Phosphodiesterases and subcellular compartmentalized cAMP signaling in the cardiovascular system

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Stangherlin A, Zaccolo M. Phosphodiesterases and subcellular compartmentalized cAMP signaling in the cardiovascular system. Am J Physiol Heart Circ Physiol 302: H000–H000, 2012. First published October 28, 2011; doi:10.1152/ajpheart.00766.2011.—Phosphodiesterases are key enzymes in the cAMP signaling cascade. They convert cAMP in its inactive form 5′-AMP and critically regulate the intensity and the duration of cAMP-mediated signals. Multiple isoforms exist that possess different intracellular distributions, different affinities for cAMP, and different catalytic and regulatory properties. This complex repertoire of enzymes provides a multiplicity of ways to modulate cAMP levels, to integrate more signaling pathways, and to respond to the specific needs of the cell within distinct subcellular domains. In this review we summarize key findings on phosphodiesterase compartmentalization in the cardiovascular system.

Adenosine 3′,5′-cyclic monophosphate; compartmentalization

Introduction

Adenosine 3′,5′-cyclic monophosphate (cAMP) is a pleiotropic second messenger that converts the signal of a large variety of extracellular stimuli into specific cellular responses. When hormones and neurotransmitters bind to G protein-coupled receptors (GPCRs) and trigger the release of the Gs α-subunit from the heterotrimeric G protein complex, Gs α-subunit activates the adenyl cyclase (AC), a plasma membrane-bound enzyme that catalyzes the conversion of ATP to cAMP. Phosphodiesterases (PDEs) are metallohydrolases that catalyze the breakdown of cAMP into the inactive 5′-AMP, thus modulating the duration and the intensity of the cAMP intracellular response.

cAMP exerts its functions through the activation of three effectors: protein kinase A (PKA), the exchange protein directly activated by cAMP (Epac), and cyclic nucleotide-gated (CNG) ion channels. The most studied cAMP effector is PKA, a heterotetramer composed of a dimer of two regulatory subunits (R) each bound to a catalytic subunit (C). Two isoforms of the R subunit (RI and RII) have been described that classify PKA into either PKA-RI or PKA-RII isoforms (129). When cAMP binds to the R subunits (two molecules of cAMP for each R subunit) the C subunits are released and trigger a series of signaling cascades via phosphorylation of serine and threonine residues. PKA mediates a variety of cellular functions via direct phosphorylation of its targets and via cross talk with other signaling pathways (40). Cell growth and differentiation, cell movement and migration, hormone secretion, cellular metabolism, and gene transcription are only some of the many biological functions that are regulated by PKA (43). In the cardiovascular system, PKA controls the vascular tone via regulation of K+ channels (57) and modulation of Ca2+ sensitivity of the contractile apparatus in smooth muscle cells (97), and vascular permeability through phosphorylation of myosin light chain in endothelial cells (92). In addition, PKA mediates the catecholaminergic control over the force and frequency of cardiac contraction (139) via phosphorylation of proteins in cardiac myocytes that are part of the excitation-contraction coupling system such as L-type Ca2+ channels (LTCCs), ryanodine receptors (RyRs), and phospholamban (PLB) (11). A second, recently described effector of cAMP is Epac (34, 59), a protein that functions as guanine nucleotide exchange factor for the small G proteins Rap1 and Rap2 (33). Two variants of Epac exist, Epac1 and Epac2, which show different tissue distributions. Epac1 is expressed ubiquitously with high expression in the heart, whereas Epac 2 is detected mainly in the brain (34, 47, 59, 61). The binding of cAMP to the amino-terminal cAMP-binding site in Epac releases an intramolecular autoinhibition and results in Epac activation (114). This promotes an accumulation of activated Rap proteins and/or R-Ras with subsequent signaling to phospholipase Cε, phospholipase D, Rap1, p38-MAPK, and phosphatidylinositol 3-kinase (PI3K) (47, 69, 119). Epac is involved in integrin-mediated cell adhesion (111) and vascular endothelial cell barrier formation (45, 63). In cardiac myocytes, Epac has been shown to regulate the assembly of GAP junctions (124) and to influence Ca2+ mobilization (99), RyR phosphorylation (103),
of structurally unrelated proteins able to bind PKA and seques-
ter it into macromolecular complexes (68, 118). PKA and
AKAPs interact via a conserved amphipatic α-helix on the
AKAP (95) and a hydrophobic groove formed by the dimeriza-
tion/docking domains located at the NH₂-terminus of PKA R
subunits (48, 62). For a long time PKA-RII was thought to be
the only isoform anchored to AKAPs since it was mainly found
in the particulate fraction in myocyte lysates (28), whereas
PKA-RI was mostly recovered in the soluble fraction. How-
ever, it was recently shown that both PKA-RI and PKA-RII
reside in physically segregated and distinct compartments
in cardiac myocytes and that a substantial fraction of PKA-RI
is also anchored to specific subcellular sites, although with lower
binding affinity than PKA-RII (36). AKAPs are therefore
classified as RI-selective, RII-selective, or dual-specificity
AKAPs, depending on the R-subunit isoforms for which they
show higher affinity of binding (52). The fact that PKA is
physically constrained in defined subcellular compartments
allows for the activation of specific subsets of PKA and the
phosphorylation of selected targets in response to specific
cAMP-raising stimuli. In the heart, overexpression of Ht31, a
peptide that competes with endogenous AKAPs for binding to
R subunits (24), was shown to affect substrate phosphorylation
by PKA and cardiomyocyte shortening, demonstrating the
functional relevance of PKA anchoring to AKAPs (41). In
cardiac myocytes a number of AKAPs that tether PKA in
proximity to critical substrate involved in the control of con-
tractility, Ca²⁺ handling, and action potential duration have
been identified. A complex involving AKAP18α (AKAP7),
PKA, and the LTCC mediates PKA-dependent phosphoryla-
tion of the channel and the subsequent control of its opening
probability and conductance (44, 49). Another AKAP, Yotiao
(AKAP9), recruits PKA and protein phosphatase 1 to the
carboxyl terminus of the slow delayed rectifier K⁺ channel
(Iₛₑ), thus controlling its phosphorylation state that is critical
for the response to sympathetic stimulation (77, 134). The
muscle-selective mAKAP (AKAP6) tethers PKA to the RyR
and allows PKA-mediated phosphorylation of the channel (78,
79). AKAP18δ (AKAP7) tethers PKA in proximity to PLB and
favors its PKA-mediated phosphorylation. The manipulation of
the AKAP18δ/PLB/PKA complex, in particular, the disruption
of AKAP18δ-PLB interaction via a competing peptide or
knockdown of AKAP18δ, were shown to significantly
decrease the speed of Ca²⁺ reuptake in the sarcoplasmic reticu-
Timely, myomelagin was found to bind PKA and to
regulate the phosphorylation of cardiac myosin binding protein
C, an event that protects myosin binding protein C against
degradation (131) and that may be important for normal sar-
comeric integrity and normal physiological cardiac function.

It should be noted that whereas the disruption of AKAP-
PKA interactions using disrupting peptides such as Ht31
has confirmed the functional relevance of PKA compartmentaliza-
tion, the use of more selective disruptors that interfere with the
anchoring of a specific AKAP to a subcellular structure selec-
tively affects the function by selectively removing PKA from
a specific target and has been proposed as a potential novel
therapeutic avenue (72).

Interestingly, evidence is also accumulating that different
anchoring mechanisms for Epac exist that mediate its spatial
regulation. Epac was found to localize at many intracellular
sites such as the nucleus, microtubules, centrosome, and mito-
chondria (109). In neonatal and adult rat ventricular myocytes,
Epac shows a specific pattern of localization at the sarcolemma and perinuclear regions (37, 87). Intracellular localization of Epac proteins is achieved by the membrane targeting disheveled/Egl-10/pleckstrin domain (33, 105) and Ras-association domain (67). Evidence is emerging that Epac is involved in many intracellular complexes and protein-protein interactions (37, 137). For example, Epac was found to interact with β-arrestin and Ca2+/calmodulin kinase II (CaMKII) (75). The stimulation of β1-adrenergic receptors (B1-ARs) was shown to mediate the recruitment of the complex at the plasma membrane where cAMP binding to Epac subsequently activates CaMKII via a RapGTP and phospholipase Cε/PKCε signaling pathway and triggers the phosphorylation of downstream targets (75).

Localized PDEs Sustain cAMP Compartmentalization

Although the anchoring of PKA at specific subcellular sites is important for its function, mechanisms that constrain cAMP diffusion appear to be necessary to generate stimulus-specific responses. On the basis of cAMP molecular weight and on cell viscosity, the diffusion constant of cAMP can be estimated to be as fast as 700 μm²/s (100). Given the organization of the sarcolemma in T tubules that run parallel to sarcomeric Z lines and are spaced only a few micrometers apart, if the spatial diffusion of cAMP were not regulated, cAMP generated at the plasma membrane by ACs would fill the entire cell within a fraction of a second. This would clearly overcome any benefit of having PKA tethered to specific targets via AKAPs, since cAMP would reach and activate all targeted PKA subsets. PDEs are the only enzymes committed to cAMP degradation and therefore are good candidates to fulfill the task of regulating the spatial diffusion of cAMP signals.

PDEs are subdivided in 11 families (PDE1–11), encoded by 21 different genes. More than 80 enzyme variants are generated from multiple promoters and as a consequence of alternative splicing (42). PDE1, PDE2, PDE3, PDE10, and PDE11 are dual-specificity PDEs since they degrade both cAMP and cGMP: PDE4, PDE7, and PDE8 specifically degrade cAMP, whereas PDE5, PDE6, and PDE9 specifically degrade cGMP (42). As a general feature, PDEs possess a highly conserved catalytic domain that harbors the cyclic nucleotide binding site and a less-conserved NH2-terminal domain that confers different regulatory properties to each family and to individual isoforms. The NH2-terminal portion of the enzyme may undergo phosphorylation/dephosphorylation events, binding of Ca2+/calmodulin, and allosteric binding of cGMP and can mediate interactions with other proteins. PDE1e, -3, -4, and -5 contain phosphorylation sites for various kinases. PDE1e also contains Ca2+-calmodulin binding sites, and stimuli that increase or decrease intracellular Ca2+ profoundly affect its activity. PDE2, PDE6, and PDE9 contain allosteric binding sites for cGMP, named GAF domains (originally identified in cGMP-binding PDEs, Anabaena AC, and Escherichia coli FhIA). The binding of cGMP to GAFB in PDE2 activates the enzyme, whereas the binding of cGMP to GAFA in PDE5 favors PKG-mediated phosphorylation and activation of the enzyme [reviewed in (42, 70)].

Interestingly, PDE isoforms show not only different tissue distributions but also different intracellular localizations, and for some of them, targeting to specific membranes is conferred by their NH2-terminal portion. For example, PDE2 enzymes are partitioned between membrane and cytosol, depending on their NH2-terminal domains. The NH2-terminus of PDE2A1 is more hydrophilic than the one of PDE2A2 or PDE2A3 and shows cytosolic distribution (1, 117). PDE2A3 was found to localize to synaptic membranes (117), whereas PDE2A2 has recently been shown to localize to mitochondria (1). PDE3 instead contains two NH2-terminal hydrophobic regions (NHR1 and NHR2) that provide for membrane association (60, 122). PDE4A1 contains a NH2-terminal domain (TAPAS-1) that interacts with phosphatidic acid and provides for membrane insertion (4), whereas PDE4A5 is targeted to the cell membrane via the upstream conserved region 2 (7). In addition to showing different membrane distributions, PDEs can also be sequestered in a number of signaling complexes with selective interactions with other proteins. For example, PDE2A is found to interact with the immunophilin XAP2 through its GAFB domain and to regulate the nuclear translocation of the aroyl hydrocarbon receptor (32). PDE4A4/5 and PDE4D4 interact with Src-homology-3 domain-containing proteins Src, Fyn, and lyn (7, 8, 85, 98). PDE3A has been reported to interact with cystic fibrosis transmembrane conductance regulator and to modulate its activity (102), whereas PDE3A interacts with specific guanine nucleotide-exchange factors BIG2 and regulates the activity of ADP-ribosylation factors that are involved in vesicular trafficking (107). Other examples of protein-protein interactions involving PDEs include PDE4D3 and myomoglobin (133), PDE4D5 and receptor for activated C-kinase 1 (16, 141), and PDE4D5 and β-arrestin (3, 104), PDE4D and gravin (AKAP12) (136). Recently, members of the PDE4 family have been shown to bind to the small heat shock protein (Hsp20) (123). Hsp20 is an intracellular chaperone that has been shown to exert cardioprotective effects. The levels of Hsp20 and its phosphorylation are increased on ischemic insults, and its overexpression protects the heart against ischemia-reperfusion injury (108). In addition, Hsp20 protects cardiomyocytes against apoptosis, and its phosphorylation at Ser16 enhances cardioprotection (96). PDE4 has been recently shown to directly bind to Hsp20 and to control cAMP levels around the Hsp20 complex. Inhibition of PDE4 or disruption of the HSP20-PDE4 complex via a specific competing peptide was shown to increase PKA-mediated phosphorylation of HSP20 and to promote its cardioprotective function in a model of β-agonist-induced hypertrophy (123).

A similar approach has been applied in a study on a cancer cell model where PDE4D5 has been shown to interact with focal adhesion kinase (FAK) and receptor for activated C-kinase. The displacement of PDE4D5 from the PDE4D5/RAK1/FAK complex resulted in a dramatic reduction of cell polarization and invasive phenotype (120).

Of note, the use of competing peptides to remove a specific PDE isoform from a particular complex allows the manipulation of cAMP levels selectively at that specific location without affecting global cAMP levels. This approach offers the possibility to overcome the current limitations of PDE inhibitors, which do not distinguish between PDE isoforms of the same family. Indeed, targeting only one specific complex and its related pathway may avoid many of the off target effects of current PDE inhibitors.

Interestingly, the interaction of some PDEs with other proteins can affect the catalytic and regulatory properties of the
enzyme. PDE1, for example, is regulated by binding of Ca2+/calmodulin to the NH2-terminus of the enzyme, which results in up to a 10-fold increase in its enzymatic activity (125). PDE4A5 interacts with XAP2 through its upstream-conserved region-2 domain, and such interaction reversibly inhibits the enzymatic activity of PDE4A5, increases the sensitivity of PDE4A5 to the PDE4 inhibitor rolipram, and attenuates the ability of PKA to phosphorylate PDE4A5 in intact Cos7 cells (17). The intracellular localization and the composition of signaling units that include PDEs may dynamically change upon specific cellular events. For instance, upon β-adrenergic stimulation, the complex PDE4D5-β-arrestin is recruited at the plasma membrane, where PDE4D5 controls PKA-mediated phosphorylation events that lead to the desensitization of the β-ARs (6, 73). Other examples are the release of PDE4D3 and PDE4C2 isoforms from disrupted-in-schizophrenia 1 (DISC1) upon elevation of intracellular cAMP levels (93), the release of PDE4D3 from the associated nuclear distribution element-like (Nudel) upon Nudel phosphorylation (27), and the binding of PDE3B to 14-3-3 proteins upon PDE3B phosphorylation (106).

PDEs, therefore, appear to be strategically positioned or recruited to specific subcellular domains according to the specific needs, and the existence of a multiplicity of PDE isoforms suggests that many different combinations of signaling complexes may coexist within the same cell to supply tailored control on localized cAMP dynamics (Fig. 1).

The involvement of PDEs in the compartmentalization of cAMP signaling was suggested in a study performed in frog ventricular myocytes. By using a whole cell patch-clamp recording technique and a double perfusion system, the authors (58) showed that local stimulation with the β-agonist Iso induced a fast increase in LTCC current nearby the site of application and had very little effect on distal regions of the cells not exposed to the stimulus. On the contrary, the unselective ACs activator forskolin (FRSK) induced a slower but homogeneous increase in cAMP in both local and distant regions. This compartmentalization was abolished by the non-selective inhibitor 3-isobutyl-1-methylxanthine (IBMX), suggesting that cAMP-degrading PDEs play a critical role in limiting the amount of cAMP diffusing from the membrane to the cytosol and allow for the accumulation of cAMP in specific subcellular domains.

The role of PDEs in compartmentalization of cAMP was better characterized after the development of a more direct approach to monitor cAMP in single living cells based on fluorescent proteins, fluorescence resonance energy transfer (FRET), and real-time imaging microscopy. This approach relies on genetically encoded sensors constituted of one or more cAMP binding domains and a FRET pair, usually cyan fluorescent protein and yellow fluorescent protein. In these sensors the distance between the fluorophores varies as a consequence of a conformational change that occurs upon cAMP binding (10, 46) (Fig. 2). Real-time imaging of cAMP...
using genetically encoded FRET optical sensors allowed for the first time a direct visualization of cAMP microdomains (146, 147). In neonatal rat ventricular myocytes (NRVMs), the stimulation of β2ARs was shown to generate localized increases in cAMP levels in correspondence of Z lines that could be directly imaged using the FRET reporters. Such cAMP gradients were dissipated by application of the PDEs inhibitor IBMX, demonstrating not only that cAMP is generated inside the cell in restricted microdomains but also that its diffusion is prevented by the activity of PDEs (146). Subsequent analysis of cAMP dynamics in NRVMs showed that different PDE isoforms are sequestered into defined intracellular locations and confirmed the role of localized PDEs in shaping cAMP gradients (88, 90). The impact of spatially confined PDE isoforms in determining local cAMP signals was further confirmed in a study in HEK293 cells (130) using a dominant negative approach (84). This approach exploits the overexpression of a catalytically inactive PDE isoform to displace its endogenous cognate and has been successfully applied to establish the importance of the specific localization of individual PDE enzymes. In fact, while the available pharmacological PDE inhibitors cannot distinguish between different isoforms within the same family, the displacement approach allows for the compartment- and subtype-specific blockade of PDEs enzymatic activity. In their paper Terrin et al. (130) showed that in HEK293 cells, PDE4B and PDE4D isoforms are spatially organized and selectively modulate the concentration of cAMP in individual subcellular compartments. PDE4B is localized at the plasma membrane, whereas PDE4D is mainly distributed in the cytosol. By the use of FRET biosensors targeted to different subcellular compartments, PGE1 was found to generate a higher cAMP response at the plasma membrane and nuclear compartments compared with the bulk cytosol. These multiple and simultaneous cAMP gradients are generated by the combined activity of PDE4B and PDE4D. In particular, PDE4D acts as a drain for cAMP and keeps the level of the second messenger low in the cytosol. Accordingly, the displacement of the endogenous PDE4D isoform disrupts the cAMP gradients generated by PGE1 (130). Similar results were obtained in a study using mouse embryonic fibroblasts isolated from PDE4B−/− and PDE4D−/− transgenic mice where the authors (14) confirmed that PDE4B and PDE4D have specific functions in controlling cAMP levels in different subcellular domains.

**PDEs and Compartmentalized cAMP Signaling in the Cardiovascular System**

In the cardiovascular system, eight PDE families are known to be expressed, PDE1, -2, -3, -4, -5, -7, -8, and -9 (42) (Fig. 3), and the expression of different variants and their relative abundance varies from species to species. PDE1 is expressed in the cardiac muscle in a number of species and was reported to be absent from cardiac myocytes, at least in rats (15). However, a large amount of Ca2+/calmodulin-stimulated cAMP hydrolytic activity was found in soluble fractions of human myocardium (50). This activity was later attributed to PDE1C isoform, which is distributed along the Z lines and M lines of human cardiac myocytes (132). In NRVMs, PDE2A represents about 1% of total cAMP hydrolytic activity and was found to localize at the plasma membrane in correspondence of the cell-to-cell junctions and at the sarcomeric Z lines (89). Despite its low contribution to the degradation of total cAMP, PDE2 is responsible for the degradation of a large portion of the cAMP pool.
cAMP generated by β-AR stimulation and mediates the negative inotropic effects of nitric oxide (NO) (89). In human myocardium, PDE3 constitutes the majority of cAMP-degrading PDE activity (91), and the impact of different isoforms on cAMP hydrolysis is different in cytosolic and microsomal fractions (50). Long forms of PDE3, PDE3A1, and PDE3B1 contain both NHR1 and NHR2 and are recovered in the microsomal fraction of human myocardium; PDE3A2 lacks one NHR domain and is present in both microsomal and cytosolic fractions, whereas PDE3A3 lacks both NHR1 domains and is primarily cytosolic (50, 135). In NRVMs, immunolocalization of PDE3 and PDE4 indicates that PDE4B and PDE4D localizes at the sarcomeric Z lines, whereas PDE3A shows a widespread and irregular intracellular staining compatible with localization on internal membranes (88). In a study in adult mouse ventricular myocytes, PDE4B was shown to localize at the transverse tubules (66). In myocytes isolated from neonatal rats, PDE3 and PDE4 account for 90% of total cAMP hydrolytic activity and possess distinct functions in the control of cAMP dynamics (88). Real-time imaging of cAMP showed that upon Iso stimulation, the inhibition of all PDE3s gives rise to a small increase in cAMP, whereas partial inhibition of PDE4 is sufficient to generate a large increase in cAMP, suggesting that PDE4 is involved in the control of a pool of cAMP generated upon activation of β-ARs. On the contrary, in the presence of FRSK, selective inhibition of PDE3 generates a larger cAMP response than selective inhibition of PDE4 (88).

In cardiac myocytes, PDE4D3 is part of at least two important macromolecular complexes organized by the AKAPs Yotiao and mAKAP, respectively. In the complex generated by Yotiao, PDE4D3 regulates PKA-mediated phosphorylation of the KCNQ1 subunit of the IKs. Phosphorylation at Ser27 on KCNQ1 regulates the Ks and the Ks. Phosphorylation at Ser2809 on RyR2 and the duration of the action potential (77). mAKAP organizes a macromolecular complex that involves PKA, PDE4D3, Epac, and the extracellular signal-regulated kinase 5 (ERK5). mAKAP is unique in its ability to bind both cAMP effectors, PKA and Epac, and to integrate these two signaling pathways (37). Within this complex, PDE4D3 is involved in two feedback control loops that rely on phosphorylation events. PKA-mediated phosphorylation of PDE4D3 at Ser54 and Ser13 strengthens the interaction between mAKAP and PDE4D3 and increases PDE4D3 enzymatic activity to achieve more rapid termination of the cAMP signal (23, 121). Conversely, ERK5-mediated phosphorylation of PDE4D3 at Ser579 inhibits PDE4D3, leads to an increase in cAMP, and triggers a signaling cascade initiated by Epac (5, 54, 74). Recently, RyR has been found in a signaling complex with PDE4D3, PKA, and mAKAP (79).

In this complex, PKA has been suggested to regulate the open probability of the channel via phosphorylation of Ser2809 (79). According to this model, PDE4D3 may serve to prevent excessive phosphorylation of the channel that may lead to diastolic Ca\(^{2+}\) leak and decreased contractility and cardiac output (65), although the impact of PKA-mediated phosphorylation on RyR function and heart failure is still controversial (31, 79, 115). For example, decreased cAMP levels have been found in failing human heart (113) and abnormal Ca\(^{2+}\) release has been found also in the presence of normal levels of phosphorylated RyRs (56). However, PDE4D\(^{-/-}\) mice show hyperphosphorylated “leaky” RyRs and develop dilated cardiomyopathy and exercise-induced ventricular arrhythmia (65).

Another important complex in the heart involves AKAP15/18, PKA, and the LTCC. PKA phosphorylates and increases LTCC activity, thereby enhancing the L-type Ca\(^{2+}\) current. The regulation of this process is fundamental for the excitation-contraction coupling, and the PDEs involved in the regulation of cAMP dynamics within this complex have been recently described. In a study using knockout models, two PDE4 enzymes, PDE4B and PDE4D, were shown to coimmunoprecipitate with Ca\(_{\text{a1.2}}\), a subunit of L-type voltage-dependent Ca\(^{2+}\) channel (66), although surprisingly only the inactivation of the PDE4B gene, but not inactivation of the PDE4D gene, appeared to be involved in the regulation of Ca\(^{2+}\) currents upon β-AR stimulation in this model (66).

Although the importance of localized PDEs in the control of compartmentalized cAMP signaling emerges mainly from studies in cardiac myocytes, PDEs are expressed also in other cellular components of the cardiovascular system, and recent studies show that they regulate important functions in these cells via similar mechanisms. For example, in aortic vascular smooth muscle cells (VSMCs), mAKAP organizes a signaling complex that includes PKA and PDE4D8 (112). This complex is enriched in pseudopodia of VSMCs where PDE4D8 seems to regulate actin cytoskeletal dynamics. Interestingly, in contrast to what has been reported in cardiac myocytes (38), PDE4D8 and not PDE4D3 was found in complex with PKA and mAKAP. These findings suggest that different cells may use distinct PDE4D variants to regulate selected pools of targeted PKA activity and that the disruption of this complex may allow for a selective manipulation of cAMP-dependent events in different cell types. In human arterial endothelial cells (HAECs), PDE3B was shown to interact with the PI3K regulatory subunit p84 and Epac (137). In this complex, PDE3B-tethered EPAC1 regulates PI3K activity, and this allows dynamic cAMP-dependent regulation of cell adhesion, cell spreading, and tubule formation. Other PKA and Epac-based signaling complexes were isolated from both HAECs and human microvascular endothelial cells (HMVECs) by using a cAMP-agarose adsorption method (94). Immunoblot analysis of the isolated complexes showed that HAECs and HMVECs contain a combination of PKA and Epac signaling units populated with either PDE3B or PDE4D (94). However, while Epac, PDE4D, or PDE3B seem to regulate cell adhesion in HMVECs, the functional link between these complexes and cell adhesion appears to be missing or to be less important in HAECs. In another study, PDE4D was shown to regulate the activity of Epac1 and to control HAECs vascular permeability (110). Indeed, the inhibition of PDE4 but not PDE3 increased the ability of cAMP raising agents to antagonize vascular endothelial growth factor-induced permeability. In addition, PDE4D was found to bind to Epac and to promote its integration into vascular endothelial cadherin adhesions and therefore to decrease vascular permeability (110). Taken together, this data suggest that multiple, nonoverlapping signaling complexes including Epac, PKA, and PDEs are expressed in vascular cells and specifically coordinate the effects of cAMP on vascular physiology.
concentrations of cGMP were found to increase the L-type Ca\(^{2+}\) current via PDE3 inhibition, whereas a higher concentration of cGMP strongly reduced LTCC current via activation of PDE2 (86). Furthermore, in endothelial cells, low doses of the cGMP-raising agents atrial natriuretic peptide (ANP) and NO donors potentiated the inhibitory effects of the unselective ACs activator FRSK on thrombin-induced permeability (128). However, the inhibitory effect of FRSK was reversed when higher doses of ANP or NO were applied. These opposing effects suggest that cGMP at lower concentrations inhibits PDE3 and thereby increases cAMP, whereas at higher concentrations it activates PDE2 and decreases cAMP levels. In NRVMs, cGMP was shown to blunt the β-adrenergic response via activation of PDE2. In this study, PDE2 was proposed to be tightly coupled to a pool of ACs activated by the β-adrenergic pathway and to shape the cAMP response to catecholamines (89). PDE2, by integrating β1/2cAMP and β3/cGMP pathways, was proposed to coordinate a feedback control loop that protects the myocytes from excessive adrenergic stimulation. More recently, it was shown that cGMP modulates the response of cardiac myocytes to catecholamines in a compartment-selective manner (126) (Fig. 4). Real-time imaging of cAMP and cGMP with FRET-based sensors targeted to different intracellular compartments showed that the impact of cGMP on cAMP signals depends on the cyclase that generates cGMP and on the PDEs associated with each compartment. cAMP and cGMP sensors were engineered to achieve selective targeting to the subcompartments in which PKA-RI and PKA-RII are normally localized (36, 126). PDE3 activity was found to be mainly coupled with the PKA-RI compartment, whereas PDE2 activity was found to be mainly coupled to the PKA-RII compartment. Stimulation of the soluble guanylyl cyclase (sGC) increased the cAMP response to catecholamine selectively in the PKA-RI compartment via inhibition of PDE3, whereas it reduced the cAMP response in the PKA-RII compartment via activation of PDE2. The effect of cGMP on the local cAMP signals was found to be different when cGMP was

**PDEs at the Crossway Between cAMP and cGMP Signaling Pathways**

Thanks to their ability to degrade both cyclic nucleotides and to be regulated by cGMP, some PDEs allow for a complex interplay between cAMP and cGMP signals. PDE1, PDE2, and PDE3 can degrade both cAMP and cGMP, although they show for the two cyclic nucleotides different affinities and different hydrolytic properties (83). In addition, cGMP can modulate the activity of these enzymes. PDE1 is mainly regulated by Ca\(^{2+}\)-calmodulin, and substrate specificity significantly differs among the different isoforms with PDE1A and PDE1B, showing a preference for cGMP, whereas PDE1C hydrolyzes the two cyclic nucleotides equally well (9). cGMP-mediated inhibition of PDE1 has been demonstrated only in vitro, and its relevance in vivo has not been established yet. PDE2 has a similar affinity for both cAMP and cGMP. cGMP also binds to the PDE2 GAFB domain, thus increasing the rate of cAMP hydrolysis by about sixfold (76). Interestingly, PDE3 has comparable affinity for both cAMP and cGMP, but because of a lower catalytic rate for cGMP than for cAMP, the enzyme is inhibited by cGMP. Therefore, cGMP, by modulating PDEs activity, can impinge on cAMP signaling cascades. The interplay between cAMP and cGMP signals through PDEs has been suggested to occur in a number of cell types (80). For example, in rat aortic endothelial smooth muscle cells, an increase in cGMP levels induced by the NO donor sodium nitroprusside potentiates the relaxation induced by Iso (81, 82). Such a mechanism, which involves the generation of cGMP and the inhibition of PDE3, was found to occur also in rat aortic rings (35) and in VSMCs (101). Of note, the effect of cGMP on dual-specificity PDEs has been shown to be concentration dependent (145). At a concentration of <50 nM, cGMP inhibits PDE3; at concentration between 200 and 500 nM, cGMP activates PDE2; and at concentrations of >1 μM, cGMP also affects PDE1 activity (145). In frog ventricular myocytes, low concentrations of cGMP were found to increase the L-type Ca\(^{2+}\) current via PDE3 inhibition, whereas a higher concentration of cGMP strongly reduced LTCC current via activation of PDE2 (86). Furthermore, in endothelial cells, low doses of the cGMP-raising agents atrial natriuretic peptide (ANP) and NO donors potentiated the inhibitory effects of the unselective ACs activator FRSK on thrombin-induced permeability (128). However, the inhibitory effect of FRSK was reversed when higher doses of ANP or NO were applied. These opposing effects suggest that cGMP at lower concentrations inhibits PDE3 and thereby increases cAMP, whereas at higher concentrations it activates PDE2 and decreases cAMP levels. In NRVMs, cGMP was shown to blunt the β-adrenergic response via activation of PDE2. In this study, PDE2 was proposed to be tightly coupled to a pool of ACs activated by the β-adrenergic pathway and to shape the cAMP response to catecholamines (89). PDE2, by integrating β1/2cAMP and β3/cGMP pathways, was proposed to coordinate a feedback control loop that protects the myocytes from excessive adrenergic stimulation. More recently, it was shown that cGMP modulates the response of cardiac myocytes to catecholamines in a compartment-selective manner (126) (Fig. 4). Real-time imaging of cAMP and cGMP with FRET-based sensors targeted to different intracellular compartments showed that the impact of cGMP on cAMP signals depends on the cyclase that generates cGMP and on the PDEs associated with each compartment. cAMP and cGMP sensors were engineered to achieve selective targeting to the subcompartments in which PKA-RI and PKA-RII are normally localized (36, 126). PDE3 activity was found to be mainly coupled with the PKA-RI compartment, whereas PDE2 activity was found to be mainly coupled to the PKA-RII compartment. Stimulation of the soluble guanylyl cyclase (sGC) increased the cAMP response to catecholamine selectively in the PKA-RI compartment via inhibition of PDE3, whereas it reduced the cAMP response in the PKA-RII compartment via activation of PDE2. The effect of cGMP on the local cAMP signals was found to be different when cGMP was

Fig. 4. cAMP/cGMP interplay in cardiac myocytes. cGMP signals can modulate cAMP in a compartment-selective manner. cGMP can modulate the activity of PDE2 and PDE3 (activation of PDE2 and inhibition of PDE3, respectively) and therefore can modulate the response to catecholamines in defined subcellular compartments. In cardiac myocytes, for example, activation of the soluble guanylyl cyclase (sGC) increases the cAMP signal generated upon β-adrenergic stimulation selectively in the PKA-RI compartment via PDE3 inhibition whereas it decreases cAMP in the PKA-RII compartment via PDE2 activation. Stimulation of particulate guanylyl cyclase (pGC) affects the PKA-RII compartment only and blunts the cAMP response via activation of PDE2.
generated by the particulate guanylyl cyclase (pGC). ANP treatment resulted in a reduction in cAMP level selectively in the PKA-RII compartment, whereas the PKA-RI compartment was not affected, indicating that the effect of cGMP on cAMP signals depends on the source of cGMP. It is interesting to note that these findings also support the notion that, similar to cAMP, cGMP signals are also compartmentalized, since cGMP signals generated by sGC and pGC do not seem to be able to reach all targets indiscriminately. The cGMP-mediated modulation of PDEs activity was shown to have a profound impact on PKA activity and on phosphorylation of downstream targets. Indeed, PKA activity was found to be reduced in the PKA-RII compartments following stimulation of both the sGC and the pGC, but it was selectively increased in the PKA-RI compartment by the activation of the sGC. Importantly, the reduced response to catecholamines in the PKA-RII compartment was paralleled by reduced phosphorylation of targets involved in excitation-contraction coupling system and by reduced myocyte contractility (126).

Conclusions

It is now appreciated that PDE activity is critical for spatial and temporal regulation of cAMP signal propagation. PDEs are strategically distributed within the cell and selectively regulate cAMP signals triggered by individual GPCRs. The existence of multiple PDE variants with different catalytic and regulatory properties permits the simultaneous generation of multiple and unique signaling complexes that generate tailored cAMP signals within distinct subcompartments. Any perturbations that may affect this tight control on cAMP signals very likely results in abnormal cAMP responses and may lead to pathological conditions. Mapping the complex network of intertwined signaling units and understanding the composition of each unit and the protein–protein interactions involved are a challenge for the future. However, recent data suggest that it is possible to selectively manipulate cAMP in distinct subcellular compartments via selectively displacing PDE-AKAP modules or PDE isoforms from specific macromolecular complexes. In addition, the possibility to modulate PDE activity, for example, by activating PDE2 or inhibiting PDE3 via cGMP, offers an alternative route to increase or decrease cAMP levels in specific subcellular domains. Although our knowledge of the precise composition of individual signaling units is still rudimentary, it is possible to envisage a future in which local manipulation of cAMP levels may be used as a therapeutic strategy to increase effectiveness and reduce off-target effects.

DISCLOSURES

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