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REVIEW

cAMP signal transduction in the heart: understanding spatial control for the development of novel therapeutic strategies

Manuela Zaccolo

Neuroscience and Molecular Pharmacology, Faculty of Biomedical and Life Sciences, University of Glasgow, Glasgow, Scotland, UK

3′-5′-cyclic adenosine monophosphate (cAMP) is a pleiotropic intracellular second messenger generated in response to activation of G_s protein-coupled receptors. In the heart, cAMP mediates the catecholaminergic control on heart rate and contractility but, at the same time, it is responsible for the functional response to a wide variety of other hormones and neurotransmitters, raising the question of how the myocyte can decode the cAMP signal and generate the appropriate functional output to each individual extracellular stimulus. A growing body of evidence points to the spatial organization of the components of the cAMP signalling pathway in distinct, spatially segregated signalling domains as the key feature underpinning specificity of response and data is emerging, indicating that alteration of spatial control of the cAMP signal cascade associates with heart pathology. Most of the details of the molecular organization and regulation of individual cAMP signalling compartments are still to be elucidated but future research should provide the knowledge necessary to develop and test new therapeutic strategies that, by acting on a limited subset of downstream targets, would improve efficacy and minimize off-target effects.
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Abbreviations: AC, adenylyl cyclase; AKAP, A kinase-anchoring proteins; C, catalytic subunit of protein kinase A; cAMP, 3′-5′-cyclic adenosine monophosphate; FRET, fluorescence resonance energy transfer; GPCR, G-protein-coupled receptor; HF, heart failure; Iso, isoproterenol; LTCC, L-type Ca^{2+} channel; NHE, sodium-hydrogen exchanger; PDE, phosphodiesterase; PGE1, prostaglandin E1; PKA, protein kinase A; PLB, phospholamban; R, regulatory subunit of PKA; RyR, ryanodine receptor; SERCA, sarcoplasmic reticulum Ca^{2+} ATPase; TnI, troponin I; TRP, transient receptor potential

3′-5′-cyclic adenosine monophosphate (cAMP) is a small and diffusible intracellular second messenger generated in response to binding of a number of hormones and neurotransmitters to G-protein-coupled receptors (GPCRs). cAMP activates a limited number of intracellular targets including protein kinase A (PKA), the exchange proteins activated by cAMP and cyclic nucleotide-gated channels, and by doing so it controls a bewildering number of cellular functions, ranging from cell growth and differentiation to cell movement and migration, from learning and memory formation to control of hormone secretion, metabolism and gene transcription (Francis and Corbin, 1994). Even at the single cell level, the cAMP/PKA signalling system is involved in a multitude of diverse functions. In cardiac myocytes, cAMP generated in response to catecholamine-mediated, β-adrenoceptors stimulation modulates excitation contraction coupling by activating PKA and the subsequent phosphoryla-

Correspondence: Manuela Zaccolo, Neuroscience and Molecular Pharmacology, Faculty of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK. E-mail: m.zaccolo@bio.gla.ac.uk
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tion of the L-type Ca^{2+} channel (LTCC) and the ryanodine receptor (RyR), thus increasing the amount of Ca^{2+} available for contraction (positive inotropic effect). In addition, β-adrenoceptors stimulation leads to PKA-mediated phosphorylation of troponin I (TnI), accelerating troponin C-Ca^{2+} off-rate and allowing faster force development and shortening during systole and faster force relaxation and re-lengthening during diastole (Bers, 2008). Catecholamines also induce PKA-mediated phosphorylation of phospholamban (PLB), a negative regulator of the sarcoplasmic reticulum Ca^{2+} ATPase (SERCA), resulting in increased Ca^{2+} re-uptake in the sarcoplasmic reticulum and myofilament relaxation (lusitropic effect) (Bers, 2008). In addition to the machinery involved in the control of excitation-contraction coupling, however, cardiac myocytes express a host of other proteins that are affected by cAMP and PKA-mediated phosphorylation, including metabolic enzymes and transcription factors (Muller *et al.*, 2001). β-adrenoceptors are far from being the only receptors signalling through cAMP generation in cardiac myocytes. As a consequence, among the plethora of chemical messages that impact on the heart at any given time, several will result in the generation of cAMP, raising the question of how the myocytes decode all these signals and avoid phosphorylation of unsuitable targets in response to a given stimulus. Catecholamine-mediated control of the contractile performance of the heart is a vital mechanism that allows the necessary adjustments to confront imminent danger or to prevent precipitous drops in blood pressure. At the same time, inappropriate or excessive activation of the adrenergic signalling pathway may contribute to disease states such as heart failure (HF) (Floras, 2003). It is therefore imperative that the cAMP signals are delivered correctly to appropriately tune the contractile response to catecholamines and that, at the same time, potential interference from the minute-by-minute hormonal fluctuations are prevented. How do myocytes achieve this? This review focuses on recent evidence unravelling the mechanisms that allow cAMP to generate specific responses thus preventing intracellular chaos.

cAMP: one messenger for multiple messages

The first evidence that not all cAMP signals are equal in terms of the downstream responses they elicit was provided almost three decades ago when studies in isolated perfused hearts showed that isoproterenol (Iso) and prostaglandin E1 (PGE1), although elevating intracellular cAMP to comparable levels and similarly affecting the PKA activity ratio, had very different effects on PKA substrates. Specifically, Iso caused phosphorylation of phosphorylase kinase (Keely, 1977), Tn I (Brunton *et al.*, 1979) and several other PKA phosphorylation substrates (Hayes *et al.*, 1979), whereas no increase in the phosphorylation of these substrates was observed upon PGE1 stimulation (Hayes *et al.*, 1979). Interestingly, it had been previously reported that cAMP can bind to sites which are both soluble and particulate in nature (Terasaki and Brooker, 1977) and up to 50% of the PKA activity was shown to be associated with the particulate fraction of heart homogenates (Corbin *et al.*, 1977), indicating that PKA is targeted to specific locations within the cell. Based on these observations the hypothesis was formulated that multiple and distinct cAMP pathways exists that are spatially segregated (Corbin *et al.*, 1977; Hayes *et al.*, 1980). The finding that perfusion with Iso results in the activation of the particulate fraction of PKA, whereas PGE1 increases the activity ratio of soluble PKA (Hayes *et al.*, 1980) supported this hypothesis and suggested that somehow selective activation of PKA subsets that are confined to distinct intracellular compartments occurs and this leads to distinct functional responses (Hayes and Brunton, 1982). Over the years, further evidence of a functional compartmentalization of the cAMP signal has accumulated. For example, by using various cAMP raising agents, it was shown that the amplitude of myocyte shortening and Ca^{2+} transients correlates better with the increase in the particulate cAMP concentration than with total cAMP levels (Hohl and Li, 1991). Another example relates to the differential functional effects of β-AR subtype stimulation. β_1-AR activation leads to phosphorylation of the LTCC, PLB, RyR, TnI and myosin binding protein C, causing the typical positive inotropic and lusitropic effects that catecholamines exert on heart function, whereas the effect of β_2-AR activation is more restricted and selectively leads to the phosphorylation of the LTCC causing a lesser positive inotropic effect and no lusitropic effect (Kuschel *et al.*, 1999). Again, the different functional response appears to correlate with differential activation of particulate versus soluble fractions of PKA (Xiao *et al.*, 1994). As another example, the gut hormone glucagon-like peptide-1 was found to increase cAMP to the same level as that generated by Iso in adult rat cardiac myocytes but failed to induce the robust positive inotropic effect and actually mildly reduced myocyte contraction (Vila Petroff *et al.*, 2001). Despite such accumulating evidence, how the activation of different GPCRs can exert distinct functional effects while generating similar amounts of cAMP has remained a classical question in the pharmacology of the heart and it is only recently that new information has emerged that sheds light on the underpinning molecular mechanisms.

Compartmentalization of PKA: the A kinase-anchoring proteins (AKAPs)

It is now clear that, at one level, the specificity of cAMP signal transduction is achieved by spatial control of its main effector PKA. As anticipated by the insightful studies performed in the late seventies, PKA has been demonstrated to be tethered to subcellular compartments via binding to AKAPs. AKAPs are a large (>50 members) family of structurally unrelated proteins that have in common the ability to bind to and thereby tether PKA (Wong and Scott, 2004). PKA is a heterotetramer formed by two catalytic (C) subunits held in an inactive state by a dimer of regulatory (R) subunits. Binding of cAMP to the R subunits induces the dissociation of the C subunits and the phosphorylation of downstream targets. Anchoring of PKA to AKAPs is achieved by interaction of a conserved amphipatic α-helix region of 14–18 amino acid on the AKAP (Newlon *et al.*, 1999) with a hydrophobic groove formed by the dimerization/docking domains located at the N-terminus of the R subunit (Gold *et al.*, 2006; Kinderman *et al.*, 2006).

AKAPs also have unique protein–lipid or protein–protein targeting domains that tether the AKAP-PKA complex to distinct subcellular locations (Dell'Acqua *et al.*, 1998; Trotter *et al.*, 1999). Crucially, AKAPs can anchor PKA in proximity to its targets, thereby leading to the preferential phosphorylation of a local pool of PKA substrates (Zhang *et al.*, 2001). As an example, AKAP250 (also known as gravin) interacts with the β-AR and targets PKA to phosphorylate the receptor, leading to aspecific feedback regulation of receptor activity (Shih *et al.*, 1999). An additional key feature of AKAPs is their ability to coordinate other signalling enzymes such as kinases, phosphatases, phosphodiesterases (PDEs), small GTPases and other regulatory proteins into multifunctional transduction complexes, thereby ensuring integration and processing of multiple signals within discrete locales (Beene and Scott, 2007).

Several AKAPs have been shown to be expressed in cardiac tissue (Ruehr *et al.*, 2004) and evidence is emerging on the role that AKAPs have in heart physiology and pathophysiology (Diviani, 2008). For example, AKAP18α has been shown to target PKA to the LTCC. The anchored PKA can thus phosphorylate the channel increasing its opening probability (Gray *et al.*, 1998; Hulme *et al.*, 2006) as confirmed by experiments using competing peptides and showing that disruption of PKA anchoring to the LTCC via AKAP18α markedly inhibits the β-AR mediated regulation of the channel (Hulme *et al.*, 2003). AKAP18δ, another splice variant of the AKAP18 gene, has recently been shown to form a supramolecular complex with PKA, PLB and SERCA2 in cardiac myocytes (Lygren *et al.*, 2007). The AKAP18δ-anchored pool of PKA phosphorylates PLB in response to adrenergic stimuli and thereby regulates SERCA2-mediated Ca^{2+} re-uptake into the sarcoplasmic reticulum. Disruption of the AKAP18δ-PLB interaction using a competing peptide or AKAP18δ knock-down by an siRNA approach abolish the effect of norepinephrine on Ca^{2+} re-uptake in the sarcoplasmic reticulum (Lygren *et al.*, 2007), thus confirming the key regulatory role of co-localizing PKA and its substrate PLB via AKAP18δ.

The AKAP Yotiao has been shown to be critical for the regulation of the activity of the slowly activating potassium current channel (Marx *et al.*, 2002). The control of the sympathetic nervous system over the duration of cardiac action potential requires PKA-mediated phosphorylation of the KCNQ1 subunit of the I_{Ks} channel with the consequent increase in I_{Ks} current, accelerated repolarization and increased heart rate. A single amino acid mutation (G589D) in Yotiao has been shown to be sufficient to disrupt its interaction with the KCNQ1 channel and has been found in patients with long QT syndrome (Fodstad *et al.*, 2004), a heart condition associated with altered repolarization of the ventricle, suggesting that anchoring of PKA to the I_{Ks} channel is necessary for proper channel activity *in vivo*.

Another cardiac AKAP, mAKAP, has been shown to interact directly with the RyR2 located at the sarcoplasmic reticulum. At this site, mAKAP has been proposed to favour PKA-mediated phosphorylation of the channel in response to β-AR stimulation (Marx *et al.*, 2000). In addition to its localization at the sarcomere, mAKAP has also been shown to localize at the nuclear envelope of cardiac myocytes (Pare *et al.*, 2005b). At this location, mAKAP appears to transduce several hypertrophic signals as indicated by experiments in which silencing of mAKAP expression strongly reduces hypertrophic gene transcription induced by Iso, phenylephrine and the leukemia inhibitor factor (Dodge-Kafka *et al.*, 2005; Pare *et al.*, 2005a). Compartmentalized PKA signalling can also potentially impinge on the regulation of other important signal transduction pathways in the heart. For example, recent evidence suggests that cAMP/PKA may regulate the activation of PKD (Haworth *et al.*, 2007) which itself has been implicated in the regulation of cardiac contractile function, through phosphorylation of cardiac TnI (Cuello *et al.*, 2007), and remodelling, through phosphorylation of histone deacetylase 5 (Vega *et al.*, 2004). A large body of evidence thus confirms that compartmentalization of PKA via AKAPs is critical for the proper functioning of cardiac myocytes at several levels.

Compartmentalization of the signalling machinery at the plasma membrane

The generation of a cAMP signal involves binding of a hormone or neurotransmitter to a specific GPCR, the subsequent activation of a G_s protein which in turn activates an adenylyl cyclase (AC), the enzyme that synthesizes cAMP from ATP. If the specific response to a given hormone relies on compartmentalization of the cAMP effector PKA close to specific targets, one would expect to find that the signalling machinery upstream of PKA is also spatially confined. If GPCRs and ACs could freely float around in the plasma membrane, cAMP would be made available ubiquitously leading to unselective activation of PKA subsets, irrespective of what targets they are coupled to.

In fact, a number of GPCRs, including β-AR, serotonin receptors, adenosine receptors, to mention only a few, have been shown to localize to specific membrane microdomains (Patel *et al.*, 2008). Lipid rafts, specialized regions of the plasma membrane enriched in cholesterol and other lipids, and caveolae, a subset of lipid rafts that form flask-shaped invaginations of the plasma membrane enriched in particular proteins (such as caveolins) appear to be the sites at which these GPCRs concentrate (Patel *et al.*, 2008). Interestingly, the PGE2 receptor EP_2 has been shown to be excluded from caveolin-rich fractions (Ostrom *et al.*, 2001), confirming that different GPCRs segregate to different membrane compartments. In addition, the localization of individual GPCR to distinct regions in the plasma membrane can be a regulated and dynamic mechanism, as suggested by the finding that β_2-AR are detected in caveolae/lipid raft membranes but egress from this compartment upon activation. On the contrary, β_1-AR are found in both caveolae and non-caveolae membranes and do not undergo a detectable translocation upon activation (Rybin *et al.*, 2000).

Not only the receptors but also the downstream effectors and regulatory molecules involved in the synthesis if cAMP appear to be associated with specialized membrane regions. G proteins have been found to localize in caveolae (Ostrom *et al.*, 2004) where coupling to specific downstream signalling pathways appears to be facilitated (Bhatnagar *et al.*, 2004; Head *et al.*, 2005). Evidence of targeting mechanisms to caveolae also exists for G protein receptor kinases (Penela *et al.*, 2003).

Adenylyl cyclases are a family of several isoforms (nine membrane bound and one soluble) that show different regulatory mechanisms and interaction with other signalling pathways (Willoughby and Cooper, 2007). Different AC isoforms have been shown to localize to distinct compartments.

All Ca^{2+}-sensitive isoforms of AC (AC1/3/5/6/8) have been found to localize in lipid rafts, whereas the Ca^{2+}-insensitive isoforms (AC2/4/7) are excluded from these membrane compartments (Willoughby and Cooper, 2007). Interestingly, the sodium-hydrogen exchanger 1 and 3 (NHE1/3) and the transient receptor potential (TRP) 1/3 channels also localize in lipid rafts (Brazer *et al.*, 2003; Willoughby *et al.*, 2005), as do LTCCs in cardiac myocytes (Balijepalli *et al.*, 2006). NHE proteins are acid extruders and regulate intracellular pH, whereas TRP channels have been recently identified as a likely component of the capacitative Ca^{2+} entry channel (Putney, 2005). Ca^{2+} sensitivity of ACs is dramatically affected by intracellular pH (Willoughby *et al.*, 2005). In addition, in non-excitable cells, Ca^{2+}-sensitive ACs have been shown to be selectively regulated by Ca^{2+} that enters into the cells by the capacitative mode (Chiono *et al.*, 1995), whereas in excitable cells, such as cardiac myocytes, Ca^{2+}-sensitive ACs can be regulated by LTCC activity in response to β-AR stimulation (Yu *et al.*, 1993). Colocalization of cyclases with NHE, TRP channels and LTCC at lipid rafts may provide an efficient way to regulate cAMP synthesis in a microdomain that is sheltered from the metabolic fluctuations continuously affecting the cell.

Several isoforms of ACs can be expressed in the same cell and in cardiac myocytes AC5 and AC6 represent the dominant isoforms (Defer *et al.*, 2000). Several studies suggest that the different AC isoforms may have distinct roles. For example, AC6 seems to exert a beneficial effect on cardiac cell survival (Roth *et al.*, 2002), intracellular Ca^{2+} handling (Tang *et al.*, 2004) and contractile function (Lai *et al.*, 2000), whereas AC5 shows opposite affects (Okumura *et al.*, 2003; Iwatsubo *et al.*, 2004). To explain such differences, it is reasonable to assume that such isoforms have a distinct localization and can thus selectively interact with specific receptors. In fact, in cardiac myocytes, AC5 appears to be the selective target of purinergic receptors (Puceat *et al.*, 1998), whereas AC6 is selectively activated upon β_1-AR stimulation (Ostrom *et al.*, 2000) but not upon PGE1 receptor stimulation (Ostrom *et al.*, 2001). Although the molecular basis for the preferential coupling of different AC isoforms with specific receptors remains to be elucidated, the multiplicity of isoforms and their selective receptor coupling appear to contribute to specificity of response.

cAMP compartmentalization

3′-5′-cyclic adenosine monophosphate is a small, hydrophilic molecule that is expected to diffuse freely within the cytosol (Bacskai *et al.*, 1993; Chen *et al.*, 1999). However, if this were the case, ligand binding to any G_s-coupled receptor would generate a cAMP signal capable of activating all PKA subsets within the cell regardless of their localization with respect to specific targets. The resulting message would be muddled and specificity would be lost.

One mechanism to protect the cell from inappropriate target phosphorylation may rely on the activity of protein phosphatases. Interestingly, serine-threonine phosphatases have been shown to directly bind to several AKAPs. The AKAP79/150 has been shown to bind to β-ARs (Gardner *et al.*, 2006) and to be involved in the regulation of various ion channels, including LTCC and M-type K^+ channels, and is known to interact with the A subunit of the $Ca2^+$/calmodulin-dependent phosphatase PP2B (Coghlan *et al.*, 1995). Other examples relevant for the heart are the binding of protein phosphatase 1 to the AKAP Yotiao (Marx *et al.*, 2002) and to AKAP220 on vesicles (Schillace and Scott, 1999), the binding of PP2B to AKAP250 (Shih *et al.*, 1999) and the binding of the phosphatase PP2A to mAKAP (Kapiloff *et al.*, 2001). The presence, within the same signalling complex, of enzymes for signal transduction and signal termination appears to be an effective solution for local control of inappropriate activation. However, a more economical strategy to selectively activate targeted pools of PKA is also in place and relies on compartmentalization of the cAMP signal itself, as demonstrated by an increasing body of evidence (Jurevicius and Fischmeister, 1996; Rich *et al.*, 2001; DiPilato *et al.*, 2004; Mongillo *et al.*, 2004; 2006; Barnes *et al.*, 2005; Zhang *et al.*, 2005; Nikolaev *et al.*, 2006). The direct demonstration of restricted diffusion of cAMP was provided by a series of experiments in which the second messenger dynamics could be monitored in real time in intact living cardiac myocytes (Zaccolo and Pozzan, 2002). The approach used in these studies took advantage of a genetically encoded sensor for cAMP (Zaccolo *et al.*, 2000) and of the phenomenon of fluorescence resonance energy transfer (FRET) (Förster, 1948) to demonstrate that β-adrenergic stimulation of neonatal cardiac myocytes generates multiple and restricted microdomains with increased concentration of cAMP that specifically activate a subset of PKA enzymes that are anchored to AKAPs (Zaccolo and Pozzan, 2002).

The mechanisms responsible for compartmentalization of cAMP are still to be fully elucidated. One hypothesis put forward is that physical diffusional barriers, possibly formed by elements of the endoplasmic reticulum and localized underneath the plasma membrane, may be involved (Rich *et al.*, 2000). Such a hypothesis was formulated to explain the limited diffusion of cAMP from the plasma membrane to the deep cytosol in HEK293 cells upon PGE stimulation (Rich *et al.*, 2001). Although cardiac myocytes are rich in physical submembrane microdomains, it is not clear how these may restrict diffusion of cAMP and yet allow efficient diffusion of Ca^{2+} from the same microdomains, a phenomenon that occurs in the millisecond timescale (Bers, 2008). Whatever the nature of the barrier may be, a reduced diffusion coefficient for cAMP appears to be particularly relevant for sub-plasma membrane compartmentalization of cAMP and much less so for generation of cAMP gradients in the inner cell (Saucerman *et al.*, 2006).

Another mechanism that has been suggested to contribute to cAMP compartmentalization is PKA-mediated buffering (Saucerman *et al.*, 2006). In this case, binding of cAMP to the R subunits of PKA may reduce diffusion of cAMP due to the low diffusivity of R subunits. This hypothesis is supported by the observation that a significant proportion of the total basal

[cAMP] may be bound to PKA (Khac *et al.*, 1973; Corbin *et al.*, 1977).

However, with no doubt, the best-established mechanism contributing to cAMP compartmentalization involves PDEs, the enzymes that degrade cAMP.

Compartmentalization of PDEs

Phosphodiesterases are a superfamily of more then 70 different isozymes that degrade cyclic nucleotides. Individual PDE enzymes exert specific functional roles as a consequence of the unique combination of regulatory mechanisms, intracellular localization and enzyme kinetics (Conti and Beavo, 2007; Baillie *et al.*, 2005) and, in the heart, multiple cAMP-degrading PDEs are expressed (PDE1, PDE2, PDE3 PDE4 and PDE8) (Lugnier, 2006).

Several studies have demonstrated that inhibition of PDE activity has a profound effect on intracellular cAMP gradients (Fischmeister *et al.*, 2006). In an early biochemical study, 45% of the cAMP generated in adult canine ventricular myocytes stimulated with Iso was recovered in the particulate fraction but the proportion of total cAMP residing in the particulate fraction declined to less than 20% in the presence of PDE inhibitors (Hohl and Li, 1991), indicating that PDE activity contributes to the compartmentation of cAMP. Subsequent analysis in intact living myocytes using both electrophysiologal (Jurevicius and Fischmeister, 1996; Rochais *et al.*, 2006) and imaging (Zaccolo and Pozzan, 2002; Mongillo *et al.*, 2004; 2006) approaches confirmed that indeed PDEs have a key role in shaping the intracellular gradients of cAMP. Again, the mechanism by which PDEs can control intracellular diffusion of cAMP appears to involve localization of PDEs to specific subcellular compartments. In a study in neonatal cardiac myocytes, a striking difference was observed between the effect of PDE3 and PDE4 inhibition in the control of [cAMP] on β-AR stimulation. In these cells, selective inhibition of about 10% of the total PDE4 activity resulted in a dramatic increase in cAMP, whereas total inhibition of PDE3 had only a marginal effect on cAMP levels (Mongillo *et al.*, 2004). These results could not be explained by different enzyme concentrations, as PDE3, although being expressed at a lower level than PDE4 in these cells, still represents a substantial 30% of the total PDE activity. Interestingly, PDE3 and PDE4 enzymes were shown by immunostaining to be localized in distinct compartments within the myocyte. In another study, PDE2 was shown to be responsible for the degradation of a large proportion of the cAMP generated by β-AR stimulation, although representing only about 1% of the total PDE activity in the neonatal rat heart (Mongillo *et al.*, 2006). Again PDE2 was shown to be localized to specific subcellular sites (Mongillo *et al.*, 2006). These data indicate that there is a functional coupling of individually localized PDEs with selected pools of AC that are activated in response to specific hormones. To further confirm this model, a study in HEK293 cells demonstrated that the specific spatial arrangement of different PDEs generates a pattern of local drains that dump cAMP in defined locales, thus resulting in the generation of multiple gradients of cAMP (Terrin *et al.*, 2006). Overexpression of dominant-negative PDEs, that is of mutant PDEs that are catalytically inactive and exert a dominant-negative effect by displacing the cognate endogenous active PDEs from their functionally relevant anchor sites (Baillie *et al.*, 2003), was shown to be sufficient to disrupt the cAMP gradients generated in response to PGE_1 in these cells (Terrin *et al.*, 2006). The efficacy of these PDE mutants in disrupting intracellular pools of cAMP confirms that the tethered PDEs are responsible for shaping the cAMP gradients.

Phosphodiesterase localization to different compartments occurs through different mechanisms involving direct binding to membrane lipids or protein–protein interactions (Lynch *et al.*, 2006; Conti and Beavo, 2007). Particularly intriguing is the ability of PDE4 isoforms to interact with AKAPs. PDE4D3, for example, has been shown to bind to mAKAP in muscle cells (Dodge *et al.*, 2001) and to AKAP 450 at the centrosome (Tasken *et al.*, 2001). A sophisticated and multilayered control of local cAMP concentration and PKA activation has been demonstrated to take place at the mAKAP signalling complex. The basal activity of mAKAP-anchored PKA is kept under control by the anchored PDE4D3 (Dodge *et al.*, 2001). Upon β-AR stimulation, cAMP concentration increases, anchored PKA becomes activated and phosphorylates PDE4D3 on Ser54 causing a two- to threefold increase in PDE activity (Sette and Conti, 1996; MacKenzie *et al.*, 2002), which reduces cAMP levels, favouring PKA holoenzyme reformation. Active PKA also phosphorylates PDE4D3 on Ser13 thus enhancing its affinity for mAKAP (Carlisle Michel *et al.*, 2004) such that, upon hormonal stimulation, PDE4D3 is drawn into the mAKAP complex and has increased activity, thereby decreasing cAMP back to basal levels. The co-localization of PKA and PDE generates through a negative feedback loop mechanism, local fluctuations of cAMP and local pulses of PKA activity, as directly confirmed by imaging studies in living cells (Dodge-Kafka *et al.*, 2005).

Different PKA isoforms sense different pools of cAMP and phosphorylate distinct downstream targets

Not only AC and PDEs are expressed in cells as a variety of different isoforms. The cAMP effector PKA also is formed by a combination of several isoforms of R and catalytic (C) subunits. Each of the three C subunit isoforms (α, β and γ) can associate with any of the two RI (RIα, RIβ) and RII (RIIα and RIIβ) subunits. Although the diversity exhibited by the C subunits has been suggested to play a role in the specificity of the PKA signal (Taylor *et al.*, 2008), all C subunits show common kinetic features and substrate specificity (Taylor *et al.*, 1992). On the contrary, R subunits possess different physical and biological properties and determine the characteristics of the PKA holoenzyme (Skalhegg and Tasken, 2000).

The features of PKA-type I ($RI\alpha_2C_2$ and $RI\beta_2C_2$) and PKA-type II ($RII\alpha_2C_2$ and $RII\beta_2C_2$) suggest that the diversity of PKA isoforms may contribute to the specificity seen in the cAMP/PKA signalling pathway. Differential expression of PKA isoforms has been demonstrated in several cells and tissues at various stages of development and differentiation (Skalhegg and Tasken, 2000). PKA isoforms localize differently within

the cell via the unique ability of the dimerization/docking domain of R subunits to bind to AKAPs. PKA-type II bind to AKAPs with high affinity (K_D = 10^{-9} M) (Carr *et al.*, 1992), whereas, in general, most AKAPs bind PKA-type I with 1000-fold lower affinity (Stokka *et al.*, 2006). As a result, PKA-type I is considered to be predominantly cytosolic, whereas PKA-type II is typically associated with cellular structures and organelles. PKA isoforms also show different biochemical properties. PKA-type I is more readily dissociated by cAMP than PKA-type II both in vitro (Dostmann *et al.*, 1990) and *in vivo* (Cummings *et al.*, 1996) and the recent structure solution of holoenzyme complexes (Kim *et al.*, 2007; Wu *et al.*, 2007) has elucidated critical isoform-specific features that specifically regulate inhibition and cAMP-induced activation of PKA-type I and PKA-type II (Taylor *et al.*, 2008) . Given the distinct biochemical properties, specific patterns of expression and subcellular localization of PKA isozymes it is not surprising that the biological role of PKA isoforms is non redundant, as demonstrated by genetic studies (Brandon *et al.*, 1995; Cummings *et al.*, 1996; Hensch *et al.*, 1998).

Recent work in cardiac myocytes has furthered our understanding of the mechanisms by which individual PKA isoforms can deliver a specific response to a given extracellular stimulus (Di Benedetto *et al.*, 2008). The first finding was that, contrary to the consensus that in cardiac myocytes, PKA-type II holoenzyme is localized, whereas PKA-type I is mainly cytosolic (Corbin *et al.*, 1977), a considerable amount of PKA-type I anchoring sites is present in these cells, as shown by fluorescence recovery after photobleaching experiments. In fact, the notion of a PKA-type I floating around freely in the cytosol would be difficult to reconcile with any selectivity in the activation of PKA isozymes, in particular if one considers that PKA-type I is more readily activated by cAMP than PKA-type II. In other tissues, dual specificity AKAPs capable of binding with high-affinity RI as well as RII have been described (Huang *et al.*, 1997) and AKAP-mediated localization of PKA-type I to the neuromuscular junction (Imaizumi-Scherrer *et al.*, 1996; Barradeau *et al.*, 2000) as well as at interphase microtubules and specific regions of the mitotic spindle (Imaizumi-Scherrer *et al.*, 2001) have been reported. Interestingly, a specific RI-binding AKAP that does not interact with RII subunits has been described in *C. elegans* (Angelo and Rubin, 1998) opening the possibility that RI-specific binding proteins may exist also in other organisms. Indeed, a specific role for localized PKA-type I in modulating T-cell receptor signalling has been demonstrated in mammalian cells (Skalhegg *et al.*, 1994).

The features characterizing PKA-type I and PKA-type II compartments were further elucidated by the analysis of cAMP dynamics *in vivo* using FRET-based reporters targeted to intracellular sites where endogenous PKA-type I and type II isoforms normally localize. These studies showed that PKA isoforms reside in physically distinct compartments within which the level of cAMP is selectively regulated by a unique subset of PDEs. Activation of the β-AR receptor generates a cAMP signal selectively in the PKA-type II compartment, whereas activation of the PG receptor generates a cAMP signal selectively in the PKA-type I compartment, implying that cAMP cannot diffuse from one compartment to the other to cross-activate PKA isozymes (Di Benedetto *et al.*, 2008) (Figure 1). The functional consequence of such compartmentalization is that Iso stimulation leads to the specific phosphorylation of PLB, TnI and β_2AR, whereas PGE1 stimulation does not affect these substrates, demonstrating that individual PKA isoforms are coupled with defined subsets of targets and that PKA isoforms activity is not promiscuous (Di Benedetto *et al.*, 2008). At present, no cardiac targets selective for type I PKA have been identified and this represents an interesting challenge for future studies. The findings described above provide a molecular mechanism for the early observation that activation of different cardiac GPCRs, although generating similar amounts of cAMP, results in distinct functional effects.

Implications for the treatment of cardiovascular disease

The organization of the cAMP/PKA signalling system into multiple distinct and independent pathways may have important implications for the treatment of heart disease. It is well-established that alterations in β-AR signal transduction are a primary determinant of the evolution towards HF (Lefkowitz *et al.*, 2000) with the primary biochemical defects consisting in the down regulation of β_1-ARs in myocardial membranes and a decrease in the functional coupling of the remaining receptors to the G_s-AC system (Port and Bristow, 2001). However, counter intuitively, the upregulation or activation of the adrenergic nervous system has been directly correlated with shortened survival (Kaye *et al.*, 2004), whereas β-blocker therapy has been shown to decrease morbidity and mortality significantly for patients with left-ventricular dysfunction and HF (Domanski *et al.*, 2003). Although significant advances have been made in the pharmacologic treatment of HF, mortality remains high, due to the inability of current treatment to effectively reverse pathologic remodelling and myocardial dysfunction in most HF patients. This certainly reflects the fact that our understanding of the full range of the signalling mechanisms involved in HF progression is still incomplete. Manipulation of the overall cAMP signal within cardiac myocytes as one can achieve with β-agonists or β-blockers may simply be too blunt to provide control of the signal transduction system with the necessary spatial accuracy and may results in deleterious off-target effects.

Evidence is emerging that spatial control of cAMP/PKA signalling is critical for the healthy heart. Interaction between AKAPs and R subunits of PKA is decreased in human HF (Zakhary *et al.*, 2000) and a polymorphism in the human dual specificity D-AKAP-2 that leads to marked differences in its affinity for PKA has been shown to be associated with cardiac rhythm defects and has been suggested as a predictor of sudden cardiac death (Tingley *et al.*, 2007). The detailed description of the spatial organization of the individual cAMP/PKA signalling subdomains and of their specific regulation in cardiac myocytes will provide a map to decipher the intricacy of cAMP signalling and may contribute to the identification of novel therapeutic strategies targeting only the relevant signalling cascades while leaving the remaining

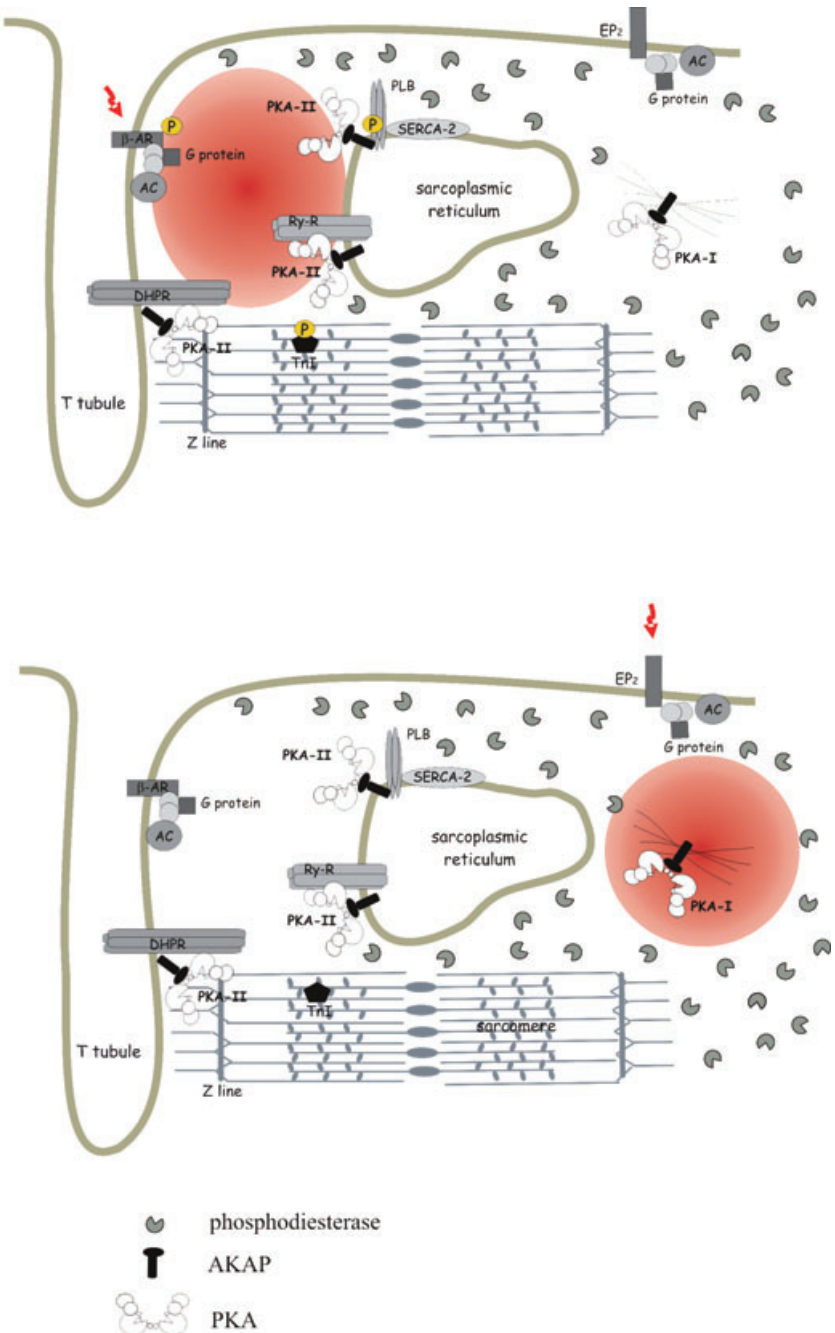

Figure 1 Compartmentalized PKA isoforms are activated by distinct pools of cAMP. Top panel: activation of β-adrenoreceptors by catecholamines leads to the generation of a spatially restricted pool of cAMP activating PKA isoforms type II and to the phosphorylation of the downstream targets β_2-AR, PLB and TnI. Lower panel: a distinct pool of cAMP is generated upon activation of the prostaglandin receptor EP_2, leading to activation of PKA isoforms type I but not to the phosphorylation of B2-AR, PLB and TnI. The boundaries of the cAMP pools are defined by the activity of spatially segregated PDEs. AC, adenylyl cyclase; AKAP, A kinase-anchoring proteins; cAMP, 3′-5′-cyclic adenosine monophosphate; PDE, phosphodiesterase; PKA, protein kinase A; PLB, phospholamban; SERCA, sarcoplasmic reticulum Ca^{2+} ATPase; TnI, troponin I.

signalling network unaffected, thus improving treatment efficacy while reducing side effects.

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Conflicts of interest

The author states no conflict of interest.

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