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PDEs create local domains of cAMP signalling

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Abstract

In the light of the knowledge accumulated over the years, it becomes clear that intracellular cAMP is not uniformly distributed within cardiomyocytes and that cAMP compartmentation is required for adequate processing and targeting of the information generated at the membrane. Localized cAMP signals may be generated by interplay between discrete production sites and restricted diffusion within the cytoplasm. In addition to specialized membrane structures that may limit cAMP spreading, degradation of the second messenger by cyclic nucleotide phosphodiesterases (PDEs) appears critical for the formation of dynamic microdomains that confer specificity of the response to various hormones. This review will cover the role of the different cAMP-PDE isoforms in this process.

1. Introduction

The cAMP pathway is one of the most common and versatile signal pathways in eukaryotic cells and is involved in regulation of cellular functions in almost all tissues in mammals [1]. In the heart, the cAMP pathway regulates cardiac contractility, relaxation, automaticity, metabolism and gene expression. It is a major pathway involved in the sympathetic regulation of heart function [2]. In this setting, noradrenaline released from intracardiac nerve terminals activates cardiac β -adrenergic receptors (β -ARs), which trigger in sequence G_s protein activation of adenylyl cyclase (AC), cAMP synthesis and protein kinase A (PKA) activation. PKA then phosphorylates several key proteins involved in excitation-contraction (EC) coupling, such as L-type Ca^{2+} channels (LTCC), phospholamban (PLB), ryanodine receptors (RyR), troponin I (TnI) and myosin binding protein-C (MyBP-C) [3]. Cyclic AMP has also PKA-independent targets in the heart, such as the small G protein exchange factor Epac (Exchange Protein directly Activated by cAMP) [4] involved in hypertrophic growth [5,6] and HCN cyclic nucleotide-gated ion channels underlying the pacemaker current, I_f [7].

There is a large body of data supporting the hypothesis that cAMP signaling in cardiac cells occurs within discrete subcellular compartments (reviewed in [8]). Early evidence for the compartmentation of cAMP signaling was provided by the observation that activation of β -ARs by catecholamines and of prostanoid EP receptors (EP-R) by prostaglandin E_1 (PGE_1) similarly increased cAMP but elicited different effects in isolated perfused hearts [9,10]. A first clue to this paradoxical observation was the identification of two PKA isozymes (PKAI and PKAII) in myocardium, differing by the nature of their

regulatory subunits (called RI and RII) and by their distribution: whereas PKAI was mostly soluble, PKAII was associated to the particulate fraction [9,11]. Whereas β -AR stimulation activated cAMP-dependent protein kinase (PKA) in both soluble and particulate fractions and caused activation of glycogenolytic enzymes and phosphorylation of the myofilament protein troponin I (TnI), EP-R stimulation only increased PKA in the soluble fraction and failed to stimulate glycogen metabolism, contractile activity and TnI phosphorylation [12-15]. Similar results were reproduced in isolated myocytes, thus indicating that the differences observed had a subcellular basis and were not due to the different cell types present in the whole heart [12]. Subsequently, β_1 -ARs and β_2 -ARs were found to elicit qualitatively different responses in isolated cardiac myocytes. In contrast to β_1 -AR stimulation, which leads to the phosphorylation of LTCC, RyR2, PLB, TnI and MyBP-C, in rat ventricular myocytes β_2 -AR signalling appears restricted to the plasma membrane and LTCC regulation, thus inducing a modest inotropic effect without hastening relaxation [16-18]. A similarly localised β_2 -AR signaling occurs in dog ventricular myocytes, although in this case β_2 -AR stimulation still accelerates relaxation [17,19,20]. However, others found that in rat ventricular myocytes, β_2 -AR stimulation enhanced PKA-dependent PLB phosphorylation and accelerated relaxation, although it failed to phosphorylate TnI and phosphorylase *a* [21]. Besides β -ARs and EP-R, a given cardiac myocyte expresses several other receptors coupled to stimulatory G proteins (G_s PCR), for instance glucagon receptors (Glu-R) and glucagon-like peptide-1 receptors (GLP1-R). Activation of these receptors also leads to cAMP elevation, however the contractile and metabolic effects of Glu-R are typically not sustained (tachyphylaxis) [22,23] whereas GLP1-R stimulation exerts a modest negative inotropic effect despite an increase in total cAMP which is comparable to that elicited by a β -AR stimulation [24]. Recently, electrophysiological and imaging approaches that resolve cAMP signals at the subcellular level in intact cardiomyocytes confirmed that β_1 -AR, β_2 -AR, EP-R, Glu-R and GLP1-R generate distinct cAMP signals that differentially regulate PKA targets such as LTCC, PLB, and TnI [25-28]. Using ratiometric fluorescence resonance energy transfer (FRET) sensors for cAMP fused with the dimerization/docking domain of the RI α and the RII β regulatory subunits of PKA, Di Benedetto et al. [27] showed that cAMP generated by isoprenaline (Iso) increases cAMP preferentially in the RII compartment, whereas GLP-1, glucagon and PGE₁ stimulation resulted in larger increases in cAMP in the RI compartment. This was associated with the phosphorylation of PLB, TnI and β_2 -AR by Iso but not by PGE₁. Altogether, these observations indicate that the cell is able to distinguish between different stimuli acting via the same second messenger, and raise the question of the mechanisms involved.

A first element to answer this question is the uneven localization of the components of the cAMP signaling pathway within discrete compartments of the cell. In ventricular cells, invagination of the plasma membrane forms a transverse tubular network whose major function is the synchronization of Ca^{2+} release during excitation-contraction coupling (ECC) [29]. Together with proteins involved in Ca^{2+} movements across the plasma membrane, such as LTCC [30,31], the $\text{Na}^+/\text{Ca}^{2+}$ exchanger [32] and the plasma membrane Ca^{2+} ATPase [33,34], several components of the cAMP pathway were found to be enriched in the T-tubules: G_s proteins [35], AC5/6 [30,35], phosphodiesterase (PDE) type 3 and 4 [36,37], PKA regulatory subunits and anchoring proteins (AKAPs) [38]. So far, G_s PCR localization in cardiac myocytes has proved difficult due to low expression levels of these proteins and insufficient antibody specificity. Using an elegant combination of scanning ion conductance microscopy with cAMP imaging by FRET, Nikolaev *et al.* recently provided evidence that β_2 -ARs are localized in T-tubules in healthy ventricular myocytes, in contrast to β_1 -ARs which are more evenly distributed throughout the plasma membrane [39]. Other microdomains of high interest for cAMP signal transduction are caveolae, where a number of components of the cAMP pathway were shown to be localized [40]. The role of caveolae in signalling compartmentation being the focus of an accompanying review, it will not be developed here.

Within the above described microdomains, cAMP signalling components are organized into multiprotein complexes, an arrangement that increases both efficiency and specificity of the transduction cascade. AKAPs play an essential role in these arrangements. AKAPs form a large family of proteins comprising >50 members whose primary function is to anchor PKA in the vicinity of its substrates, thus ensuring the preferential phosphorylation of a limited number of targets [41]. Importantly, AKAPs not only bind PKA but act as scaffold proteins for other signalling components including phosphatases 1 and 2 [42,43], Epac [44], adenylyl cyclase (AC) type 5/6 [45] and PDE4D [44,46,47]. More details about AKAPs in cardiac signalling can be found in an accompanying review in this issue of *JMCC*.

How is the specificity of the response to the various hormones maintained when they share a small diffusible molecule such as cAMP? Localized cyclic nucleotide signals may be generated by the interplay between discrete production sites and restricted diffusion within the cytoplasm. Restricted diffusion of cAMP may be achieved by several means. A first possibility is that physical barriers are created by specialized membrane structures within the cytoplasm. This was initially proposed to explain the differences in cAMP concentration elicited by PGE_1 at the plasma membrane and in the bulk cytosol of HEK293 cells, although

an experimental proof that this actually occurs is still lacking [48]. However, another important mechanism that limits cAMP diffusion is cAMP buffering by PKA regulatory subunits [39,49] and degradation of the second messenger by PDEs, which appears critical for the formation of dynamic microdomains that confer specificity of the response [2,41].

2. PDEs and cAMP compartmentation

At least 11 PDE families including 20 genes and about 50 isoforms are identified on the basis of primary amino acid (AA) sequence as well as kinetic and regulatory properties [50]. All PDEs harbor a C-terminal catalytic domain of about 270 AA, of which 20-25% are identical in isozymes belonging to different subfamilies. On the other hand, the N-terminal regulatory domain of PDEs has a unique AA sequence in each PDE subfamily, and it contains dimerization domains, binding sites for specific regulators of isozyme activity as well as phosphorylation sites targeted by protein kinases, and membrane-associated domains anchoring PDE isozymes to discrete subcellular compartments [50,51]. Classically, 4 of these 11 PDE families are responsible for cAMP degradation in heart [52]: PDE1, PDE2, PDE3 and PDE4. While PDE1 and PDE2 can hydrolyse both cAMP and cGMP, PDE3 preferentially hydrolyses cAMP and PDE4 is specific for cAMP. PDE1 is activated by Ca^{2+} /calmodulin complex; PDE2 is stimulated by cGMP binding to amino terminal allosteric regulatory sites known as GAF domains; PDE3 has a similar K_m for cAMP and cGMP but a higher V_{max} for the former, making it a cGMP-inhibited cAMP-PDE; and PDE4 is activated by PKA [51]. PDE1 is encoded by 3 genes (*PDE1A-C*), PDE2 by one gene (*PDE2A*), PDE3 by 2 genes (*PDE3A* and *PDE3B*) and PDE4 by 4 genes (*PDE4A-D*). Cardiac myocytes express 2 forms of PDE2A [53], 3 forms of PDE3A [54] and more than 5 forms of PDE4 encoded by *PDE4A*, *PDE4B* and *PDE4D* [55-57]. A fifth PDE, PDE8A, specifically hydrolyzes cAMP and was shown recently to control ECC in mouse cardiac myocytes [58].

Many studies investigated PDE activities in heart homogenates or isolated cardiomyocytes. While the four PDE isoenzymes (PDE1, PDE2, PDE3 and PDE4) are present in mammalian cardiac tissue, their level of expression is highly dependent on the species investigated. In rats and mice, PDE4 represents the major cAMP-PDE, contributing for up to 60% of total cAMP-hydrolytic activity, whereas PDE3 accounts for 20 to 30% [59-62], and PDE2 and PDE1 representing the remaining activity. The prevalence of PDE4 is further exaggerated because PKA phosphorylates and activates the long forms of these enzymes [63,64]. In rat cardiac myocytes, this provides an important negative feedback loop

by which PKA limits excessive cAMP accumulation during β -AR stimulation [46,61,65]. In the heart of other rodents such as rabbits, guinea pigs or hamsters, PDE1 was found to be predominant followed by PDE2 and PDE3, with less activity represented by PDE4 [66-68]. In human heart, PDE4 activity represents only 10% of total PDE activity [62,62,66-68] while other PDE families represent the vast majority of the hydrolytic cAMP activity [62,69] distributed between PDE1, PDE2 and PDE3 [62,69,70].

An early evidence of the contribution of PDEs to intracellular cyclic nucleotide compartmentation was obtained by comparing the effects of the non selective β -AR agonist Iso, or the nonselective PDE inhibitor, 3-isobutyl-1-methylxanthine (IBMX), or the PDE3 inhibitor, milrinone, in guinea pig perfused hearts. Whereas each of these treatments increased intracellular cAMP and produced positive inotropic and lusitropic effects, differences in the phosphorylation pattern of PLB, TnI and MyBP-C by PKA were observed [71]. These results were attributed to a functional cellular compartmentation of cAMP and PKA substrates due to a different expression of PDEs at the membrane and in the cytosol [72]. In canine ventricular myocytes, increase in particulate but not total cAMP correlated with increase in Ca^{2+} transient amplitude and decay kinetics [73]. In response to β -AR stimulation, about 45% of the total cAMP was found in the particulate fraction but this fraction declined to <20% when IBMX was added to Iso, although cAMP production was up to 3-4 fold greater. These results show that cAMP-PDEs reside predominantly in the cytoplasm, where they prevent excessive cAMP accumulation upon β -AR activation. Thus PDEs appear important to maintain the specificity of the β -AR response by limiting the amount of cAMP diffusing from membrane to cytoplasm. Similar results were obtained when studying the impact of PDE inhibition on LTCC current ($\text{I}_{\text{Ca,L}}$) regulation by local application of Iso in frog ventricular myocytes [74]. In the absence of IBMX, application of Iso to half of the cell increased $\text{I}_{\text{Ca,L}}$ half maximally, corresponding to activation of the channels located in the same part of the cell as the β -AR agonist. When IBMX was added with Iso on one half of the cell, the effect of Iso was greatly potentiated because in this condition, LTCCs in the remote part of the cells could be recruited. Thus, these results suggest that PDE activity is important for the definition of local cAMP pools involved in the β -AR stimulation of LTCCs. Subsequent studies using ratiometric FRET biosensors to directly monitor cAMP have shown that the second messenger increases preferentially in discrete microdomains corresponding to the dyad region under β -AR stimulation, and that cAMP diffusion is limited by PDE activity [75,76]. Subsequent studies using recombinant cyclic nucleotide gated channels to measure cAMP generated at the plasma membrane identified specific functional coupling of

individual PDE families to β_1 -AR, β_2 -AR, PGE₁-R and Glu-R as a major mechanism enabling cardiac cells to generate heterogeneous cAMP signals in response to different hormones [61].

The use of selective inhibitors for each PDE family has allowed evaluating the contribution of these enzymes in the compartmentation of cAMP signaling pathways in cardiac myocytes. However, because of the lack of a commercially available selective PDE1 inhibitor, the role of this PDE family in cardiac function has been limited to few studies [70,77].

3. Role of PDE isoforms in cAMP compartmentation

3.1. PDE2 and cAMP compartmentation

In rat and frog cardiomyocytes, PDE2 is found preferentially in microsomal fractions [78,79] in association with functional membrane structures: plasma membrane, sarcoplasmic reticulum (SR), Golgi, nuclear envelope [80]. Although PDE2 activity is relatively small compared to other cardiac PDEs, PDE2 tightly controls the activity of LTCCs in various species including humans [81-86]. The involvement of PDE2 in the cyclic nucleotide compartmentation has been also examined on frog cardiomyocytes [84] where it is the most abundant PDE isozyme [78]. The authors have shown that a stimulation of the soluble guanylyl cyclase leads to a dramatic local decrease in cAMP in the vicinity of LTCCs due to the activation of PDE2, and only a slight decrease in cAMP in the rest of the cell. This phenomenon is explained by the existence of a microdomain between the β -AR, the LTCC and PDE2 [84]. More recently, a study realized on neonatal rat cardiomyocytes using the FRET-based imaging technique has demonstrated that PDE2 restricts cAMP signals, and hence inotropism, generated after a β -AR stimulation by Iso. This effect might involve the β_3 -AR and the NO/cGMP pathway [79]. PDE2 is not only involved in the modulation of subsarcolemmal cAMP concentration, but also controls the concentration of cGMP in that compartment [87,88]. A recent study showed that localized cAMP responses are profoundly modulated by cGMP, under β -AR stimulation [89]. The authors have identified PDE2 and PDE3 as effectors of such modulation and have demonstrated that cGMP alters the local activation of PKA isoforms (PKA-RI and PKA-RII) localized to different subcellular compartments, modifying the phosphorylation of downstream targets and cell contraction. In their model, PDE2 activity is preferentially coupled to PKA-RII compartment and PDE3 to PKA-RI compartment in the presence of catecholamines, and cGMP-mediated activation of

PDE2 and inhibition of PDE3 are responsible for the opposing effects of cGMP on local cAMP signals [89].

3.2. PDE3 and cAMP compartmentation

That PDE3 plays a role in cAMP compartmentation is anticipated because of its intracellular distribution. Furthermore, because PDE3 is inhibited by cGMP, localized production of cGMP participates in shaping cAMP signals produced during β -AR stimulation, leading to restricted increases in cAMP concentration [89]. PDE3 is present in membrane and cytosolic fractions of cardiomyocytes with a species- and tissue-specificity [80,90]. For instance, in dog heart, where PDE3 inhibitors exert a potent positive inotropic effect, PDE3 activity revealed in membrane fraction is associated with the SR membrane [36,72,91]. Thus, PDE3 inhibition leads to a local increase of cAMP levels and PKA activation, responsible for the phosphorylation of PLB [92]. In contrast, in guinea pig and rat, PDE3 is mainly found in the soluble fraction and PDE3 inhibitors have more modest effects [72]. Two genes encode two PDE3 subfamilies, namely PDE3A and PDE3B. PDE3A is expressed in rat, guinea-pig, dog and human myocardium [54,57,93,94] and both PDE3A and PDE3B were found to coexist in mouse ventricular myocytes [95] (but see [92]). In human myocardium, three isoforms of PDE3A appear to be generated by alternative splicing of the PDE3A gene and post-transcriptional processing, giving rise to isoforms with different N-terminus length with singular association domains as well as phosphorylation sites by PKB and PKA [54,69], the latter representing an important feedback to control cAMP levels in cardiomyocytes [65]. This allows preferential targeting of PDE3A in different compartments of the cell, and hence cAMP compartmentation. In mouse heart, PDE3B is found in a complex with PI3K γ at the level of sarcolemmal membrane [95]. In this study, the authors have demonstrated that PI3K γ activates PDE3B in a kinase-independent manner, by acting as an anchoring protein which recruits PDE3B into a membrane compartment where cAMP homeostasis shapes the chronic sympathetic drive [95]. More recently, these authors have discovered that PI3K γ anchors PKA through a site in its N-terminal region, and that anchored PKA activates PDE3B to enhance cAMP degradation [96]. This mechanism may represent an important negative feedback regulation of β -AR stimulation of LTCCs by PDE3 activity [97]. However, this finding was recently challenged by a study showing that PI3K γ is only necessary for the PDE4 activity regulating the SERCA2a-PLB function and SR Ca²⁺ content,

but not for the control of the cAMP/PKA dependent phosphorylation of LTCC and RyR2 [92].

3.3. PDE4 and cAMP compartmentation

Unlike PDE2 and PDE3, PDE4 is specific for cAMP and is not regulated by cGMP. In **adult** ventricular myocytes from rat and mice, PDE4 has no effect on basal cAMP levels but becomes crucial to hydrolyze cAMP generated during β -AR stimulation [25,37,60,61,65,98] and for the control of PKA phosphorylation of key proteins involved in ECC such as LTCC [61,99], RyR2 [100] and PLB [92]. As a consequence, PDE4 inhibition strongly potentiates the inotropic effects of β -AR stimulation in these species [101-104]. However, although less studied, the role of PDE4 appears more modest in larger mammals and in humans. In human atrium, β -AR inotropic responses are predominantly regulated by PDE3 [105,106]. *In vivo*, a positive inotropic effect of PDE4 inhibitors was reported in dog, which was abolished by β -blockers, suggesting a presynaptic action on norepinephrine release [72]. [56]The PDE4 family is encoded by four different genes (*A*, *B*, *C* and *D*) but only *PDE4A*, *PDE4B* and *PDE4D* are expressed in cardiac tissue [55]. These three genes give rise to multiple isoforms generated through alternative splicing and by the use of different promoters [107]. A constantly growing body of literature concerns the PDE4D subfamily, with PDE4D3, PDE4D5, PDE4D8 and PDE4D9 being detected as active proteins in rat heart [56]. Less is known concerning a possible role for PDE4A and PDE4B isoforms to regulate cAMP levels and cardiac function, yet their activity has been detected in rodents and to a lesser extent in human myocardium [62]. However, PDE4B was shown to regulate cAMP levels near the plasma membrane in HEK293 cells [108] and to control β -AR signal termination in neonatal cardiomyocytes [37]. In a recent study, we have shown that both PDE4B and PDE4D can associate with the principal subunit of LTCC, but only PDE4B regulates $I_{Ca,L}$ during β -AR stimulation in mouse ventricular myocytes. In ventricular myocytes from mice lacking PDE4B (*PDE4B*^{-/-}) challenged with Iso, enhanced $I_{Ca,L}$ leads to augmented Ca^{2+} transients and shortening, but also to an increase in spontaneous Ca^{2+} release events. Upregulation of $I_{Ca,L}$ and abnormal Ca^{2+} handling may explain the increased susceptibility of *PDE4B*^{-/-} mice to ventricular arrhythmias under β -AR stimulation. These results emphasise the importance of strategically located phosphodiesterase isoforms for adequate control of cardiac ECC and arrhythmias [109].

Subcellular localization of PDE4 isoforms is mediated by their unique N-terminal region which represents the postcode for their cellular distribution [110]. For instance,

PDE4A1 contains a lipid-binding domain, TAPAS, with a specificity for phosphatidic acid that serves to target this enzyme to specific cellular membranes [111]. In the heart, PDE4D3 is localised in the sarcomeric region of the myocytes via an anchoring to myomegalin [112] and in the perinuclear region by an association with the muscle AKAP (mAKAP/AKAP6) [44,46]. This isoform is also found at the level of the dyads, associated with RyR2 [100], and in the sarcolemmal region associated with the KCNQ1/KCNE1 potassium channel responsible for I_{Ks} [47]. It has been suggested that the cAMP compartment localized near the nucleus could control the import of the catalytic subunit of PKA into the nucleus and hence gene regulation [46,113]. The mAKAP not only binds PDE4D3 and PKA, but also Epac1 and the Erk5 kinase [44]. The 3 enzymes, PKA, PDE4D3 and Epac1 respond to cAMP in different concentration ranges: PKA responds to nanomolar concentrations while PDE4D3 and Epac1 are activated at micromolar cAMP concentrations and their inactivation would precede PKA holoenzyme reformation [44]. Furthermore, PDE4D3 phosphorylation by PKA on serine 54 leads to an increased activity [56,114] and phosphorylation on serine 13 increases its affinity for mAKAP [115]. In contrast, phosphorylation by Erk2 and 5 on serine 579 suppresses the activity of this enzyme [44,116]. When Epac1 is activated by cAMP, it recruits Rap1 which allows the suppression of Erk5 activation and relieves the inhibition of PDE4D3. This complex allows a spatial control of PKA activity by its anchoring to mAKAP, and a temporal regulation of cAMP signals by the presence of PDE in the immediate vicinity [1,46]. Interestingly, the expression and activity of PDE4D3 in the RyR2 complex is decreased in human heart failure [100]. These results highlight the importance of cAMP microdomains and suggest that a deregulation of a specific compartment may lead to pathological situations.

More recently, it has been demonstrated that PDE4D3, PKA and phosphatase 1 (PP1) are recruited at the KCNQ1/KCNE1 potassium channel via Yotiao/AKAP9 and contribute to the cAMP-dependent regulation of the potassium current I_{Ks} [47]. PDE4D5 and the scaffold β -arrestin protein are part of a complex organised around β_2 -AR [117]. β -arrestins initiate desensitization of the receptors by their translocation from the cytosol to the plasma membrane. β -arrestins are able to form stable complexes with all PDE4 subfamilies, but PDE4D5 has a unique N-terminal region which confers a preferential interaction with β -arrestins. β_2 -AR activation leads to cAMP production and AKAP79 (AKAP5)-anchored PKA activation [118]. PKA in turn phosphorylates the β_2 -AR, which triggers a shift in its coupling from G_s to G_i , hence activating Erk kinase through a Src-regulated pathway [119]. The recruitment of β -arrestin-PDE4D5 complex stops the β_2 -AR phosphorylation by PKA and

prevents the shift to G_i -signaling cascade; conversely, disruption of this complex enhances PKA phosphorylation of the β_2 -AR, leading to a dramatic change in its function [119,120].

PDE4D5, PDE4D8 and PDE4D9 have been shown to interact directly or indirectly via β -arrestin with β -AR receptors in neonatal rat cardiomyocytes [119,121,122]. Whereas PDE4D5 is recruited to β_2 -AR upon agonist stimulation, PDE4D8 is constitutively associated with the β_1 -AR and dissociation occurs upon receptor activation [121]. More recently, β_2 -AR was shown to interact also with PDE4D8 and with PDE4D9 [122]. β_2 -AR stimulation induces the dissociation of PDE4D9 from the receptor and the recruitment of PDE4D8. These complex modes of interaction of β -AR subtypes with PDEs may shape divergent cAMP signals leading to specific physiological responses [122].

4. cAMP-PDEs and Heart Failure

A number of studies demonstrated a reduction in cAMP PDE expression and/or activity in various models of cardiac hypertrophy and/or HF: cardiomyopathic hamsters [68], failing dog hearts [123,124], mouse [125] and rat heart [57] subjected to transverse aortic constriction, including human HF [126]. On the contrary, PDEs that hydrolyze cGMP, such as PDE1A, PDE2 and PDE5, seem upregulated in hypertrophy [77,127,128]. Because PDEs are crucial orchestrator of cAMP signaling in cardiomyocytes [2], decreased cAMP PDE activities may lead to decompartmentation and loss of specificity of cAMP. PDE3 inhibition, initially described as a therapeutically relevant strategy to treat HF, has been associated with increased mortality in failing patients (see e.g. [129]). Such deleterious effects could be explained by the induction of the proapoptotic factor ICER (Inducible Cyclic AMP Early Repressor) occurring in a CREB dependent manner when PDE3 is inhibited [125,129]. The loss of a specific isoform, PDE3B at the plasma membrane, produces also dramatic cardiac damages in a chronic pressure overload animal model [95]. The decrease in PDE4 isoforms could promote electrophysiological remodelling by increasing the LTCC activity during hypertrophy, as reported in human failing ventricular myocytes [130] or prolongation of the action potential duration by increasing KCNQ1/KCNE1 potassium channel phosphorylation by PKA [47,131]. Furthermore, in mice deficient for the *PDE4D* gene, RyR2 hyperphosphorylation by PKA and abnormal Ca^{2+} release result in increased sensitivity to exercise-induced arrhythmias and the development of a late onset dilated cardiomyopathy [100]. In addition, as shown above, PDE4D3 participates in a complex involving ERK5 and Epac1, two proteins involved in cardiomyocyte growth [5,44].

5. Future directions

In the light of the knowledge accumulated over the years, it becomes clear that cAMP is not uniformly distributed within cardiomyocytes and that PDEs play a crucial role in its confinement. A tenet in this field of research is that cAMP compartmentation is required for adequate processing and targeting of the information generated at the membrane. The compartmentation hypothesis relies on two central postulates [8]. The first is that not all PKA molecules phosphorylate all possible substrates in the cell. This condition is verified by the existence of AKAPs which specifically target PKA to its effectors [132]. The second postulate is that cAMP generated by a given AC does not activate all cellular PKAs. This condition may be verified by specific locations of cAMP-PDEs near ACs and cAMP effectors. Unveiling the details of this subcellular organization will require molecular, cellular and animal models to explore, in particular i) the cAMP signalosomes generated by AC5 and AC6, the two major adenylyl cyclases expressed in adult ventricular myocytes [133] whose specific function is yet unknown, ii) the role of PDE isoforms in shaping the cAMP compartments that specifically control the function of major effectors in cardiac ECC, iii) the role of cellular architecture. The compartmentation hypothesis needs also to be explored in other cardiac cell types, such as vascular cells where cAMP controls vasodilatation, cell growth and proliferation [134].

Another direction concerns cardiac pathophysiology and the difference between acute and chronic effects of cAMP. Although the cAMP pathway is beneficial on heart function under acute conditions, and is essential for the chronotropic, inotropic and lusitropic effects during the ‘fight-or-flight’ response, chronic activation of the cAMP pathway generally (but not always) induces hypertrophic growth and ventricular dysfunction [135], which ultimately leads to the development of chamber dilatation and HF [136]. Despite the description of a number of alterations in the cAMP pathway during hypertrophy and HF, the exact mechanisms accounting for the cardiotoxicity of this pathway are not fully understood. As discussed above, several cAMP PDEs are down regulated in an animal model of cardiac hypertrophy [57]. Does this lead to a disorganization of the cAMP compartmentation? To a loss of hormonal specificity? To cardiotoxic effects of cAMP? Does this contribute to the development of HF?

Finally, can a new therapeutic strategy emerge from the concept of cAMP compartmentation? A number of studies have demonstrated that PDEs are integral

components of macromolecular complexes formed around specific target proteins, such as LTCCs [2,25,74] or RyR2 [100]. Activation of these PDEs may therefore reduce chronic PKA phosphorylation of these proteins, reduce intracellular Ca^{2+} concentration, and prevent arrhythmias and sudden cardiac death. On the contrary, inhibition of PDE3A-136 associated with PLB in the SR membrane might allow preserving intracellular calcium fluxes and contractility in patients treated by β -blockers, without any concomitant arrhythmogenic effects [54,137]. To test these hypotheses, animal models with cardiac overexpression or silencing of specific PDE isoforms need to be generated and their cardiac function explored, under normal and stressed conditions. Such experiments will allow to test whether restoring a proper cAMP signaling might stop or reverse the development of HF.

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