Roles of A-Kinase Anchoring Proteins and Phosphodiesterases in the Cardiovascular System

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Received: 31 January 2018; Accepted: 18 February 2018; Published: 20 February 2018

Abstract: A-kinase anchoring proteins (AKAPs) and cyclic nucleotide phosphodiesterases (PDEs) are essential enzymes in the cyclic adenosine 3′-5′ monophosphate (cAMP) signaling cascade. They establish local cAMP pools by controlling the intensity, duration and compartmentalization of cyclic nucleotide-dependent signaling. Various members of the AKAP and PDE families are expressed in the cardiovascular system and direct important processes maintaining homeostatic functioning of the heart and vasculature, e.g., the endothelial barrier function and excitation-contraction coupling. Dysregulation of AKAP and PDE function is associated with pathophysiological conditions in the cardiovascular system including heart failure, hypertension and atherosclerosis. A number of diseases, including autosomal dominant hypertension with brachydactyly (HTNB) and type I long-QT syndrome (LQT1), result from mutations in genes encoding for distinct members of the two classes of enzymes. This review provides an overview over the AKAPs and PDEs relevant for cAMP compartmentalization in the heart and vasculature and discusses their pathophysiological role as well as highlights the potential benefits of targeting these proteins and their protein-protein interactions for the treatment of cardiovascular diseases.

Keywords: cAMP; compartmentalization; A-kinase anchoring proteins (AKAP); cyclic nucleotide phosphodiesterases (PDE); PDE inhibitors

1. Introduction

Cardiovascular diseases (CVD) represent the leading cause of death worldwide and hypertension is the main risk factor for such conditions [1]. Treatments targeting the causes of cardiovascular diseases such as hypertension or heart failure are rare [2].

The second messenger cyclic adenosine 3′-5′ monophosphate (cAMP) is ubiquitous and functions as a signal transducer of many extracellular cues [3]. It regulates a variety of biological processes that are essential for, among others, proper cardiac function and it is involved in disease [4,5]. cAMP exerts its effects via activation of downstream effector proteins, i.e., cAMP-dependent protein kinase A (PKA), exchange proteins activated by cAMP (Epac) and cyclic nucleotide-gated ion channels (CNG), hyperpolarization-activated cyclic nucleotide-gated channels (HCN) and the recently identified Popeye domain containing (POPDC) proteins [6–8].

The plethora of extracellular signals, the limited number of intracellular cAMP effectors and the requirement of a specific biological response to each of the external signals imply a tight control of the intracellular signaling. This is achieved through signaling in defined cellular compartments. Local cAMP pools are established by the interplay of essentially four processes within the cell, namely cAMP synthesis, its diffusion, formation of multi-protein signaling complexes and cAMP degradation. In the heart, stimulation of β-adrenoceptors (β-ARs) triggers the activation the α-subunits of the stimulatory G proteins (Gαs), which in turn stimulate adenylyl cyclases (ACs) to convert ATP to
cAMP [9]. The formation of multi-protein signaling complexes in the cAMP signaling pathway is orchestrated by the family of A-kinase anchoring proteins (AKAPs), which act as scaffolds and engage in direct protein-protein interactions, including with PKA, and target them to defined subcellular compartments [10–15]. AKAPs play essential roles both in the heart and vascular physiology by coordinating complexes involved in the regulation of various processes including endothelial-barrier function [16,17] cardiac contraction and relaxation [18–21] and action potential duration [22,23]. AKAPs apparently play a role in several pathophysiological conditions in the cardiovascular system, e.g., in the heart their dysregulation is associated with heart failure [24,25].

Termination of cAMP signaling is predominantly achieved by hydrolysis of the phosphodiester bond within the second messenger, a reaction catalyzed by cyclic nucleotide phosphodiesterases (PDEs) [26]. Various PDE families regulate different aspects of cardiac and vascular muscle functions [27], such as the endothelial barrier function [28,29], the Ca^{2+} handling and thus contractility [30] and the basal pacemaking activity [31]. PDEs are also involved in the pathological cardiac remodeling and dysfunction [4,32–35].

The aim of this review is to provide an overview over the AKAPs and PDEs that are relevant in the compartmentalization of cAMP signaling in the cardiovascular system, to discuss their role in physiology and pathophysiology and the potential of these proteins and their protein-protein interactions as pharmacological targets in cardiovascular diseases.

2. A-kinase Anchoring Proteins (AKAPs)

AKAPs are a family of over 40 different scaffolding proteins and are key players in the spatio-temporal control of cAMP-dependent signaling by targeting PKA and additional signaling proteins including ACs, PDEs, further protein kinases and phosphatases to specific subcellular compartments [11,36–38]. PKA is the major downstream effector of cAMP. It is a serine/threonine kinase with broad specificity that controls many cellular processes, e.g., metabolism, cell growth, cell division and cardiac myocyte contraction [39]. It is a heterotetramer that consists of two catalytic subunits (C\(\alpha\), C\(\beta\) or C\(\gamma\)) kept in an inactive state by two regulatory RI (RI\(\alpha\) or RI\(\beta\)) or RII (RII\(\alpha\) or RII\(\beta\)) subunits that are organized as homodimers in the holoenzyme [40]. Upon cAMP binding to the R subunits, the C subunits are released and thus activated and subsequently phosphorylate local substrates [39]. This view was recently confirmed by quantitative mass spectrometric analyses [41]. However, PKA holoenzyme can also be active, as indicated by early biochemical experiments [42,43]. This notion was supported by recent fluorescence resonance energy transfer (FRET) imaging-based experiments, which suggested that physiological cAMP levels promote only minimal dissociation of the C subunits from the holoenzyme, thereby limiting the range of PKA action to the substrates in the immediate proximity [44]. Thus, it appears that both PKA holoenzyme and/or the dissociated C subunits can be active.

The structural feature that all AKAPs share is their ability to bind PKA via their A-kinase binding domains (AKBs), a structurally conserved amphipathic helix of 14–18 amino acids that docks into the hydrophobic groove formed by the N-terminal dimerization/docking (D/D) domains upon R subunits’ dimerization (Figure 1) [45–48]. Despite the fact that most AKAPs bind to PKA-RII subunits [37,49], there are the so-called dual specific AKAPs [50] that can bind both RI and RII subunits as well as AKAPs that specifically bind RI subunits (e.g., sphingosine kinase interacting protein (SKIP) and small membrane (sm) AKAP) [51–53]. Recently, hydrophilic anchor points have been identified within and outside the amphipathic helix forming the AKB that are involved in determining the affinity of the binding between an AKAP and the D/D domain. These observations suggest that targeting the amino acids that act as anchor points could lower the binding affinity or even prevent the interaction, making them candidates for pharmacological targeting. Moreover, targeting the anchor points makes the development of selective inhibitors of specific AKAP-PKA interactions feasible [48]. Selective inhibitory agents would be valuable tools for the investigation of cellular functions of individual AKAP-PKA interactions and could be starting points for drug development efforts.
Figure 1. Schematic representation of A-kinase anchoring proteins (AKAP)-protein kinase A (PKA) interactions displayed at two different angles. The amphipathic AKB helix of AKAPs docks into the hydrophobic groove formed by the dimers of the N-terminal D/D domains of regulatory subunits of PKA. AKB A-kinase-binding domain; D/D dimerization and docking domain.

2.1. AKAP Subcellular Localization

Targeting of AKAPs to specific subcellular compartments is essential for a coordinated cAMP-dependent signaling response, including accurate PKA-catalyzed substrate phosphorylation [54]. AKAPs can be directed to various cellular compartments, including the plasma membrane (PM, e.g., AKAP18α, AKAP18β, AKAP79 [55–57]), the sarcoplasmic reticulum (SR, e.g., AKAP18δ [20]), the cytosol (e.g., SKIP, GSKIP [51,58–61]), the cytoskeleton (e.g., gravin, ezrin [62]), the mitochondria (e.g., D-AKAP1 [63]) and the nucleus (e.g., pericentrin and AKAP350 [64,65]).

2.2. AKAPs in the Cardiovascular System

Several AKAPs are expressed in the cardiovascular system (Table 1). They regulate a variety of processes and are key proteins in maintaining the homeostatic functioning of the heart and vasculature [66]. For instance, gravin and AKAP220 are involved in maintaining the vascular integrity [16,17]. Homeostasis of the vascular tone is achieved through tight control of the balance between contraction and relaxation of vascular smooth muscle cells (VSMC), processes in which AKAP79 is involved [67,68]. Ca^{2+} handling and thus cardiac myocyte contractility is regulated by several macromolecular protein complexes whose platforms are AKAPs, e.g., AKAP18α, γ and δ, mAKAPβ [19–21,69]. The AKAP Yotiao is the key player in cardiac myocyte repolarization that follows contraction [22]. Several AKAPs are involved in stress response-induced cardiac myocyte hypertrophy, including AKAP-Lbc and mAKAPβ [70,71]. AKAP79 and gravin are important for the recycling of β1-ARs and β2-ARs, respectively [72,73].

Table 1. Overview of AKAPs expressed in the heart and vasculature and of the cardiovascular processes that they regulate.

| Common Name | Gene Name | Alternative Name | Regulated Cardiovascular Process |
|-------------|-----------|------------------|----------------------------------|
| D-AKAP1     | AKAP1     | AKAP121/         | Cardiac stress response          |
|             |           | AKAP149/AKAP84   |                                  |
| D-AKAP2     | AKAP10    | -                | Cardiac repolarization           |
| AKAP9 (long isoform) | AKAP9 | -                | Endothelial barrier function     |
| AKAP18α     |           |                  |                                  |
| AKAP18β     | AKAP7     |                  | Excitation-contraction coupling  |
| AKAP188     |           |                  |                                  |
Table 1. Cont.

| Common Name    | Gene Name       | Alternative Name | Regulated Cardiovascular Process                                                                 |
|----------------|-----------------|------------------|--------------------------------------------------------------------------------------------------|
| AKAP79         | AKAP5           | AKAP75/AKAP150   | Vascular tone; Excitation-contraction coupling; β-AR desensitization/resensitization cycle       |
| AKAP220        | AKAP11          | -                | Endothelial barrier function                                                                     |
| AKAP-Lbc       | AKAP13          | Brx-1/Proto-Lbc/ Ht31 | Cardiac stress response                                                                      |
| mAKAPβ         | AKAP6           | AKAP100          | Excitation-contraction coupling; Cardiac stress response                                       |
| AKAP Yotiao    | AKAP9           | GC-NAP           | Endothelial barrier function; β-AR desensitization/resensitization cycle Cardiac stress response |
| Gravin         | AKAP12          | AKAP250          |                                                                                                 |
| SKIP           | SPHKAP          | -                |                                                                                                 |

2.2.1. AKAPs Regulating the Endothelial Barrier Function

The vascular endothelium lining the intima of blood vessels consists of a layer of endothelial cells tightly adherent to each other through cell-cell junctions. A healthy endothelium plays an essential role in the proper functioning of the vascular system. It regulates macromolecular permeability and anti-inflammatory, anti-thrombotic and anti-hypertrophic responses. Inflammatory conditions trigger pathological changes in the vascular system that lead to endothelial dysfunction, a state in which pathologically activated endothelial cells lose their barrier properties and initiate expression of pro-inflammatory adhesion molecules on their surface [74]. This results in increased vascular permeability allowing the infiltration of various molecules such as lipoproteins into the sub-endothelial space, and of circulating immune cells (e.g., monocytes). Ultimately, this leads to severe pathological conditions including atherosclerosis, allergy and sepsis [75,76].

AKAP-mediated PKA compartmentalization is essential for the maintenance of proper endothelial barrier function [16,17,77]. The vascular endothelial integrity is mainly dependent on tight junctions (TJs), important in sealing space between adjacent cells, and on adherens junctions, which assure direct contacts with the actin cytoskeleton of neighboring cells, thus providing mechanical strength. AKAP220 associates with PKA, β-catenin and the endothelial adherens junctions protein VE-cadherin, tethering PKA in close proximity to the cell-cell junctions [16]. Gravin (also known as AKAP12 or AKAP250) promotes vascular integrity by regulating the actin cytoskeleton via p21-activated kinase family proteins 2 (PAK2), an actin cytoskeletal regulator and afadin (AF6), a linker of the actin cytoskeleton with intercellular adhesion molecules [17]. Rac1 is a member of the Rho family of small GTPases, which upon activation strengthens the adherens junctions and the cortical actin skeleton, thereby preserving the endothelial barrier [78]. Simultaneous depletion of gravin and AKAP220 inhibited cAMP-mediated Rac1 activation, underlining the importance of these AKAPs in preventing endothelial dysfunction [16].

One other member of the AKAP family is involved in maintaining vascular integrity, the long isoform of AKAP9. Following Epac1 activation, AKAP9 contributes to microtubule growth regulation and is essential for preserving the endothelial barrier [79].

2.2.2. AKAPs Regulating the Vascular Tone

Homeostasis of the vascular tone is maintained by a tight balance between dilation and constriction of blood vessel endothelium; the main regulator is the renin-angiotensin-aldosterone system (RAAS). The main effector molecule of this system is angiotensin II (AngII), which exerts most of its effects via angiotensin type I receptors (AT1R). For instance, arterial smooth muscle contraction is induced by AngII-dependent stimulation of AT1R, localized at the sarcolemma, and subsequent activation of phospholipase C (PLC), which catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) to diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3). DAG activates
protein kinase C (PKC), which in turn phosphorylates L-type Ca\(^{2+}\) (Cav1.2) channels, thereby increasing their open probability and increasing Ca\(^{2+}\) entry into the cytosol [75].

In arterial smooth muscle cells, the activity of a specific subpopulation of Cav1.2 channels is regulated by AKAP79 (AKAP5/AKAP75/AKAP150)-dependent targeting of PKA\(\alpha\) to the sarcolemma, which facilitates phosphorylation of the channels and increases their open probability [80]. By affecting the opening probability of specific Cav1.2 channels, the AKAP79 complex regulates the so-called "Cav1.2 sparklets", which refers to local elevations of intracellular Ca\(^{2+}\) pools that directly induce contraction of the VSMCs. The sparklets increase the vascular tone [67]. In addition, AKAP79 facilitates and most probably stabilizes the coupling of small clusters of adjacent Cav1.2 channels, which can then open synchronously and generate large Cav1.2 sparklets, thus increasing the contractile force [81,82]. Prolonged Cav1.2 channel activity and thus persistent Cav1.2 sparklets could lead to vascular dysfunction and eventually contribute to AngII-induced hypertension [80].

Transient receptor potential vanilloid 4 (TRPV4) channels are Ca\(^{2+}\) permeant channels that unlike the Cav1.2 channels, promote relaxation upon activation. Both Cav1.2- and TRPV4-mediated Ca\(^{2+}\) influxes activate adjacent ryanodine receptors (RyR), leading to release of Ca\(^{2+}\) from the SR into the cytosol in the form of Ca\(^{2+}\) sparks. While the Cav1.2-mediated Ca\(^{2+}\) influx increases contraction, the local TRPV4-generated Ca\(^{2+}\) sparks activate the large-conductance, Ca\(^{2+}\)-activated K\(^+\) (BK) channels, which promote membrane hyperpolarization and closure of the Cav1.2 channels, ultimately resulting in relaxation [83,84]. In the arterial smooth muscle cells, AngII increased TRPV4 activity via PKC, which is tethered to the sarcolemma in close proximity of the channel by AKAP79, thus opposing the Cav1.2 channel-induced vasoconstriction [68].

In conclusion, AKAP79 plays an essential role in the control of arterial vascular tone by regulating two opposing processes, contraction and relaxation of arterial myocytes.

2.2.3. AKAPs Controlling Excitation-Contraction Coupling

The cycling of Ca\(^{2+}\) between the cytosol and the SR is at the basis of cardiac contraction and relaxation. Key players in these processes are L-type Ca\(^{2+}\) Cav1.2 channels, RyR2, SR Ca\(^{2+}\) ATPase 2 (SERCA2) and the Na\(^+\)/Ca\(^{2+}\) exchanger. More specifically, upon sarcolemma depolarization, Cav1.2 channels located at the T tubules open allowing Ca\(^{2+}\) influx into the cardiac myocyte. This causes Ca\(^{2+}\)-induced Ca\(^{2+}\) release from the SR into the cytosol through RyR2 located at the SR. The Ca\(^{2+}\), upon interaction with troponin T located on the thin myofibers, promotes contraction. Relaxation occurs via SERCA2-mediated Ca\(^{2+}\) re-uptake into the SR and through Ca\(^{2+}\) transport out of the cell by Na\(^+\)/Ca\(^{2+}\) exchangers [85]. SERCA2 is activated upon the phosphorylation and subsequent dissociation of phospholamban (PLN), a SR phosphoprotein [20,86,87].

\(\beta\)-ARs introduce a further layer into the regulation of cardiac myocyte contractility. Their stimulation induces PKA-dependent phosphorylation of several proteins involved in Ca\(^{2+}\) handling, e.g., the Cav1.2 channels, RyR2 and PLN. These phosphorylations are facilitated by distinct AKAPs.

AKAP18\(\alpha\) is a membrane-associated scaffolding protein and is the smallest AKAP7 gene transcript, comprising 81 amino acids. AKAP18\(\alpha\) promotes cardiac contractility by mediating the PKA-dependent phosphorylation of Cav1.2 channels at Serine 1928 (Ser1928) on its \(\alpha\) subunit and at multiple sites on its \(\beta\) subunit, which enhances the open probability of the channel and increases the Ca\(^{2+}\) current [69,88]. The activity of a subset of Cav1.2 channels associated with caveolin-3 (Cav3) is regulated by PKA phosphorylation of the specific channel subpopulation mediated by an AKAP79 (AKAP5/AKAP75/AKAP150)-based macromolecular complex consisting of \(\beta\)-AR, PKA, AC5/6 and protein phosphatase calcineurin (PP2B) [89]. The muscle selective AKAP, mAKAP\(\beta\) (a short version of mAKAP) associates with RyR2 at the SR and thereby facilitates the PKA phosphorylation of the channel, leading to enhanced opening of the channel and subsequent enhanced Ca\(^{2+}\) release from the SR into the cytosol [19]. In addition, mAKAP\(\beta\) interacts with the Na\(^+\)/Ca\(^{2+}\) exchanger 1 at the sarcolemma and promotes the PKA-dependent activation of the exchanger, resulting in increased Ca\(^{2+}\).
efflux [90,91]. AKAP18δ (rat heart) and AKAP18γ (human heart) facilitate the PKA phosphorylation of PLN and promote its dissociation from SERCA2 and hence activation of the ATPase, thus enhancing the re-uptake of Ca$^{2+}$ into the SR [20,21,92].

2.2.4. AKAPs Regulating Cardiac Repolarization

The cardiac repolarization phase is initiated by the slow heart potassium current ($I_{Ks}$) moving outwards through the $I_{Ks}$ potassium channel, a macromolecular complex consisting of a pore-forming α subunit (KCNQ1) and a regulatory β subunit (KCNE1) along other intracellular proteins [93]. The AKAP Yotiao, the smallest transcript of the AKAP9 gene, is essential for cardiac repolarization since it mediates the PKA-dependent phosphorylation of KCNQ1 and therefore regulates the activity of the $I_{Ks}$ potassium channel [22]. Mutations in the KCNQ1 subunit or Yotiao increase the duration of the action potential and lead to type I long-QT syndrome (LQT1), a channelopathy that can elicit fatal arrhythmia [94]. Another AKAP that contributes to the regulation of cardiac action potentials is the dual specific D-AKAP2 (AKAP10). A single-nucleotide polymorphism (SNP) in its PKA binding domain causes a decrease in the PR interval in the electrocardiogram, which in turn can cause arrhythmias and sudden cardiac death [54,95–97].

2.2.5. AKAPs Involved in Cardiac Stress Response

Cardiac hypertrophy is a stress-induced adaptation to maintain normal heart function [23,25]. At the cellular level, it is characterized by the upregulation of specific genes that promote the non-mitotic growth of cardiac myocytes [98]. AKAP-Lbc encodes in addition to its AKAP function for a guanine nucleotide exchange factor (GEF) that directly binds and activates the GTP-binding protein RhoA [99–102]. The interaction is involved in both cardiac development [103] and pathological cardiac myocyte hypertrophy [70]. α₁-AR stimulation enhances the RhoGEF activity of AKAP-Lbc, which in turn activates RhoA, contributing to a pathological increase in the hypertrophic response [70]. PKA-mediated phosphorylation at Ser1565 of AKAP-Lbc leads to the recruitment of 14-3-3 proteins, which inhibit the Rho-GEF activity of the anchoring protein [104]. Also, an AKAP-Lbc-dependent signalosome mediates the activation and cytosolic release of activated protein kinase D (PKD), which has been shown to promote cardiac hypertrophy by facilitating the nuclear export of histone deacetylase 5 (HDAC5) [105,106].

Another AKAP that plays a central role in modulating stress signal-induced hypertrophic pathways is mAKAPβ. It coordinates a variety of cAMP-responsive enzymes. This anchoring protein is targeted to the nuclear envelope of cardiac myocytes via an interaction with nesprin-1α [107]. At the SR it can integrate and transduce a variety of hypertrophic signals [71]. For instance, mAKAPβ-mediated PKA phosphorylation and subsequent activation of RyR2 located at the nuclear envelope promotes the activation and nuclear translocation of the pro-hypertrophic transcription factor nuclear factor of activated T cells (NFAT) [108]. In addition, a mAKAPβ-based signalosome consisting of PKA, PDE4D3, Epac1, ERK5 and PP2A promotes ERK5-induced cardiomyocyte hypertrophy [71,109]. Cardiac remodeling can also be regulated by hypoxia, a process in which a mAKAP-based protein complex consisting of hypoxia-inducible factor 1α (HIF-1α), prolyl hydroxylase domain protein (PHD), the von Hippel-Lindau protein (pVHL) and the E3 ligase designated seven in absentia homolog 2 (Siah2) plays a role. More specifically, when oxygen levels are reduced, mAKAP promotes the degradation of PHD and thereby facilitates an increase in HIF-1α levels, which regulates transcription of genes that promote cell survival [110].

Other AKAPs that are thought to be involved in the cardiac stress response are D-AKAP1 and SKIP [111,112]. D-AKAP1 is a scaffolding protein of the outer mitochondrial membrane, which is protective against cardiac hypertrophy since its overexpression leads to cardiac myocyte cell size reduction and inhibition of the β-AR agonist isoproterenol-induced hypertrophy [111]. Moreover, D-AKAP1 expression maintains the mitochondrial structure and function in the heart and reduces
the infarct size, cardiac remodeling and mortality under conditions of ischemia, i.e., after myocardial infarction [113]. SKIP plays an important role in the generation of the cardioprotective and anti-apoptotic lysophospholipid sphingosine-1-phosphate (S1P) produced upon myocardial ischemia-reperfusion injury [112]. It is involved in the regulation of sphingosine kinase type 1 (SPHK1), which upon activation phosphorylates sphingosine to form S1P [114].

2.2.6. AKAPs Involved in the β-ARs Desensitization/Resensitization Cycle

Upon activation, β-ARs are phosphorylated and subsequently bind β-arrestin, which prevents further ligand binding leading to receptor desensitization. The phosphorylated β-ARs are internalized and reach the early endosomes where they undergo resensitization after PP2A-mediated dephosphorylation. Upon resensitization, the non-phosphorylated receptors are recycled to the plasma membrane where they can bind further ligands. Therefore, β-AR desensitization and resensitization are essential processes in maintaining the proper functioning of the receptor [115]. Gravin and AKAP79 are important in the desensitization/resensitization cycle [72,73]. A gravin-based complex consisting of PKA, PKC, PP2B, β-arrestin and G protein-linked receptor kinase 2 (GRK2) is essential for the desensitization and resensitization of the β2-ARs, with which it interacts at their C-terminal tail [72]. AKAP79 mediates the PKA-dependent phosphorylation of the β1-ARs by also binding to the C terminus of the receptor, leading to their recycling and resensitization [73].

2.3. Aberrant cAMP Compartmentalization Can Be Visualized

Dysregulation of local cAMP signaling is associated with cardiovascular diseases, e.g., maladaptive cardiac remodeling and heart failure [116,117]. FRET-based imaging using genetically encoded sensors (cAMP-binding and PKA activity reporters) is utilized to visualize local cAMP signaling components and real-time changes in cAMP levels with high spatio-temporal resolution [118–121]. Such sensors can be targeted to various subcellular locations including in cardiac myocytes in close proximity of sarcolemmal ion channels and SR proteins involved in Ca$^{2+}$ handling, e.g., RyR2 and SERCA2a [122–125]. In addition, the FRET-based reporters can be used for cAMP imaging in intact cardiac tissue as well as in ex vivo and in vivo hearts [126]. For monitoring activities of signaling molecules in their cognate microdomains, FRET approaches have also been combined with other techniques such as scanning ion-conductance microscopy (SICM), a non-optical method that allows the imaging of both cell membrane morphology and functional parameters at resolutions in the nanometer range [127–130].

3. Cyclic Nucleotide Phosphodiesterases (PDEs)

Hydrolysis by PDEs is the main route for lowering of intracellular levels of cAMP and cGMP and is essential for the spatio-temporal regulation of cyclic nucleotide-dependent signaling [26]. The PDE superfamily consists of 21 genes that give rise to more than 100 proteins due to differential transcription initiation sites and alternative splicing. PDEs are classified into 11 families (PDE1–PDE11). They differ in their primary structures, substrate specificities, mechanisms of regulation and kinetic properties [131]. Some PDE families selectively hydrolyze cAMP or cGMP, while others, the so-called dual specific PDEs, catalyze the hydrolysis of both second messengers (Figure 2) [132].

PDEs display a common general structure consisting of three components: a family-specific N-terminal regulatory domain, a conserved catalytic domain (25–52 % homology) and a C-terminal domain that can be either phosphorylated by the mitogen-activated protein kinase (MAPK) or prenylated [131,133–135]. The regulatory domains contain various structural features involved in the regulation (i.e., sites for covalent modifications, e.g., phosphorylation), binding of regulatory molecules (e.g., Ca$^{2+}$-binding protein calmodulin), localization (targeting domains and protein-protein interaction motifs, e.g., AKAP-binding motifs) and dimerization (e.g., GAF domains) of the enzymes [131,136,137].
The catalytic domains feature in the active site a Zn\(^{2+}\) binding motif and an additional divalent metal binding site that is most probably occupied by Mg\(^{2+}\), but could also correspond to Mn\(^{2+}\) and Co\(^{2+}\) [138].

Figure 2. Substrate specificity of individual phosphodiesterase (PDE) families.

3.1. PDE Subcellular Localization

The subcellular localization of PDEs is key in achieving compartmentalized cyclic nucleotide signaling and, therefore, in the generation of specific physiological responses [136]. PDEs are located at various intracellular locations, e.g., the cytosol (e.g., PDE3A3 and PDE5 [139,140]), plasma membrane (e.g., PDE2A, PDE3A1, PDE6\(\alpha\), PDE6\(\beta\) [139,141,142]), the Golgi–centrosome (e.g., PDE7A1 [143]) and nuclear regions (e.g., PDE9A1, PDE9A16, PDE9A17 [144]).

3.2. PDEs in the Cardiovascular System

Members from most PDE families are expressed in the cardiovascular system and regulate a variety of processes essential for the proper functioning of the heart and vasculature (Table 2). The PDE families 2, 3, 4 and 5, for example, regulate the endothelial barrier function and are, therefore, of utmost importance in maintaining vascular integrity [28,145,146]. Several members of the PDE families 2, 3, 4, 5 and 8 are involved in the control of cardiac contractility [30,120,147–150]. In addition, the basal pace-making activity of the sinoatrial (SA) node of the heart is regulated by two PDE families, namely PDE3 and PDE4 [151]. In addition, various PDE family members, PDE1A, PDE3A, PDE4B, PDE4D, PDE5 and PDE9A, are implicated in the cardiac stress response, which triggers pathological cardiac remodeling and ultimately cardiac dysfunction (e.g., heart failure and arrhythmias) [34,152–156].

Table 2. Overview of the PDE families expressed in the cardiovascular system and the corresponding cardiovascular processes that they regulate.

| PDE Family | PDE Gene     | Substrate Specificity | Regulated Cardiovascular Process                                      |
|------------|--------------|-----------------------|-----------------------------------------------------------------------|
| PDE1       | PDE1A, PDE1B, PDE1C | cAMP, cGMP            | Cardiac stress response                                                |
| PDE2       | PDE2A        | cAMP, cGMP            | Endothelial barrier function; Excitation-contraction coupling          |
| PDE3       | PDE3A, PDE3B | cAMP, cGMP            | Endothelial barrier function; Excitation-contraction coupling; Basal pacemaking activity of the SA node; Cardiac stress response |
| PDE4       | PDE4A, PDE4B, PDE4C, PDE4D | cAMP            | Endothelial barrier function; Excitation-contraction coupling; Basal pacemaking activity of the SA node; Cardiac stress response |
### Table 2. Cont.

| PDE Family | PDE Gene | Substrate Specificity | Regulated Cardiovascular Process |
|------------|----------|-----------------------|----------------------------------|
| PDE5       | PDE5A    | cGMP                  | Endothelial barrier function;    |
|            |          |                       | Excitation-contraction coupling; |
|            |          |                       | Cardiac stress response          |
| PDE8       | PDE8A    | cAMP                  | Excitation-contraction coupling  |
|            | PDE8B    |                       |                                  |
| PDE9       | PDE9A    | cGMP                  | Cardiac stress response          |

#### 3.2.1. PDE3A and Autosomal Dominant Hypertension with Brachydactyly (HTNB)

PDE3A along with PDE3B belongs to the PDE3 family, also known as the cGMP-inhibited cAMP PDE family, which is able to hydrolyze both cAMP and cGMP in a competitive manner. PDE3A is highly expressed and plays important roles in VSMCs, cardiac myocytes, platelets and oocytes, whereas PDE3B is mainly expressed in adipose and soft tissue. Upon alternative splicing, three PDE3A isoforms are generated, namely PDE3A1 (136 kDa), PDE3A2 (118 kDa) and PDE3A3 (94 kDa) (Figure 3A). They are located in different cellular compartments. PDE3A1 is the main isoform found in human cardiac myocytes and is predominantly located at membranes. It contains two N-terminal hydrophobic regions (NHR), of which the first one consists of four transmembrane domains. PDE3A2, which lacks the first but contains the second NHR can be both membrane-associated and cytosolic and is the main variant found in VSMCs. PDE3A3 is found only in the cytosol, since it lacks both previously mentioned hydrophobic regions. All three isoforms possess the same catalytic region and present high similarities regarding their catalytic activity and inhibitor sensitivity (Figure 3A) [157,158].

Mutations in genes encoding for distinct PDE family members can have detrimental effects and cause specific human diseases. One such example is represented by the Mendelian syndrome Autosomal-dominant hypertension with brachydactyly type E (HTNB), caused by missense mutations in the gene encoding for PDE3A [159]. The syndrome is characterized by an age-dependent progressive hypertension, brachydactyly type E and blood vessel hyperplasia [159]. If untreated, blood pressure increases by 50 mm Hg and patients die from stroke before age 50 years. Surprisingly, hypertension-associated end organ damage such as cardiac hypertrophy, kidney damage or hypertensive retinopathy is low [160,161].

Eight mutations in \( PDE3A \) were discovered in eight unrelated families from Turkey, France, the United States, South Africa, Canada, Netherlands and Japan. All these mutations were missense, gain of function mutations and found in close proximity to each other. The identified mutations cause amino acid substitutions in a region between amino acids 445 and 449 and increases of PKA-mediated phosphorylation of serine residues 428 and 438 of PDE3A1 and PDE3A2. The region is not present in PDE3A3 (Figure 3A) [159]. The substitutions lead to increased cAMP affinity and hydrolytic activity of the enzymes (Figure 3B). In addition, the hyperactive enzyme is erroneously localized in microsomal fractions from HeLa cells, suggesting that aberrant compartmentalization is detrimental in the cardiovascular system [159,161]. VSMCs from patients expressing the hyperactive version of the enzyme with the T445N substitution display higher proliferation rates, explaining the vascular phenotype.

In the human heart, PKA-mediated phosphorylation of PDE3A1 induces its recruitment to an AKAP18-based signalosome in the heart that controls the \( \text{Ca}^{2+} \) reuptake into the SR and thereby participates in the control of cardiac relaxation [21]. An extended overview over functions of PDE3 in the heart was provided in recent reviews (e.g., [162,163]).
Figure 3. Schematic representation of the PDE3A gene, PDE3A protein isoforms and the hyperphosphorylation caused by the identified mutations. (A) Eight mutations have been identified in families from the countries indicated by the flags. The mutations cluster within a region of the gene encoding amino acids 445 and 449. The mutations cause hyperphosphorylations of Ser428 and Ser438. The N-terminal hydrophobic region (NHR) 1 of PDE3A1 comprises four transmembrane domains, while NHR2 contains no typical transmembrane region but a cluster of hydrophobic amino acids. PDE3A2 contains only NHR2 and PDE3A3 lacks all N-terminal hydrophobic regions (for details see text). (B) The hyperphosphorylation increases cyclic adenosine 3′-5′ monophosphate (cAMP) hydrolysis, causing low cAMP levels.

3.3. PDE Inhibitors

Due to their essential physiological and pathological roles in cyclic nucleotide signaling, PDEs are considered pharmacological targets for a variety of cardiovascular diseases, including atherosclerosis, hypertension, heart failure and intermittent claudication [26,164–166]. Several inhibitors of PDE3, 4 and 5 are approved as drugs, some of which are used for the treatment of cardiovascular diseases.

3.3.1. PDE3

The PDE3 inhibitor cilostazol is an antiplatelet agent with vasodilatory and antiproliferative properties. It has been widely studied in a number of cardiovascular diseases including coronary and peripheral artery diseases and cerebrovascular disease [167]. Cilostazol is administered for the treatment of peripheral arterial circulatory disorders and also used as an antiplatelet agent in patients that underwent carotid artery stenting [168,169]. In addition, it is also approved for the treatment
of intermittent claudication-induced symptoms [170–172]. Cilostazol appears to be a promising therapeutic agent for secondary prevention of stroke and was shown to improve right ventricular systolic function as well as to decrease pulmonary artery pressure [167,173]. PDE3 inhibitors inhibit neointima formation in a rat balloon double-injury model displaying neither cytotoxicity nor effects on VSMC migration, and thus are considered targets in preventing acute re-occlusion after angioplasties, e.g., percutaneous transluminal coronary angioplasty (PTCA) [174].

Milrinone is another PDE3 inhibitor. It has inotropic and vasodilatory properties, and is widely used in patients with end-stage heart failure in order to temporarily improve cardiac contractility (positive inotropic effect) and decrease vascular resistance. Taking into account that long-term administration of milrinone can induce apoptosis of cardiac myocytes, cardiac arrhythmias, hypotension and increases cardiovascular mortality, it is only used in a selected group of patients [170,175–177]. A potential explanation for the long-term PDE3 inhibitor therapy-induced mortality could be the fact that PDE3A inhibition induces cardiac myocyte apoptosis via a PDE3A-inducible cAMP early repressor (ICER) feedback loop. More specifically, PDE3A inhibition leads to PKA activation and ICER protein stabilization, which, in turn, promotes cardiac myocyte apoptosis. Therefore, therapeutic strategies that would diminish PDE3A activity without affecting the PDE3A-ICER feedback loop could promote the beneficial effects while by-passing the side effects [152,162]. Current research aims at determining the effects of milrinone on pulmonary hypertension and right ventricular failure, where it is believed to be particularly helpful [176].

3.3.2. PDE4

The PDE4 family is encoded by four genes, PDE4A, PDE4B, PDE4C and PDE4D [178] and was shown to be involved in the excitation-contraction coupling regulation, especially in rodents. It has been recently suggested that PDE4 inhibitors could be beneficial in treating sepsis in infants with cardio-renal syndrome (CRS) since they are effective in improving cardiac function in a rat model suffering from sepsis-induced acute cardiac dysfunction and kidney injury [179]. In addition, PDE4 depletion stabilized the endothelial barrier by reducing the atrial natriuretic peptide (ANP)-induced vascular permeability and, therefore, was efficient in maintaining the plasma volume [180]. Despite the fact that PDE4A, PDE4B and PDE4D are expressed in the human and rodent heart, with PDE4D being the predominant isoform found in the human heart [181], there is no approval for a PDE4 inhibitor for the treatment of cardiovascular diseases. A highly selective PDE4 inhibitor, roflumilast, has been approved in various countries for the treatment of chronic obstructive pulmonary disease (COPD), a chronic inflammatory lung disease characterized by heavily breathing due to obstructive airflow from the lungs as well as a decline of lung function over time [182–184]. Another inhibitor, apremilast is employed for the treatment of psoriasis [185].

3.3.3. PDE5

PDE5A is the sole gene coding for the PDE5 family, which plays an essential role in the cardiovascular system. PDE5 expression is low in the healthy cardiac tissue, whereas it is upregulated in the diseased heart [155,186]. PDE5 inhibition counteracts cardiac remodeling and fibrosis of isolated cardiac fibroblasts via repression of transforming growth factor (TGF)-β1-induced Smad signaling [187]. PDE5 depletion inhibits left ventricular remodeling induced by hypertrophic and pro-fibrotic stimuli [188]. Reduction in PDE5 expression was beneficial for chronic heart failure patients by enhancing the endothelium-dependent, flow-mediated vasodilation [189]. In addition, high PDE5 expression was identified in the hypertrophic human right ventricle and its inhibition enhanced contractility, particularly important for pulmonary hypertension [186]. PDE5 inhibitors such as sildenafil, vardenafil and tadalafl are approved for the treatment of erectile dysfunction and pulmonary hypertension but are not yet approved for the treatment of other cardiovascular diseases [26]. Nevertheless, recent studies suggest potential therapeutic benefits for PDE5 inhibitors,
i.e., sildenafil and tadalafil in the treatment of myocardial infarction, ischemia/reperfusion injury, endothelial dysfunction, cardiac hypertrophy and heart failure [190,191].

3.3.4. Potential for PDE1, PDE2, PDE8 and PDE9 Inhibitors

PDE1, PDE2, PDE8 and PDE9 inhibition is considered a therapeutic opportunity for the treatment of cardiovascular diseases but inhibitors are not approved.

The PDE1 family is encoded by three distinct genes, PDE1A, PDE1B and PDE1C, and is the only PDE family activated by calcium/calmodulin (Ca\(^{2+}\)/CaM) binding [192,193]. Due to their potential to dilate coronary arteries, inhibition of PDE1 enzymes may be beneficial for the treatment of coronary artery disease (CAD) and angina pectoris [194]. Nuclear PDE1A is important for the proliferation of VSMCs and, therefore, could contribute to neointima formation in diseases, e.g., atherosclerosis and restenosis [195]. Thus, diminishing its expression could decrease pathological neointima development. In addition, inhibition of the PDE1 family might improve cardiopathy and pulmonary arterial hypertension since it decreases the structural remodeling process underlying these two conditions [196].

PDE2A is the only gene coding for the PDE2 family and plays a central role in the cardiac Ca\(^{2+}\) current regulation. The expression is up-regulated in human failing hearts [197,198]. Inhibition of PDE2 had a positive inotropic effect in dogs and mice, whereas its overexpression decreased the heart rate in mice. Interestingly, in a heart-specific PDE2-transgenic mouse model, increased PDE2 abundance prevents ventricular arrhythmias by inhibiting Ca\(^{2+}\) leak from the SR and helps in maintaining the contractile function of the heart after myocardial infarction [199]. On the contrary, a recent study in patients that had experienced an acute myocardial infarction (AMI) suggests that inhibition of endothelial PDE2A could have a beneficial effect and improve the clinical outcome. Hypoxia and pro-inflammatory cytokines such as tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) promote PDE2A activation, which results in diminished submembrane cAMP levels and endothelial barrier disruption. This facilitates the extravasation of activated neutrophils and leads to inflammation in the early post-myocardial infarction phase [29].

The PDE8 family comprises two members, PDE8A and PDE8B and regulates excitation-contraction coupling in ventricular myocytes. More specifically, it has been suggested that PDE8A controls at least one cAMP pool involved in the cardiac myocyte-dependent Ca\(^{2+}\) cycling regulation. It was also observed that PDE8A deletion caused both increased RyR\(_2\) leak as well as enhanced Ca\(^{2+}\) refilling of the SR [150].

The PDE9 family is encoded by a single gene, PDE9A, and consists of more than 20 different splice variants. PDE9A expression was identified in human and rodent hearts, where its expression increased upon hypertrophy and heart failure development [156,200]. PDE9A depletion had a protective effect for the heart against pathological remodeling caused by pressure overload and it reversed a previously established heart disease without requiring the activity of NO synthase [156].

4. Concluding Remarks

Compartmentalized cyclic nucleotide signaling is found at the basis of precision of cellular signaling and its dysregulation is associated with various pathological conditions including several cardiovascular diseases. Local pools of cAMP are established by the interplay of cAMP synthesis, diffusion, degradation as well as positioning of the relevant signaling proteins. AKAPs and PDEs are essential players in these processes since they orchestrate the formation of multi-protein signaling complexes and terminate local cAMP signaling, respectively. This interplay ensures the spatio-temporal regulation of cyclic nucleotide-dependent signaling. Despite the fact that both molecules are key elements in the cAMP signaling pathway, very little is known with respect to their direct interaction or their interplay in the cardiovascular system. However, a few PDE-containing AKAP complexes have been identified; examples are the SERCA2/AKAP18 signalosome, which incorporates PDE3A1 upon its phosphorylation and is important for cardiac contractility, and the
PDE4D3 containing mAKAPβ-based signalosome involved in cardiomyocyte hypertrophy regulation ([21,71,109]). Alterations in AKAP expression and their protein-protein interactions are associated with various cardiovascular diseases [12,24]. Hence the development of pharmacological agents targeting such dysregulated signaling components for evaluating their relevance as pharmacological targets is needed. First examples show that targeting AKAPs and their protein-protein interactions with small molecules is possible. For instance, an AKAP-PKA interaction inhibitor, FMP-API-1 [201] was identified. Recently, a novel small molecule, Scaff10-8, was developed, which inhibits the interaction of AKAP-Lbc and RhoA and prevents the AKAP-Lbc-mediated RhoA activation, an event pathologically activated in models of cardiac hypertrophy [102]. Further molecules directed against the AKAP-Lbc-RhoA interface have recently been identified and may serve to guide to further preclinical drug development efforts [202,203].

Approved inhibitors of PDEs target the catalytic activities of PDEs. However, the catalytic domains of the various members of individual families are identical and inhibition of one inhibits all. This lack of selectivity presumably explains PDE inhibitor therapy-associated side effects, which are frequent and dramatic over long-term administration [158]. PDE isoform-selective inhibition may be achieved through disruption of specific protein-protein interactions. and therefore the displacement of particular PDE isoforms from their subcellular compartments [204].

In conclusion, targeting proteins directing compartmentalized cAMP signaling, in particular AKAPs and PDEs, not only serves to understanding their role in heart and vascular physiology and pathophysiology but also has therapeutic potential for the treatment of a wide range of cardiovascular diseases.

Acknowledgments: This work was supported by grants from the Else Kröner-Fresenius-Stiftung (2013_A145), the German-Israeli Foundation (G.I.F. I-1210-286.13/2012), the German Centre for Cardiovascular Research (DZHK 81X210012 and B18-005 SE), the Deutsche Forschungsgemeinschaft (DFG KL1415/7-1) and the Bundesministerium für Bildung und Forschung (BMBF; 16GW0179K) to EK.

Conflicts of Interest: The authors declare no conflict of interest.

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