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cAMP signaling in subcellular compartments

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ABSTRACT

In the complex microcosm of a cell, information security and its faithful transmission are critical for maintaining internal stability. To achieve a coordinated response of all its parts to any stimulus the cell must protect the information received from potentially confounding signals. Physical segregation of the information transmission chain ensures that only the entities able to perform the encoded task have access to the relevant information. The cAMP intracellular signaling pathway is an important system for signal transmission responsible for the ancestral 'flight or fight' response and involved in the control of critical functions including frequency and strength of heart contraction, energy metabolism and gene transcription. It is becoming increasingly apparent that the cAMP signaling pathway uses compartmentalization as a strategy for coordinating the large number of key cellular functions under its control. Spatial confinement allows the formation of cAMP signaling "hot spots" at discrete subcellular domains in response to specific stimuli, bringing the information in proximity to the relevant effectors and their recipients, thus achieving specificity of action. In this report we discuss how the different constituents of the cAMP pathway are targeted and participate in the formation of cAMP compartmentalized signaling events. We illustrate a few examples of localized cAMP signaling, with a particular focus on the nucleus, the sarcoplasmic reticulum and the mitochondria. Finally, we discuss the therapeutic potential of interventions designed to perturb specific cAMP cascades locally.

Contents

1. Introduction

More than half a century after its discovery (Rall & Sutherland, 1958) 3′–5′-cyclic adenosine monophosphate (cAMP) remains the object of intense scientific interest (Beavo & Brunton, 2002; Scott et al., 2012; Perera & Nikolaev, 2013). Cyclic AMP is known to regulate many diverse and at times opposing cellular functions including, among others, gene transcription (Yamamoto et al., 1988), cell migration (Burdyga et al., 2013; Zimmerman et al., 2013), mitochondrial homeostasis (Di Benedetto et al., 2013a; Valsecchi et al., 2013), cell proliferation (Stork & Schmitt, 2002) and cell death (Suen et al., 2008; Andersen & Kornbluth, 2013). The cAMP signaling pathway comprises multiple components. G-protein coupled receptors (GPCRs) are activated at the plasma membrane on ligand binding. An active G-protein is released

Abbreviations: cAMP, 3′–5′-cyclic adenosine monophosphate; GPCRs, G-protein coupled receptors; EPAC, Guanine-nucleotide exchange proteins activated by cAMP; PKA, cAMP-dependent protein kinase; PDEs, Phosphodiesterases; FRET, Fluorescence Resonance Energy Transfer; G proteins, Heterotrimeric guanosine-binding proteins; βAR, β adrenergic receptors; AKAPs, A kinase-anchoring proteins; R, PKA regulatory subunits; C, PKA catalytic subunits; CREB, Cyclic AMP-responsive element binding protein; PLN, Phospholamban; SERCA2, Sarcoplasmic reticulum (SR) Ca^{2+}-ATPase; Ca^{2+}, Calcium; SR, Sarcoplasmic reticulum; OMM, Outer mitochondrial membrane; IMS, Inter membrane space.

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and in turn activates a membrane bound adenylyl cyclase (AC) to generate cAMP from ATP. cAMP can then bind and activate three main effector proteins: the cyclic-nucleotide-gated ion channels (CNG) (Matulef & Zagotta, 2003), the guanine-nucleotide exchange proteins activated by cAMP (EPAC) (Kawasaki et al., 1998) and the cAMP-dependent protein kinase (PKA) (Walsh et al., 1968; Taylor et al., 2013). The cAMP signal is then terminated by the actions of the cAMP-degrading enzymes phosphodiesterases (PDEs) (Manganiello & Degerman, 1999) while phosphatases can turn off the effects of PKA-mediated phosphorylation (Sim & Scott, 1999; Heijman et al., 2013). As the effects of hormone signaling via cAMP were uncovered shortly after its discovery, it became obvious that the original view that receptors specify the spectrum of hormonal sensitivity of a cell and the substrates available for phosphorylation determine the response was too simplistic. It became clear instead that the same cell can simultaneously express multiple receptors that signal via cAMP as well as multiple targets of PKA, the phosphorylation of which can trigger very different functional outcomes. With this realization, it also became clear that a linear cascade—cAMP generation in response to external stimuli followed by activation of an effector and termination via degradation of the messenger by the phosphodiesterases (PDEs)—is inadequate to explain the ability of cAMP to convey the appropriate information with high fidelity in response to a multitude of extracellular stimuli. Studies conducted in the early eighties in the heart clearly demonstrated that cells can use cAMP to transduce the signal delivered by different hormones into distinct cellular functions. The experimental evidence in essence was that treatment with either prostaglandin E_1 (PGE_1) or the β-adrenergic receptor agonist isoproterenol (ISO) caused cAMP elevation in the heart, but only ISO triggered the expected effect (i.e. positive inotropy) whereas PGE_1 had no effect on contractility, despite both stimuli produced similar amounts of second messenger and comparable levels of PKA activation. To explain these findings the hypothesis was put forward that the components of the cAMP signaling pathway are organized in such a way that cAMP is generated into specific intracellular spaces (Hayes et al., 1980).

These seminal studies first in whole tissue (Hayes et al., 1979, 1980) and then in cell preparations (Buxton & Brunton, 1983), provided the first indirect evidence of compartmentalized cAMP signals. Direct evidence for the existence and functional relevance of subcellular cAMP signaling events (Zaccolo & Pozzan, 2002; Nikolaev et al., 2006) came nearly 20 years later and consolidated the model of compartmentalized cAMP signaling (Zaccolo & Pozzan, 2002; Di Benedetto et al., 2008). The development of molecular tools for the detection of cAMP changes in real time in intact living cells (Berrera et al., 2008; Gesellchen et al., 2011; Stangherlin et al., 2014) greatly contributed to establish the notion of cAMP microdomains. Cyclic AMP and PKA-sensitive probes based on Fluorescence Resonance Energy Transfer (FRET) revolutionized the studies on cAMP signaling. These sensors take advantage of the cAMP binding domains present in PKA (Zaccolo et al., 2000; Zaccolo & Pozzan, 2002) or EPAC (Nikolaev et al., 2004; Ponsioen et al., 2004) and combine sensitivity of detection within the physiological levels with very high spatial and temporal resolution. Moreover, being genetically encoded, these tools can be easily targeted to different cellular compartments making it possible to detect cAMP at specific intracellular sites in real time. The high resolution of these probes allowed to establish that cAMP achieves specificity of action by virtue of a precise organization of the molecular components of its pathway in complex "signalosomes" located at defined cellular locations (Stangherlin & Zaccolo, 2012).

2. Compartmentalization of the cAMP/PKA signaling pathway components

While the evidence in support of compartmentalized cAMP signaling is growing, the newly developed technologies are unveiling an unanticipated level of sophistication of the mechanisms that generate and regulate cAMP functional compartments. In fact, it is now clear that generation of cAMP hotspots in subcellular microdomains involves a complex coordination of events at every step of the cAMP signaling cascade (Zaccolo, 2009; Houslay, 2010; Mika et al., 2012; Perera & Nikolaev, 2013).

2.1. G-protein coupled receptors

At any given moment cells are exposed to a myriad of extracellular stimuli, which act as "first messengers" and are detected by G protein-coupled receptors (GPCRs) at the plasma membrane. Upon ligand binding, GPCR undergo a conformational change resulting in activation of their associated heterotrimeric guanosine-binding proteins (G proteins). On activation, the Gα subunit of the trimeric G-protein is released and is free to associate and modulate the activity of ACs. Depending on the type of their associated Gα subunit, GPCRs can activate ($G\alpha_s$) or inhibit ($G\alpha_i$) cAMP production.

The large number of GPCRs confers significant level of diversity and specificity to cAMP signaling. The cell-specific pattern of GPRC expression certainly defines the ability of a particular cell to respond to extracellular stimuli as well as the intensity of the response. However, there is evidence that, within the same cell, distinct localization of individual GPCR at the plasma membrane may contribute to specificity of response. For example, in cardiac myocytes β_1 adrenergic receptors (β_1AR) are found in both caveolar and non-caveolar membrane fractions, whereas β_2AR predominantly localize to caveolae. β_2AR also appear to be able to exit caveolae upon activation (Rybin et al., 2000), thus adding a further potential element of dynamic regulation to the system (Patel et al., 2008; DiPilato & Zhang, 2009). An elegant study by Nikolaev et al. combining cAMP-sensitive FRET probes with scanning ion conductance microscopy showed that β_1AR are evenly distributed on the plasmalemma of cardiac myocytes, whereas β_2AR are found mainly in T-tubules and are absent from non-tubular areas of the membrane. This study also showed that β_1AR generate a diffuse cAMP signal whereas the signal generated by β_2AR is highly confined (Nikolaev et al., 2010). It is therefore tempting to speculate that the different localization of these receptors at the plasmalemma together with their ability to generate intracellular cAMP signals with distinct properties may contribute to the functional differences observed on selective activation of these receptors, with persistent β_1AR stimulation evoking toxic effects, including myocyte apoptosis and hypertrophy, and persistent β_2AR stimulation resulting in protective effects on the myocardium (Talan et al., 2011).

In addition to differential expression and compartmentalization of receptors at the plasma membrane, the recently described ability of at least some GPCRs to signal after internalization (Calebiro et al., 2009, 2010; Irannejad et al., 2013) provides a novel exciting facet to the model of compartmentalized cAMP signaling. The canonical view is that internalization of GPCRs is part of the receptor desensitization process and involves receptor phosphorylation by G protein-coupled receptor kinases, recruitment of beta-arrestin and consequent GPCR internalization (Kamal et al., 2012). Once internalized, GPCRs can be re-exposed, in their inactive state, at the plasma membrane or, alternatively, can be targeted for degradation (Zhang & Eggert, 2013). Recent studies, however, have challenged this dogma by showing that internalized GPCRs maintain their ability to trigger cAMP production (Calebiro et al., 2009; Ferrandon et al., 2009; Irannejad et al., 2013). Calebiro et al. used intact thyroid follicles from a transgenic mouse with ubiquitous expression of a cAMP FRET-based sensor to demonstrate that internalized thyroid-stimulating hormone receptors (TSHR) produce cAMP signals that are distinct from those generated via activation of TSHR exposed at the cell surface (Calebiro et al., 2009). A further confirmation that GPCR-mediated signal transduction cascades are not triggered exclusively at the cell surface was provided by studies where a series of conformation-specific single-domain antibodies (nanobodies) able to discriminate between the active and inactive states of the β_2-AR

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