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Review

cAMP: Novel concepts in compartmentalised signalling

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ABSTRACT

Cyclic adenosine 3,′5′-monophosphate (cAMP) is the archetypal second messenger produced at the membrane by adenylyl cyclase following activation of many different G protein-coupled receptor (GPCR) types. Although discovered over fifty years ago, the notion that cAMP responses were compartmentalised was born in the 1980s. Since then, modern molecular techniques have facilitated visualisation of cellular cAMP dynamics in real time and helped us to understand how a single, ubiquitous second messenger can direct receptor-specific functions in cells. The aim of this review is to highlight emerging ideas in the cAMP field that are currently developing the concept of compartmentalised cAMP signalling systems.

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Contents

1. Introduction

3′,5′-Cyclic adenosine monophosphate (cAMP) is the archetypal second messenger, produced at the cell membrane by adenylyl cyclase (AC) following G protein-coupled receptor (GPCR) ligation to activate a small number of cAMP effector proteins that trigger functional cellular processes [1]. Ever since the observation of Brunton and co-workers in the 1980s that a number of GPCR activators could elevate cellular cAMP to equivalent levels but at the same time produce distinct physiological outcomes [2], the idea that cAMP signalling is compartmentalised has gained credence. Unequivocal evidence that receptor-specific responses are underpinned by cAMP spatial microcompartmentation was obtained following development of genetically encoded cAMP reporters that used Förster resonance energy transfer (FRET) to measure cAMP

Abbreviations: cAMP, cyclic adenosine 3′,5′-monophosphate; GPCR, G protein-coupled receptor; EC coupling, excitation–contraction coupling; FRET, Förster/fluorescence resonance energy transfer; CFP, cyan fluorescent protein; YFP, yellow fluorescent protein; AKAR, A-kinase activity reporter; SR, sarcoplasmic reticulum; SERCA2, sarcoplasmic reticulum Ca^{2+}-ATPase; PDE, phosphodiesterase; IBMX, isobutyl-methylxanthine; mAKAP, muscle-specific A-kinase anchoring protein; RyR, Ryanodine receptor; RyR2, cardiac ryanodine receptor; AC, adenylyl cyclase; PDE4, cAMP-specific phosphodiesterase-4; PP, protein phosphatase; Epac, exchange protein directly activated by cAMP; CaN, calcineurin; PTHR, parathyroid hormone receptor; PIP2, phosphatidylinositol-4,5-bisphosphate; TSH, thyroid-stimulating hormone; PTHrP, PTH-related peptide; ERM, Ezrin Radixin Moesin; siRNA, small interfering RNA; DNAPK, DNA protein kinase; VEC, vascular endothelial cells; GRK, G protein-coupled receptor kinase.

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dynamics in real time [3–5]. It is now clear that the precise location of proteins that manufacture, degrade and are activated by cAMP is crucial to maintain coherent downstream signalling events that are triggered by specific extracellular cues [6]. As phosphodiesterase (PDE) enzymes are the only route by which cAMP can be degraded in the cell [7], compartmentalisation of these proteins is particularly relevant to regulation of the magnitude and duration of cAMP-dependent events in defined compartments [8]. Current research using cAMP reporters targeted to different intracellular regions describes areas of high PDE expression as cAMP "sinks" that act to locally drain cAMP [9,10]. This model is attractive as it can explain why many contiguous cAMP gradients can be formed simultaneously following a single GPCR activation. Presumably, the correct positioning of cAMP effectors such as cAMP-dependent protein kinase (PKA) and exchange proteins directly activated by cAMP (Epacs) within these gradients allow them to be exposed to cAMP concentrations above their threshold of activation to drive downstream signalling events only at times when cAMP is raised. Conversely, during times of low, basal cAMP, phosphodiesterase activity should prevent inappropriate activation of cAMP effectors.

Compartmentalised cAMP signalling has been the subject of a number of recent reviews looking at the role of phosphodiesterases [7], A-kinase anchoring proteins (AKAPs) [11,12], Epac [13], adenylyl cyclases [14,15] and cAMP itself via cAMP reporters [4,5]. The aim of this review, however, is to cover emerging concepts in cAMP signalling that involve compartmentalised responses. Recent noteworthy advances that have extended and contradicted established tenets of the cAMP field include the concept of sustained cAMP signalling from internalised receptors, compartmentalisation of Epac-directed signals and real time direct visualisation of cAMP gradients within intact cardiac myocytes.

2. New advances in measuring cAMP gradients in the heart

The concept of intracellular compartmentalisation of cAMP arose almost two decades ago from seminal studies performed in cardiac myocytes. Activation of β-adrenergic and prostaglandin receptors was found to result in similar increases in cellular cAMP, however, whereas β-adrenergic stimulation coupled to myocyte contraction and PKA phosphorylation of downstream effectors such as troponin I, prostaglandin receptor stimulation did not [16,17]. These puzzling differences could only be explained if cAMP was compartmentalised within the cell. More recently, the use of genetically encoded cAMP sensors has allowed the real time direct visualisation of cAMP gradients within intact cardiac myocytes [18,19]. Though important questions remain, significant advances have been made in discovering how cAMP signals are organised and integrated with those from other upstream messengers in the heart. In this section, we focus on how the use of cAMP sensors has advanced our understanding of compartmentalised cAMP signalling in myocytes in recent years.

With the introduction and increasing acceptance of the concept of cAMP compartmentalisation came a need to measure real time spatial and temporal changes in cAMP at a subcellular level. This need has been largely addressed by novel fluorescent microscopic techniques, which rely on the phenomenon of fluorescence resonance energy transfer (also known as Förster resonance energy transfer [20]), or FRET. FRET describes the process of energy transfer between two fluorophores, a donor and an acceptor, which are often cyan- and yellow-fluorescent proteins (CFP and YFP). Excitation of the donor by a particular wavelength will lead to a characteristic emission by the donor. However, if the donor and acceptor are in close proximity, and their emission and excitation spectra overlap, intermolecular FRET occurs, and the acceptor emission is predominantly detected. FRET can be used to measure protein–protein interactions, when the donor and acceptor fluorophores are linked to a pair of interacting proteins, and to monitor conformational changes in a single protein [3]. Real time imaging of FRET sensors has enabled the direct visualisation of compartmentalised cAMP signals in living cells with high spatial and temporal resolution [21]. The actions of cAMP are mediated by three types of effector: PKA, cyclic nucleotide-gated channels, and Epacs [22]. To date, a variety of FRET sensors based on PKA and Epac have been used to monitor cardiac cAMP dynamics. In fact, the first direct observation that diffusion of cAMP was restricted in the heart was made using a genetically encoded PKA-based cAMP sensor. Zaccolo and Pozzan fused CFP and YFP to the R and C subunits of PKA respectively (illustrated in Fig. 1A) [19]. Thus, in conditions of low cAMP, the PKA holoenzyme is intact and FRET can occur. When cAMP rises, it binds to the R subunits, leading to a conformational change that releases active C subunits. The distance between CFP and YFP is increased, and FRET is abolished. Using this technique, β-adrenergic stimulation of neonatal cardiac myocytes was shown to generate multiple discrete microdomains of high cAMP which activated a subset of PKA within the cells. Moreover, treatment with a non-specific phosphodiesterase (PDE) inhibitor, isobutylmethylxanthine (IBMX), destroyed these gradients, indicating that PDEs limit the diffusion of cAMP and help to shape these cAMP gradients [19].

2.1. FRET techniques illustrate compartmentalisation of PKA and β-adrenergic signals

PKA can be classified as type I or type II, depending on the presence of different regulatory (R) subunit isoforms. FRET-based studies have helped to determine that PKA-type I and PKA-type II are tethered in separate subcellular compartments in cardiac myocytes. Novel cAMP-responsive sensors were generated by fusing the unique dimerisation/docking domains that mediate PKA RI and RII binding to AKAPs to a FRET sensor incorporating the cAMP-binding domain of Epac1 sandwiched between CFP and YFP [21]. Binding of cAMP to Epac results in a conformational change which increases the distance between the two fluorophores, reducing the FRET signal (see Fig. 1B) [23]. Using these sensors, RI and RII localisation was shown to be mediated by binding to distinct AKAPs, and could be disrupted by treatment with the RI and RII-selective AKAP-competing peptides RIAD and Super-AKAP-IS respectively. This contradicted a previously accepted notion that whereas RII was associated with particulate fractions, RI was cytoplasmic and freely diffusible [24]. Addition of specific PDE family inhibitors resulted in different responses within the two compartments, therefore different PDEs appear to regulate cAMP around RI and RII. Interestingly, application of different GPCR agonists led to measurable cAMP changes in different compartments. Activation of β-adrenergic receptors with isoproterenol resulted in a larger change in fret ratio measured by the RII sensor, corresponding to cAMP generation predominantly in the RII compartment. Conversely, stimulation of prostaglandin receptors led to greater cAMP generation in the RI compartment. Functionally, the RII pool of cAMP was associated with PKA phosphorylation of contractile proteins such as troponin I and phospholamban, whereas RI cAMP was not [21]. Physical compartmentalisation of PKA isoforms therefore allows cells to specifically respond to the generation of distinct pools of cAMP produced by stimulation of diverse G protein-coupled receptors, and couples them to different downstream effectors.

In cardiac myocytes, both β1 and β2-adrenergic receptors couple to the stimulatory G protein Gs [25]. However, selective stimulation of the receptor subtypes results in different physiological and pathophysiological effects. β1- but not β2-stimulation leads to PKA phosphorylation of cardiac contractile proteins, and is

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