MINIREVIEW

Contributions of Protein Kinase A Anchoring Proteins to Compartmentation of cAMP Signaling in the Heart

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Received April 10, 2002; accepted May 8, 2002

This article is available online at http://molpharm.aspetjournals.org

ABSTRACT

The cAMP-dependent protein kinase (PKA) transduces signals in the heart initiated by β₁-adrenergic, G-protein-coupled receptors after norepinephrine, sympathetic stimulation. Signaling through this pathway results in a characteristic set of cellular responses, including increases in ion fluxes and contractile strength, mobilization of energy stores, and changes in gene expression. Not all receptors that activate adenylate cyclase and increase cAMP levels, however, cause the cardiac myocyte to react in this manner. Research in the field of signal transduction over the last 25 years has addressed this issue of specificity in signaling by diffusable second messengers. PKA is in part targeted to discrete cellular locations by A-kinase anchoring proteins. Through anchoring and formation of multi-enzyme complexes, specific, localized signal transduction is possible. I discuss in this review recent advances in the understanding of PKA signaling complexes in the cardiac myocyte.

Evidence for Localized PKA Signaling

The first model for compartmentation of PKA signaling in the heart was published in 1977, when Corbin et al. (1977) recognized that there were both particulate and soluble fractions of PKA. Soon after, it was found that prostaglandin E1 (PGE₁), which could increase cAMP levels, activated only soluble PKA, without phosphorylation of the PKA substrates troponin I and glycogen phosphorylase (Keely, 1977; Hayes et al., 1980; Brunton et al., 1981). However, not all extracellular agonists that induce cAMP and PKA phosphorylation have the same effects on cardiac function (Steinberg and Brunton, 2001). Consequently, an important question in the field of signal transduction has been: how can a broad-specificity kinase activated by a diffusable second messenger participate in differential signaling? Specificity in PKA signaling is conferred in part by the binding of PKA to A-kinase anchoring proteins (AKAPs) that are targeted to specific intracellular locations. AKAP binding sequesters the PKA with individual substrates, where it may be activated locally by cAMP (Colledge and Scott, 1999). There have recently been several excellent reviews on AKAPs and localized signaling (Colledge and Scott, 1999; Pawson and Nash, 2000; Skalhegg and Tasken, 2000; Feliciello et al., 2001; Michel and Scott, 2002). This minireview, therefore, will focus on the evidence supporting a role for localized PKA signaling in the heart.

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ABBREVIATIONS: PKA, protein kinase A; AKAP, A-kinase anchoring protein; PG, prostaglandin; ISO, isoproterenol; mAKAP, muscle A-kinase anchoring protein; GFP, green-fluorescent protein; I_{Kr}, delayed rectifier K⁺ current; RNV, rat neonatal ventricular myocyte; SR, sarcoplasmic reticulum; PP, protein phosphatase; R-subunit, regulatory subunit; C-subunit, catalytic subunit; RyR, ryanodine receptor; PDE, phosphodiesterase; ARVD, arrhythmogenic right ventricular dysplasia.
caused phosphorylation of the contractile protein troponin I and activation of glycogen phosphorylase. β-Adrenergic agonists, but not prostanoids, were positive inotropic agents. Glucagon-like peptide-1 also raises cAMP levels in cardiac myocytes (Vila Petroff et al., 2001). In contrast to PGE₁ and ISO, glucagon-like peptide-1 exerts a negative inotropic effect on cardiac myocytes (Vila Petroff et al., 2001). These results illustrate how different receptors that signal through the same diffusible second messenger can result in the specific activation of different cellular processes, presumably through some sort of segregation of the signaling mechanism.

More recently, this concept has been extended by investigations revealing that cAMP signaling can occur within a discrete region of an individual cell. Patch-clamp analysis showed that local application of ISO to one side of a cardiac myocyte induced local increases in cAMP and Ca²⁺ currents through the L-type Ca²⁺ channel, which is a PKA substrate (Jurevicius and Fischmeister, 1996). In addition, measurement of fluorescence resonance energy transfer within PKA holoenzyme subunits fused to cyan and yellow variants of green fluorescent protein (GFP) has allowed direct assessment of local cAMP levels in norepinephrine-stimulated rat neonatal ventricular myocytes (RNV) (Zaccolo and Pozzan, 2002). β-Adrenergic receptor activation generated higher cAMP levels at areas near sarcomeric Z-lines and transverse tubules and junctional sarcoplasmic reticulum (SR) membranes than in the cytosol. Fluorescence resonance energy transfer showed that cAMP could act within pools as small as 1 μm and that free diffusion of the cAMP was limited by the activity of phosphodiesterases (Zaccolo and Pozzan, 2002).

Discrimination in second messenger signaling may be achieved through local, compartmentalized activation of membrane-bound enzyme pools (Pawson and Scott, 1997; Colledge and Scott, 1999; Steinberg and Brunton, 2001). In this model, spatial segregation of signaling pathway components, including enzymes and substrates, confers specificity by enhancement of the effective concentration of both upstream activators and substrates. This increase in effective concentration overrides the intrinsically broad substrate specificity of many signaling enzymes and avoids global increases in second messenger that might trigger enzymes throughout the cell. In general, PKA is divided into particulate and soluble fractions by the binding of the particulate fraction of PKA holoenzyme molecules to A-kinase anchoring proteins (AKAPs) (Colledge and Scott, 1999; Skalhegg and Tasken, 2000; Feliciello et al., 2001).

**A-Kinase Anchoring Proteins**

PKA is a heterotetramer composed of two regulatory (R) and two catalytic (C) subunits. There are four R-subunit genes (RIα, RIβ, RIIα, and RIIβ) and three C-subunit genes (Ca, Cβ, and Cγ) in mammals (Scott, 1991; Beebe, 1994). RIα, RIIα, Ca, and Cβ are the predominant subunits expressed in the heart (Krall et al., 1999). Upon activation of adenylate cyclase, each R-subunit will bind two molecules of cAMP and release active catalytic subunit (Scott, 1991). With some prominent exceptions, the PKA RI-type subunits bind AKAPs with much lower affinity than the RII-type subunits (Burton et al., 1997). As a result, in rodents and to a less extreme degree in humans, the type I and type II holoenzymes are restricted in the heart to soluble and particulate fractions, respectively (Krall et al., 1999). Most AKAPs bind PKA through the interaction of the hydrophobic surface of an AKAP amphipathic helix and the hydrophobic surface of the X-type, four-helix bundle formed by the N-terminal domains of the RII homodimer (Newlon et al., 2001).

RII-subunits will bind AKAPs in a modified Western blot procedure called the RII overlay (Carr and Scott, 1992). The RII overlay assay has permitted cloning of multiple AKAPs from expression libraries of varying source tissues. The presence of 10 to 20 AKAPs, each located differently within an individual cell, affords much complexity and specificity to PKA signaling (Fig. 1). This includes the possibility of separ-

![Fig. 1. Cardiac AKAPs. AKAPs that have been found in the heart are shown at their respective locations. There is evidence that AKAPs are associated with ion channels including L-type Ca²⁺ channels (LCa⁺, ), the KCNQ1 delayed rectifier potassium channel (KvLQT1), RyR, and the cystic fibrosis transmembrane conductance regulator (CFTR), with the β₂ adrenergic receptor, phosphatases including protein phosphatase 1 (PP1), 2A (PP2A), and calcineurin (PP2B), large and small G proteins including G₁α, G₁β, and rho, and sarcomeric proteins such as troponin T (TnT) and myosin binding protein-C (MBP-C). See Table 1 for references. G, and adenylyl cyclase are not indicated in the drawing, although it should be understood that PKA activation is presumably a consequence of the activation of those molecules.](image-url)
rate activation of distinct subsets of PKA pools by different extracellular signals (Table 1). The importance of anchoring in cardiac signaling through particulate PKA has been demonstrated by expression of a peptide (Ht31) that can compete RII-subunit binding to AKAPs in cardiac myocytes (Fink et al., 2001). Global disruption of PKA anchoring affected the kinetics of the myocyte contractile cycle and decreased the ISO-dependent phosphorylation of two sarcomeric proteins, including troponin I. Disruption of PKA targeting did not affect the phosphorylation of all PKA substrates, including, for example, phospholamban.

Cardiac AKAPs

Published research concerning cardiac AKAPs had focused on AKAP18/15 (Fraser et al., 1998; Gray et al., 1998; Hulme et al., 2001; yotiao (Potet et al., 2001; Marx et al., 2002), and mAKAP (Dodge et al., 2001; Kapiloff et al., 1999, 2001; Marx et al., 2000, 2001). Discussed below, these three AKAPs, targeted by distinct mechanisms to different intracellular compartments, are all involved in the regulation of ion channels by PKA. Several other AKAPs have been found in the heart (Table 1). AKAP-lbc is an example of an anchoring protein for which the function in the cardiac myocyte remains uncertain. AKAP-lbc, a fragment of which is the Ht31 peptide, is expressed in many tissues, although most abundantly in the heart (Diviani et al., 2001). AKAP-lbc is a rho-selective guanine nucleotide exchange factor. It is activated by Gα12 and Gα13 but not by Gα11, Gα12/13, and Gαq, and promotes the formation of actin stress fibers in fibroblasts when induced by lysophosphatidic acid through rho-signaling (Diviani et al., 2001). This AKAP may be important to the induction of cardiac hypertrophy and, in particular, to hypertrophic gene expression (Thorburn et al., 1997). Activation of Gα13 will cause an increase in myocyte size and atrial natriuretic gene expression, potentially in a rho-dependent manner (Fin et al., 1999). Another intriguing AKAP is gravin, which binds the β2-adrenergic receptor, the phosphatase calcineurin, protein kinase C, and PKA (Fan et al., 2001). In cardiac myocytes, activation of the β2 receptor increases L-type Ca2+ channel currents and inotropy in a PKA-dependent manner without affecting the phosphorylation of phospholamban, troponin I, and phosphorylase kinase (Kuschel et al., 1999). Gravin may mediate this specific effect of the β2 receptor, which stands in contrast to the broader functions of the more abundant β1 receptor.

There is evidence for the presence of multiple unidentified AKAPs in the heart. The AKAP(s) responsible for ISO-mediated phosphorylation of the sarcomeric proteins myosin binding protein C and troponin I is unknown (Fink et al., 2001). There are also data to suggest that the AKAP responsible for PKA-mediated phosphorylation of the ryanodine receptor at the SR remains unidentified (Kapiloff et al., 2001) (see mAKAP and the Ryanodine Receptor).

**AKAP 18/15 and the L-Type Ca2+ Channel**

The action potential in the contracting cardiac myocyte is initiated by depolarization of the plasma membrane (Marban, 2002). Depolarization from −90 mV to greater than +40 mV starts with inward Na+ channel currents and is maintained by inward Ca2+ channel currents. The L-type Ca2+
channel is the major voltage-dependent Ca\textsuperscript{2+} channel in the cardiac myocyte (Bers, 2002). Responsible for the inward current that contributes to the plateau phase of the action potential, this channel triggers adjacent ryanodine receptors (RyRs) at sarcolemmal-SR junctions during excitation-contraction coupling. Although the L-type Ca\textsuperscript{2+} channel is primarily voltage-dependent, its conductance is potentiated by PKA-catalyzed phosphorylation that is PKA anchoring-dependent (Gao et al., 1997). This regulatory event is a crucial part of the inotropic action of \beta-adrenergic agonists.

AKAP18/15 is an 81-amino acid anchoring protein that binds the L-type Ca\textsuperscript{2+} channel at the plasma membrane of cardiac and skeletal muscle myocytes (Fraser et al., 1998; Gray et al., 1998). AKAP18/15 is targeted by covalently attached lipid moieties that may insert into the plasma membrane (Fraser et al., 1998; Gray et al., 1998). Amino acid residues Gly-1, Cys-4, and Cys-5 on AKAP18/15 are modified by myristoylation and dual palmitoylation. The interaction between AKAP18/15 and the Ca\textsuperscript{2+} channel was recently shown to be mediated through a leucine zipper-type interaction involving a potential helix adjacent to the PKA-binding site on AKAP18/15 (Hulme et al., 2001). Coil-coil interactions mediate the interactions of a large number of proteins including transcription factors, cytoskeletal proteins, and enzyme subunits (Kohn et al., 1997). As will become apparent from the discussion below, several AKAPs are bound by coiled-coil interactions to the PKA substrate in the complex.

**Yotiao and Long-QT Syndrome**

Repolarization of the plasma membrane occurs during termination of the myocyte action potential and during the QT interval of the electrocardiogram (Marban, 2002). The ion channels responsible for repolarization consist mainly of potassium channels. \(I_\text{ks}\) (delayed rectifier K\textsuperscript{+} current) is active at negative potentials and contributes to the maintenance of the resting potential. The slow component of the delayed rectifier K\textsuperscript{+} current in cardiac myocytes is regulated by PKA in a manner blocked by the Ht31 peptide, implying that the \(I_\text{ks}\) channel is also associated with an AKAP (Potet et al., 2001). This channel is clinically important because it is mutated in Long-QT syndrome (Marban, 2002). This human disease is characterized by a prolonged electrocardiogram QT interval and is associated with syncope and ventricular arrhythmias, such as torsades de pointes and fibrillation (Keating and Sanguinetti, 2001).

KCNQ1 (KvLQT1) was recently discovered to bind yotiao, a 210-kDa AKAP previously shown to bind the NMDA receptor and protein phosphatase 1 in the brain (Westphal, 1999; Marx et al., 2002). The binding of a kinase and phosphatase permits balanced regulation of an ion channel in a signaling complex. Yotiao-binding to the channel is required for PKA phosphorylation of hKCNQ1 Ser-27 and activation of channel currents (Marx et al., 2002). Association of the K\textsuperscript{+} channel with yotiao, PKA, and protein phosphatase 1 is blocked by a single amino acid mutation (G589D) found in patients with Long-QT syndrome. This mutation lies within a potential "leucine zipper" coiled-coiled motif on KCNQ1 responsible for binding yotiao (Marx et al., 2002). This is the first genetic and in vivo evidence that PKA targeting is necessary for proper cAMP signaling and serves as proof of principle for the PKA targeting hypothesis. Although not yet demonstrated, yotiao levels are presumably normal in the heart of these patients. The defect in cardiac function should be solely a result of the lack of association of PKA with its targeting locus and substrate.

**mAKAP and the Ryanodine Receptor**

mAKAP is a 255-kDa AKAP present in heart, skeletal muscle, and brain that can target PKA to the nuclear envelope of differentiated cardiomyocytes (Kapiloff et al., 1999, 2001). mAKAP was initially called AKAP100 (McCartney et al., 1995) and was renamed when full-length clones were later isolated and it became evident that the protein was much larger (250 kDa) than previously appreciated (Kapiloff et al., 1999). This anchoring protein, like yotiao, is an AKAP that serves as a scaffolding protein, bringing together members of different signaling pathways and allowing the integration of different upstream signals (Fig. 2). In addition to binding PKA, mAKAP was the first PKA anchoring protein shown to bind a phosphodiesterase, the cAMP-specific phosphodiesterase type 4D3 (PDE4D3) (Dodge et al., 2001). The mAKAP complex also includes a phosphatase (PP2A) and the Ca\textsuperscript{2+}-activated, Ca\textsuperscript{2+} channel ryanodine receptor (Kapiloff et al., 2001; Marx et al., 2001).

The structure of the mAKAP complex is beginning to be understood (Fig. 3A). Binding sites for PDE4D3 and RyR on mAKAP have been preliminarily defined as indicated in Fig. 3A by glutathione S-transferase pull-down assays of mAKAP fragments (Dodge et al., 2001; Marx et al., 2001). A fragment of mAKAP that can associate with RyR includes a potential leucine zipper at amino acids 1217–1242 (Marx et al., 2001). mAKAP binds PKA although a potential amphipathic \(\alpha\)-helix found at amino acid residues 2055–2072 (Kapiloff et al., 1999). The binding site for PKA has been confirmed by assay of the protein product of a full-length cDNA. mAKAP containing a point mutation designed to disrupt that potential helical structure (I2062P) does not bind RII in the overlay.
assay (Kapiloff et al., 1999). The sequences that are required to target mAKAP to the nuclear envelope were defined by deletion mapping using expression of GFP fusion proteins in actively contracting, RNV primary cultures (Fig. 3)(Kapiloff et al., 1999). mAKAP contains sequences similar to the repeated units of spectrin (Kapiloff et al., 1999). These repeated units are also found in actinin, utrophin, and dystrophin (Brown, 1997) and can participate in protein-protein interactions (Li and Bennett, 1996; Xia et al., 1997). Expression of GFP fusion proteins in RNV suggests that either the first (residues 772–882) or second (residues 952-1059) spectrin-like repeat is required for targeting to the nuclear envelope, thus defining two independent and sufficient targeting domains (Kapiloff et al., 1999). A unique aspect of mAKAP targeting is that endogenous mAKAP can be displaced when a fusion protein containing the targeting domains (residues 585-1286) is over-expressed at levels high enough to saturate the targeting mechanism (Kapiloff et al., 1999).

The RyR is a substrate for PKA, and RyR conductance is increased by PKA-mediated phosphorylation (Fig. 2) (Bers and Perez-Reyes, 1999). Thus, local Ca\(^{2+}\) and cAMP will contribute to further increases in ambient Ca\(^{2+}\) levels. To turn off the signal, PP2A can reverse the action of PKA by catalyzing the dephosphorylation of PKA substrates (Schonthal, 1998). In addition, after activation by PKA-phosphorylation, PDE4D3 catalyzes the degradation of cAMP (Dodge et al., 2001). This serves as a negative feedback loop to modulate PKA activation by cAMP (Dodge et al., 2001). Although no adenylyl cyclase has been identified that binds mAKAP, one might speculate that if the mAKAP complex is activated by a nuclear envelope-resident adenylyl cyclase (Yamamoto et al., 1998), then PDE4D3 might serve as well to prevent the spread and generalization of a cAMP-signal specific to the space near the nuclear envelope (Zaccolo and Pozzan, 2002).

The presence of RyR in the mAKAP complex may be initially surprising, because the RyR is better known as the channel at the SR responsible for release of Ca\(^{2+}\) from intracellular stores during excitation-contraction coupling (Franzini-Armstrong and Protasi, 1997; Bers, 2002). RyR channel opening is stimulated primarily by Ca\(^{2+}\) influx thought the L-type Ca\(^{2+}\) channel, a process known as “Ca\(^{2+}\)-induced Ca\(^{2+}\) release”(Bers, 2002). RyR conductance can be inhibited by the plant alkaloid ryanodine and can be potentiayed by caffeine and the endogenous ligand cyclic ADP ribose, which is produced by ADP-ribosyl cyclase, a pool of which, incidentally, is localized at the inner nuclear membrane (Adebanjo et al., 1999). There have been reports suggesting that mAKAP may also be present at the SR, where it contributes to the regulation of RyRs and release of stored Ca\(^{2+}\) (Yang et al., 1998; Marx et al., 2000).

Although mAKAP is enriched at the nuclear envelope, it is possible that a small population of mAKAP molecules targets PKA to RyRs at the SR. PKA-dependent phosphorylation and activation of the RyR at the SR has been well studied. Orthophosphate labeling of myocytes (RNV) has revealed that β-adrenergic stimulation increases phosphorylation of the RyR (Yoshida et al., 1992). RyR is hyperphosphorylated in transgenic mice over-expressing PKA catalytic-subunit in the heart (Antos et al., 2001) and in human heart failure (Marx et al.,

![Fig. 3.](molpharm.aspetjournals.org) Spectrin repeat-like sequences are required for mAKAP targeting (Kapiloff et al., 1999). A, a schematic diagram presenting a family of GFP-tagged human mAKAP protein fragments that were expressed in RNV to map the targeting domain. The first and last residues of each fragment are indicated. PKA (Kapiloff et al., 1999), PDE4D3 (Dodge et al., 2001), and RyR-binding sites (Marx et al., 2001) are indicated. B, control cell showing the immunofluorescence detection of endogenous mAKAP. C, control cell showing fluorescence detection of GFP. D to I, detection of mAKAP-GFP fragments expressed in RNV. RNV were dissociated, placed into culture, transfected with mammalian expression plasmids encoding GFP-fusion proteins, and induced to hypertrophy by phenylephrine. Cella selected for study had minimal expression of the GFP-fusion protein. Note that mature rodent cardiac myocytes are binucleate and that actively contracting RNV may be either mono- or binucleate (Kapiloff et al., 1999).
Recent results, however, show that it is highly unlikely that mAKAP is stoichiometrically bound to RyRs at the SR (Kapiloff et al., 2001). By subcellular fractionation and immunocytochemistry, mAKAP was found exclusively in fractions containing nuclei, whereas RyR was found in fractions that would contain both SR and nuclei (Kapiloff et al., 2001). A strength of this study was the care given to ensure that nuclei would remain intact and that nuclear fragments would not contaminate SR preparations, as is often the result with most fractionation protocols (Meissner, 1974; Tata, 1974). These results suggest that another AKAP at the SR facilitates RyR phosphorylation. By RII overlay, at least four bands can be detected using purified SR, all smaller than mAKAP (unpublished observations).

The function of mAKAP is not as obvious as that of AKAP18/15 and yotiao due to its location and larger size. Over the last 7 years, it has become appreciated that functional RyRs are present not only at the SR, but also at the nuclear envelope (Gerasimenko et al., 1995; Malviya and Rogue, 1998). Ca$^{2+}$ is stored within both the SR and the perinuclear space located between the outer and inner membranes of the nuclear envelope. These stores are separately regulated despite the continuity of the outer nuclear membrane with the SR (Badminton et al., 1998). Although in phase with fluctuations in cytoplasmic Ca$^{2+}$ levels during the contractile cycle, nucleoplasmic Ca$^{2+}$ fluxes in cardiomyocytes and other cell types exhibit different kinetics than cytoplasmic Ca$^{2+}$ fluxes (Abrenica and Gilchrist, 2000; Bootman et al., 2000). In addition, in situ Ca$^{2+}$ imaging has been used to demonstrate that nucleoplasmic Ca$^{2+}$ levels in cultured cardiomyocytes and isolated nuclei can be affected autonomously by nuclear envelope RyR channels (Adebanjo et al., 1999, 2000; Abrenica and Gilchrist, 2000). Perinuclear Ca$^{2+}$ may affect nuclear import (Malviya and Rogue, 1998) or nuclear Ca$^{2+}$/calmodulin-dependent protein kinase (Chawla et al., 1998; Heist and Schulman, 1998).

Interestingly, the human gene for mAKAP is on chromosome 14q (Kapiloff et al., 1999), within a linkage region for familial arrhythmogenic right ventricular dysplasia (ARVD), a cause of sudden death in athletic adolescents and adults (Severini et al., 1998; Heist and Schulman, 1998). Mutations have recently been found in the cardiac-specific type II RyR gene for one of the seven other identified ARVD linkage groups (Tiso et al., 2001). Modulation of cAMP- and Ca$^{2+}$-dependent signaling contributes to the changes in gene expression and contractile machinery characteristic of hypertrophy, which is the common cardiac response to stress (Sugden and Clerk, 1998; Post et al., 1999; Frey et al., 2000). Although cardiac hypertrophy is usually discussed in terms of disease, it may affect nuclear import (Malviya and Rogue, 1998) or nuclear Ca$^{2+}$/calmodulin-dependent protein kinase (Chawla et al., 1998; Heist and Schulman, 1998).

Conclusions

Early studies concerning cAMP signaling in the heart showed that a diffusible second messenger could participate in the specific activation of different enzyme pools. This paradox, de-