitivity to exercise-induced arrhythmias and a late onset dilated cardiomyopathy (Lehnart et al. 2005). PDE4D isoforms are localized in multiple compartments of the cardiomyocyte. For instance, PDE4D3 is localized at the perinuclear region, where it is part of a macromolecular complex organized by the scaffold protein mAKAP and comprising Epac1 and the kinase ERK5 to regulate cardiomyocyte hypertrophy (Dodge-Kafka et al. 2005). This isoform is also present at the sarcolemma, where it associates through another AKAP with slowly-activating delayed rectifier potassium channels controlling cardiac repolarization (Terrenoire et al. 2009) and at myofilaments, in association with another scaffold protein, myomegaline (Verde et al. 2001). In addition, as indicated above, distinct PDE4D isoforms were shown to interact with β_1 -ARs and β_2 -ARs, either directly or indirectly through β -arrestin, and to shape specific physiological or pathophysiological responses (Baillie et al. 2003; Berthouze-Duquesnes et al. 2013; De Arcangelis et al. 2009; Richter et al. 2008; Richter et al. 2013). Finally, similarly to PDE3A, PDE4D also associates with the PLB/SERCA2 complex and regulates the SERCA pump activity in the mouse heart (Beca et al. 2011) (Figure 1).

A role for PDE4B in the heart emerged recently when it was identified as an integral component of the LTCC complex and the main PDE regulating the LTCC current during β -AR stimulation (Figure 1). PDE4B KO mice, as PDE4D KO, have an increased susceptibility to ventricular arrhythmias during catecholamine stimulation which may be due to enhanced Ca^{2+} influx through LTCC (Leroy et al. 2011). Although RyR2 phosphorylation by PKA did not seem to be affected in adult hearts from PDE4B KO mice (Leroy et al. 2011), a recent study indicates that it was increased in neonatal myocytes lacking PDE4B (Mika et al. 2014)

suggesting that altered RyR2 regulation may also contribute to this arrhythmic phenotype. In a recent study in rat ventricular myocytes, we showed that under β -AR stimulation, inhibition of PDE4 (as well as inhibition of PDE3) exerted inotropic effects via PKA but led to spontaneous diastolic Ca^{2+} waves via both PKA and CaMKII, suggesting the potential use of CaMKII inhibitors as adjuncts to PDE inhibition to limit their pro-arrhythmic effects (Bobin et al. 2016).

As stated above, phosphorylation of certain PDE3 and PDE4 isoforms by PKA activates these enzymes, and this constitutes a powerful negative feedback for cAMP signals in cardiomyocytes (Leroy et al. 2008; Rochais et al. 2004). This regulation has been shown to be facilitated by spatial proximity of PKA and PDEs assembled by the perinuclear mAKAP (Dodge-Kafka et al. 2005) or by PI3K γ , which in addition to its lipid kinase function also acts as an AKAP facilitating phosphorylation of PDE3B, PDE4A and PDE4B by PKA (Ghigo et al. 2012).

Although these studies underline the critical role of PDE4 in controlling β -AR stimulation in rodents, this family contributes less to the regulation of cardiac contractility in humans, where PDE3 predominates (Molenaar et al. 2013). However, in human atrial strips, inhibition of PDE3, but also of PDE4 potentiate the arrhythmogenic effect of β -AR stimulation, and PDE4 activity tends to decrease in the atria of patients with atrial fibrillation (Molina et al. 2012). A further understanding of the role of PDE4 in humans may also be important for the pro-arrhythmic effect of PDE3 inhibitors since PDE3 inhibitors such as milrinone and enoximone may also inhibit PDE4 in cardiac preparations (Bethke et al. 1992; Shakur et al. 2002).

In cardiac hypertrophy and HF, there are profound modifications in the major components of the cAMP pathway. These include a decreased density of β_1 -ARs, an uncoupling of β_2 -ARs from G_s , an increase in G_i and in the G-protein coupled receptor kinase GRK2, and in certain models, a decrease in adenylyl cyclase activity (Lohse et al. 2003). In a model of pathological hypertrophy induced by pressure overload in rats, we found that the expression and activity of PDE3A, PDE4A and PDE4B were decreased, and this was associated with a blunted regulation of subsarcolemmal cAMP generated by β -ARs by PDE3 and PDE4 (Abi-Gerges et al. 2009). In contrast, in a model of cardiac hypertrophy induced by angiotensin II, an increased PDE4 activity was observed, accompanied by an increase in the 69-kDa-PDE4A isoform and a decrease in expression of 52- and 76-kDa PDE4D isoforms (Mokni et al. 2010). These results suggest that the level of expression of the isoforms of PDE3 and PDE4 is specifically regulated by the type of stimulus used to induce cardiac hypertrophy and the stage

of the disease. Whereas an increase in cAMP-PDE can participate in desensitization of the β -AR pathway, a decrease could represent a compensatory mechanism to restore cAMP levels and inotropism. However, lower PDE activity also alters the degree of cAMP confinement, which could lead to illegitimate or excessive activation of certain pools of PKA or Epac, hence promoting maladaptive remodelling and rhythmic disturbances. This is supported by the results of a recent study showing that the specific PDE4D5 isoform regulates activation of hypertrophic program by Epac1 upon stimulation of β_2 -AR (Berthouze-Duquesnes et al. 2013) receptors.

HF is also accompanied by modifications of the microarchitecture of the myocytes, including that of the T-tubular system, manifested as T-tubule loss or as reorganization depending on the studies (Louch et al. 2010). These alterations in the T-tubular network not only affect the synchrony of Ca^{2+} release (Heinzel et al. 2002), but are also associated with modifications in the distribution of CN signaling components and therefore, CN compartmentation. In a rat model of HF, it was shown that β_2 -ARs redistribute from their normal T-tubular localization to the peripheral sarcolemma, leading to diffusive as opposed to constrained cAMP signals generated by these receptors (Nikolaev et al. 2010). In a more recent study, the local regulation of cAMP by PDEs in the vicinity of SERCA2 was compared in transgenic mice with cardiac-specific expression of a PLB-targeted cAMP biosensor, and modifications induced by transverse aortic constriction were analysed (Sprenger et al. 2015). In agreement with their known localization within the SERCA2 complex (Beca et al. 2013; Beca et al. 2011), both PDE3 and PDE4 were found to regulate cAMP in this microdomain. Interestingly, during hypertrophy and early HF, there was a specific rearrangement of the PDEs regulating this specific cAMP pool, with a decreased contribution of PDE4 and an increased contribution of PDE2 (Sprenger et al. 2015). These results indicate that PDE alterations in cardiac disease include redistribution of PDE variants in discrete microcompartments of cardiomyocytes, as shown for PDE2 and PDE3 at the membrane (Perera et al. 2015).

The dual specific PDE2 represents a minor part of cAMP-hydrolytic activity in the normal heart, but the cAMP-hydrolytic activity of this PDE is stimulated 5 to 30-fold by cGMP, and this was shown to inhibit cardiac LTCC in various species including humans (Fischmeister et al. 2005). Subsequently, measurements with FRET-based sensors in neonatal rat cardiomyocytes showed that by decreasing the level of cAMP, PDE2 counteracts the effects of a β -AR stimulation downstream of β_3 -ARs (Mongillo et al. 2005). In contrast to PDE3 and

PDE4, which expression and activity are generally decreased in pathological hypertrophy and HF (Abi-Gerges et al. 2009; Ding et al. 2005; Osadchii 2007), we found recently that PDE2 is increased in animal models as well as in human HF (Mehel et al. 2013). PDE2 inhibition partially restores β -AR responsiveness in diseased cardiomyocytes, suggesting that PDE2 enhancement in HF constitutes a protective mechanism against excessive β -AR stimulation. This hypothesis was confirmed in a more recent study using a transgenic mouse model with a cardiac-specific overexpression of PDE2 (Vettel et al. 2016). Transgenic increase in PDE2 abundance in mice lowers heart rate, but preserves cardiac output due to greater cardiac force. Increased PDE2 abundance was found to be cardioprotective *in vivo* in acute catecholaminergic stress and after myocardial infarction without compromising contractile performance (Vettel et al. 2016). However, according to another recent study, PDE2 could exert a pro-hypertrophic effect by blunting PKA-mediated phosphorylation of NFAT (Zoccarato et al. 2015). Further studies are needed to fully understand the role of PDE2 in HF (Wagner et al. 2016; Zoccarato et al. 2016).

Similarly to PDE2, PDE1 and PDE5 were reported to be upregulated in pathological hypertrophy and HF (Miller et al. 2009; Pokreisz et al. 2009; Vandeput et al. 2007). Because PDE1 and PDE5 preferentially (PDE1A) or specifically (PDE5) degrade cGMP, their increase in HF can clearly be seen as maladaptive. Accordingly, transgenic mice with cardiac-specific overexpression of PDE5 are predisposed to adverse remodelling after myocardial infarction (Pokreisz et al. 2009), whereas on the contrary, pharmacological inhibition of PDE1 (Miller et al. 2009) or PDE5 (Takimoto et al. 2005) reduces hypertrophy and improves cardiac pressure and volume overload. Numerous animal studies have shown that PDE5 inhibitors protect against ischemia/reperfusion (I/R) injury, doxorubicin cardiotoxicity, ischemic and diabetic cardiomyopathy, and Duchenne muscular dystrophy (Das et al. 2015). However, it remains controversial whether significant levels of PDE5 are expressed in the myocardium, raising the possibility that the beneficial effects of PDE5 inhibitors involve other mechanisms including inhibition of PDE1 (Degen et al. 2015; Lukowski et al. 2014). In patients with systolic HF, sildenafil decreased pulmonary vascular pressure and increased peak oxygen consumption and cardiac index (Lewis et al. 2007). Sildenafil also improved left ventricular diastolic function, cardiac geometry, and clinical status in patients with systolic HF (Guazzi et al. 2011) and improved diabetic cardiomyopathy (Giannetta et al. 2012). However, despite encouraging results in an initial mono-center study (Guazzi et al. 2011), chronic therapy with sildenafil was not associated with clinical benefit in patients with diastolic HF in a larger,

multi-center study (Redfield et al. 2013). Ongoing trials with PDE5 inhibitors include testing for the gender response to tadalafil in left ventricular hypertrophy associated to diabetic cardiomyopathy (NCT01803828).

Two other PDEs were recently proposed to participate in cGMP degradation in the heart. Experiments performed in isolated cardiomyocytes from transgenic mice expressing a FRET-based cGMP biosensor have suggested that PDE3, which is classically known to degrade preferentially cAMP, may also be involved in the control of cGMP levels (Gotz et al. 2014). In addition, the cGMP-specific PDE9 was found to be expressed in rodent and human heart and to be up-regulated in hypertrophy and HF (Lee et al. 2015). PDE9 genetic ablation or pharmacological inhibition appears to protect the heart against pathological remodeling during pressure-overload. Moreover, PDE9 inhibition reverses pre-established heart disease in a NO synthase (NOS) activity independent manner, whereas PDE5 inhibition requires active NOS, which is decreased in HF. This is because PDE9 seems to hydrolyze specifically cGMP generated by natriuretic peptides, whereas PDE5 controls cGMP generated by NO (Castro et al. 2006; Takimoto et al. 2007). We had shown previously that PDE2 is critical to regulate subsarcolemmal cGMP levels in response to pGC activation in adult cardiomyocytes (Castro et al. 2006), thus raising the question of whether PDE2 and PDE9 exert a redundant or distinct regulation of natriuretic peptide signaling.

PDEs as therapeutic targets in ischemia reperfusion injury?

Manipulation of PDE activity may also prove protective in the context of ischemia-reperfusion (IR) injury. Indeed, PDE5 inhibitors were shown to reduce infarct size in rabbits and mice. They also decreased cell death in isolated cardiomyocytes, suggesting that part of this effect is independent of vasodilation. Several mechanisms appear to be involved in these effects, including increased NO synthase expression, cGMP elevation, PKG activation and opening of mitochondrial K_{ATP} and Ca^{2+} -activated K^+ channels (Das et al. 2015). PDE3 inhibitors have also been reported to reduce infarct size when applied before sustained ischemia, thus mimicking the cardioprotection conferred by ischemic preconditioning (Fukasawa et al. 2008; Sanada et al. 2001; Tosaka et al. 2007). A recent study using KO mice for either PDE3A or PDE3B strongly suggests that PDE3B is the isoform mediating the cardioprotective effect of PDE3 inhibitors in this context. Indeed, PDE3B KO mice, but not PDE3A KO mice, were protected against ischemia-reperfusion injury. This protective effect appears to involve cAMP/PKA mediated opening of mitochondrial Ca^{2+} -activated K^+

channels and assembly of ischemia-induced caveolin 3 enriched fractions (Chung et al. 2015). Somehow at odds with the above mentioned cardioprotective effect of PDE3 inhibitors, mice with cardiac-specific overexpression of PDE3A1 were protected during ischemia-reperfusion injury (Oikawa et al. 2013). In addition to regulating SERCA2, PDE3A1 also acts as a negative regulator of cardiomyocyte apoptosis, by controlling the expression of the transcriptional repressor and pro-apoptotic factor, ICER (inducible- cAMP early repressor) (Yan et al. 2007). Inhibition of this mechanism in mice with cardiac-specific overexpression of PDE3A1 was associated with protection during ischemia-reperfusion (Oikawa et al. 2013). Collectively, these studies suggest that PDE3A and PDE3B may play an opposite role during ischemia-reperfusion, which may be linked to their differential localization and the control of discrete cAMP pools in cardiomyocytes (Chung et al. 2015).

Concluding remarks

Soon after the discovery of cAMP and cGMP by Sutherland and colleagues more than 50 years ago, an enzymatic activity that could degrade these second messengers was described (Sutherland and Rall 1958). Since then, the large diversity and complexity of the PDE superfamily has been unveiled, and the critical role of these enzymes in the cardiovascular system demonstrated. After several disappointments in the development of PDE3 and PDE4 inhibitors due to their adverse effects (mortality and emesis, respectively), the success in PDE5 inhibitors to treat erectile dysfunction associated with an increasing understanding of PDE biology has raised new hopes that manipulating PDE activity with greater specificity is possible and should yield therapeutic benefits. However, the targeting of individual PDE isoforms located in distinct subcellular compartments to regulate local cAMP and/or cGMP concentrations and a specific cell function is challenging. Knowledge of the protein partners and of the molecular mechanisms that govern this specific localization allows to envisage the displacement of specific PDE pools by small molecules or peptides that disrupt protein-protein interaction, with potentially fewer adverse effects than global inhibition of an entire PDE family. Recent examples of such strategy include the disruption of PDE4 from heat shock protein 20 (HSP20) in cardiomyocytes, resulting in hyper-phosphorylation of HSP20 and attenuated cardiac hypertrophic growth (Martin et al. 2014; Sin et al. 2011) and the disruption of Epac1-PDE3B and Epac1-PDE4D complexes involved in the control of endothelial cell adhesion, spreading and permeability (Rampersad et al. 2010; Wilson et al. 2011). Another interesting strategy is the development of allosteric inhibitors of PDEs, for

instance targeting the GAF domain of PDE5 to block enzyme activation but not its basal activity (Schultz et al. 2011) or exploiting sequence differences outside the active site to reach isoform selectivity in the case of PDE4B and PDE4D (Burgin et al. 2010; Fox et al. 2014). The allosteric mode of regulation could also allow the discovery of small molecules acting as activators of specific PDEs, which may actually be useful in correcting the detrimental effects of excessive β -AR stimulation observed in HF.

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Conflict of interest

None.

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Figure legends

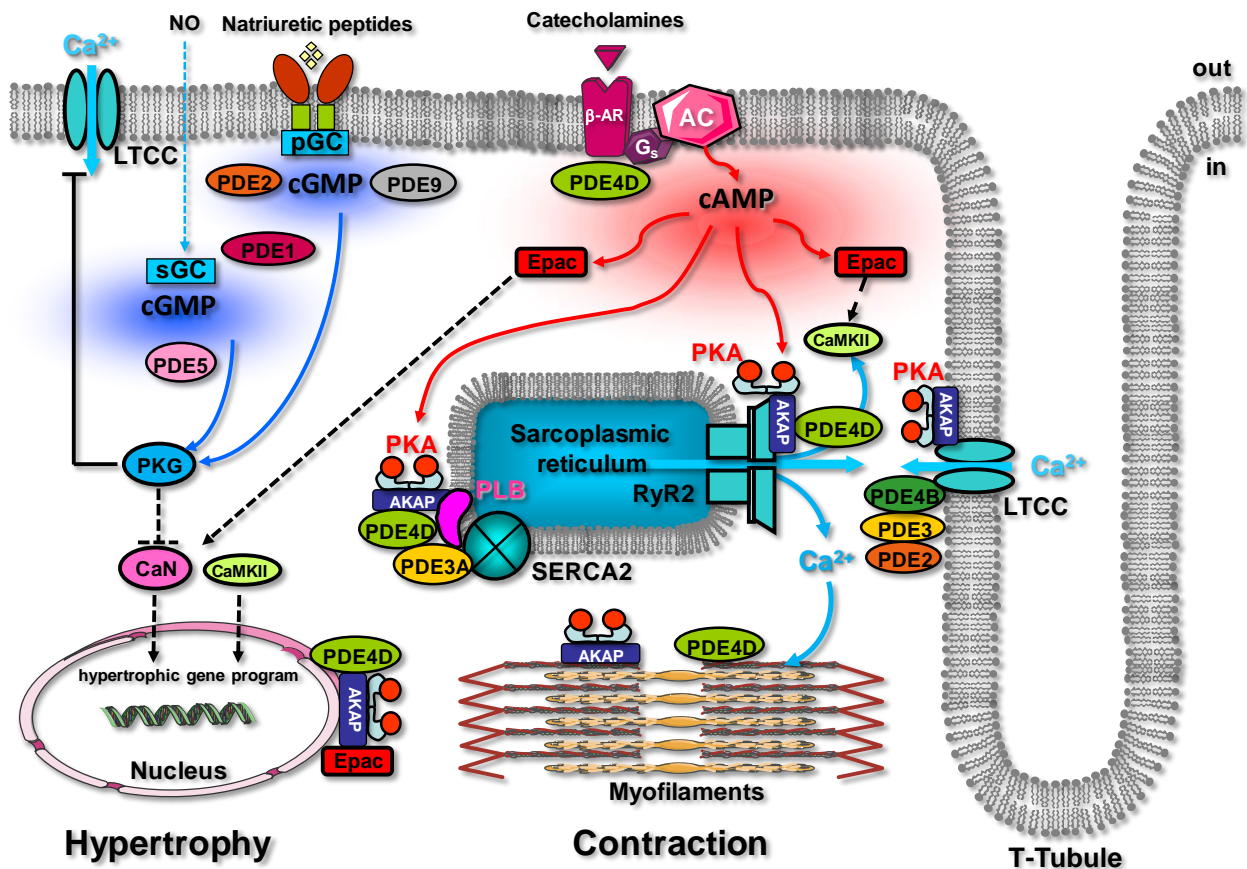


Figure 1: Cyclic nucleotides metabolism in cardiac myocytes.

The major PDEs expressed in cardiac myocytes are indicated together with their subcellular localization in relation to their role in regulating hypertrophic growth and excitation-contraction coupling. For simplicity, the PDE family and – when relevant – the gene are indicated but not the specific splice variants. AC, adenylyl cyclase; AKAP, A-kinase anchoring protein; CaMKII, Ca²⁺-Calmodulin kinase II; CaN, calcineurin; GPCR, G-protein coupled receptor; pGC, particulate guanylyl cyclase; sGC, soluble guanylyl cyclase.

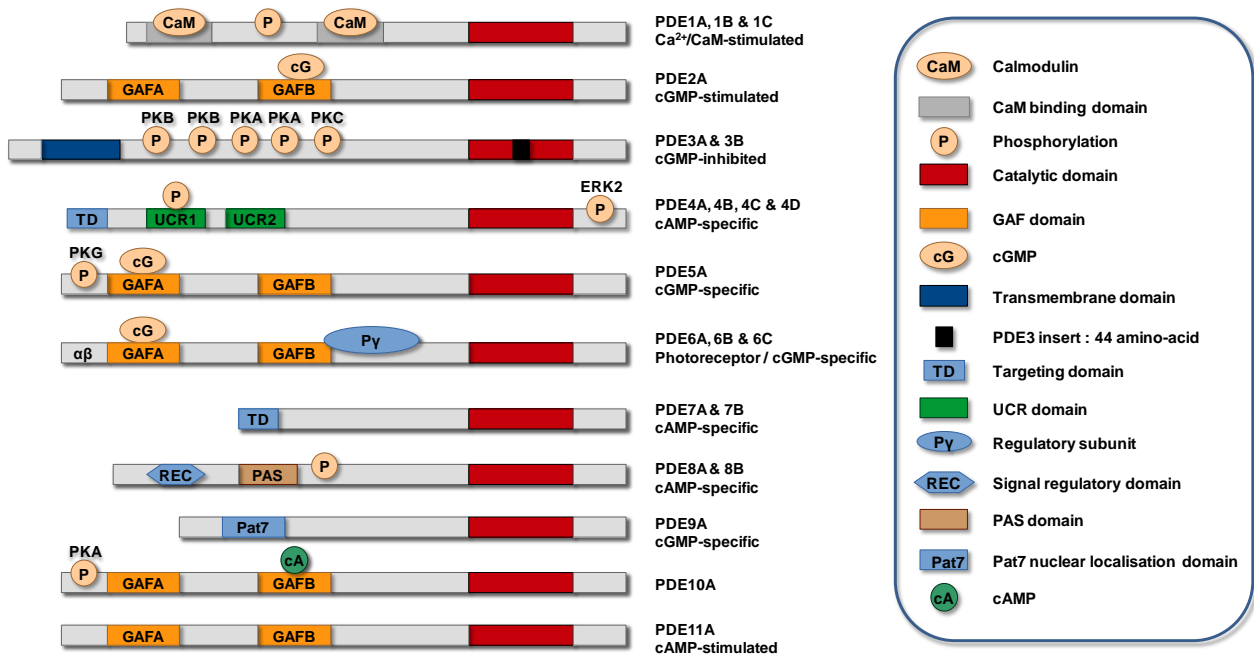


Figure 2: The eleven mammalian PDE families. The conserved catalytic domain (shown in red) is located in the carboxy-terminal portion of the PDEs. The catalytic domain of PDE3 contains a unique 44-amino-acid insert (shown in black). Many of the PDE families contain amino-terminal subdomains (such as GAF domains, transmembrane domains, targeting domains, upstream conserved regions (UCRs), PAS domains and REC domains) and N-terminal hydrophobic regions that are important in subcellular localization, in the incorporation of PDEs into compartmentalized signalosomes, in interactions with signalling molecules and molecular scaffolds, and in the regulation of PDE activity. GAF domains regulate the allosteric binding of cGMP (to PDE2, PDE5, PDE6 and PDE11), the allosteric binding of cAMP (to PDE10) and the regulation of catalytic activity (in PDE2, PDE5 and PDE6). (Modified from ref.(Maurice et al. 2014))