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cAMP specificity in the heart

Compartmentation of cAMP in cardiomyocytes

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Summary

The second messenger cAMP is responsible for β -adrenergic receptor (β -AR) stimulation of cardiac function by catecholamines, but is also elevated in response to several other receptors which exert distinct functional effects on the heart. Evidences that spatial segregation of cAMP signalling may explain these observations are reviewed in this chapter. In particular, the role of cell architecture and the molecular mechanisms that restrict cAMP signals will be detailed.

Key words: hormone-heart-cAMP-compartmentation-phosphodiesterase

Introduction

The concept of compartmentalized signaling stems from studies of the cAMP pathway in the heart (reviewed in Steinberg & Brunton, 2001 [1]). In cardiac myocytes, intracellular cAMP levels can be augmented by catecholamine as well as by a large number of hormones and circulating factors. These different “first messengers” act through specific heptahelical receptors coupled to heterotrimeric G_s proteins, which activate cAMP synthesis by adenylyl cyclases (AC). In some cases, such as catecholamine or isoprenaline (ISO) binding to β_1 -adrenergic receptors (β_1 -AR), cAMP elevation causes a dramatic increase in cardiac beating frequency (positive chronotropy), in cardiac force of contraction (positive inotropy) and in cardiac relaxation speed (positive lusitropy). These effects are mainly due to the concerted phosphorylation of key proteins of the excitation-contraction coupling (ECC) by cAMP-dependent protein kinase (PKA): L-type Ca^{2+} channels (LTCC), phospholamban (PLB), ryanodine receptors (RyR) and troponin I (TnI) (Figure 1) [2]. However, in other cases, such as prostaglandin E1 (PGE_1) binding to prostanoid receptors (EP-R), a comparable elevation of cAMP does not lead to phosphorylation of ECC proteins and does not produce the robust functional response that characterizes β_1 -AR stimulation. A first clue to this paradoxical observation was the identification of two PKA isozymes (PKAI and PKAII) in myocardium, differing by the nature of their regulatory subunits (called RI and RII) and by their distribution: whereas PKAI was mostly soluble, PKAII was associated to the particulate fraction [3,4]. It was shown subsequently that ISO increased cAMP and PKA in both the soluble and particulate fraction, whereas PGE_1 increased cAMP and PKA in only the soluble fraction [5,6]. These results constitute the initial evidence that the cAMP cascade is confined in distinct intracellular compartments in cardiac cells. Recently, electrophysiological and imaging approaches that resolve cAMP signals at the

subcellular level in intact cardiomyocytes confirmed that cAMP generated by EP-R stimulation has no access to PKAII and does not regulate PKAII targets [7-9]. Moreover, other G_s protein-coupled receptors (G_s PCRs) that increase cAMP were shown to elicit specific responses in cardiomyocytes. This is the case of β_2 -ARs (see below), glucagon receptors (Glu-Rs) [10] and glucagon-like peptide-1 receptors (GLP₁-Rs) [11]. The current view is that each receptor acts through a specific macromolecular complex that transmits the signal to the final effector(s) [12]. Scaffold proteins, and in particular A-kinase anchoring proteins (AKAPs), that tether PKA in the vicinity of its substrates, are key organizers of these complexes (for further information on this topic, see chapter by John Scott *et al.*) [13,14]. Nevertheless, such organization would not be sufficient to ensure specificity if cAMP would freely diffuse inside the cell. In addition to specialized membrane structures that influence the organization of the cAMP cascade, degradation of cAMP by cyclic nucleotide phosphodiesterases (PDEs) appears critical for the formation of dynamic cAMP microdomains. In this chapter, we will briefly summarize these aspects of cAMP signalling compartmentation in heart cells.

Cell architecture

Signal transduction cascades cannot be dissociated from the surrounding cell architecture which determines their organization. This is particularly true in large adult ventricular myocytes, which developed the transverse tubular network to increase the efficiency of ECC. T-tubules are invaginations of the plasma membrane occurring at the sarcomeric Z-line, whose major function is the synchronization of Ca^{2+} release [15]. Together with proteins involved in cellular Ca^{2+} cycling, several components of the cAMP pathway were shown to localize in T-tubules, including G_s proteins, AC type 5 and 6, and AKAPs [16-19]. However, ECC takes place mostly in specialized regions called dyads where T-tubule and SR meet [2]. To our knowledge, the truly

relevant question in terms of cAMP signalling compartmentation, *i.e.* whether β_1 -ARs are specifically enriched in dyads whereas, for instance, prostanoid receptors would be excluded from these domains is still unanswered.

Other microdomains of interest for signal transduction are caveolae. These small invaginations of the plasma membrane enriched in cholesterol and sphingolipids, are defined by their principal structural protein, caveolin 3 in striated muscles [20]. A number of components of the cAMP pathway including GPCRs, G proteins, AC5/6 isoforms and PKA regulatory subunits were shown to segregate between caveolar and non caveolar fractions of the membrane [21-27]. For instance, in canine heart, caveolae contain the type II regulatory subunit of PKA, but not the type I [22]. Also excluded from caveolae, are the prostanoid E2 receptors [24] and this location was suggested to account for the lack of effect of PGE₁ on L-type Ca²⁺ channel current ($I_{Ca,L}$) and cAMP measured with PKAII-based FRET sensors [8]. Ostrom *et al.* (2001) [24] also found both β_1 -ARs and β_2 -ARs in caveolae, together with AC6. However, the group of Steinberg proposed that segregation of β_1 -ARs and β_2 -ARs subtypes, respectively in non caveolar and caveolar domains of the membrane, underlies the functional differences between β -AR subtypes [25]. Indeed, in contrast to β_1 -AR stimulation, in some species activation of β_2 -ARs appears restricted to the plasma membrane and LTCC phosphorylation, thus inducing a modest inotropic effect without hastening relaxation [28,29] (but see [30]). In line with these findings, co-immunoprecipitation experiments identified a macromolecular signalling complex linking β_2 -ARs to LTCCs in cardiomyocyte caveolae [12]. Caveolae disruption with cholesterol binding agents selectively enhances β_2 -ARs but not β_1 -ARs responses [12,26,31,32]. In particular, caveolae disruption allows β_2 -AR stimulation to phosphorylate PLB and TnI, thus accelerating relaxation [32]. Collectively, these observations support the idea that receptor location in distinct

microdomains of the plasma membrane is an important determinant of functional compartmentation.

Receptor coupling to G_i

GPCRs positively coupled to cAMP may not act exclusively through the classical G_s/cAMP/PKA cascade. In particular, certain GPCRs such as histamine, serotonin, and glucagon can also couple to G_i proteins in cardiac cells [33]. However, the best example of such coupling is the β₂-AR, and this peculiarity has been proposed to explain the distinct effects of β₁-ARs and β₂-ARs in cardiomyocytes [34-36]. Indeed, G_i inhibition with pertussis toxin (PTX) specifically potentiates β₂-AR stimulation of Ca²⁺ transients and contraction [31,32,34,37], and allows β₂-AR to phosphorylate PLB and accelerate relaxation [36]. Thus, β₂-AR coupling to G_i appears to circumvent the concurrent G_s/cAMP/PKA activation and to prevent global actions of β₂-ARs. Surprisingly however, this does not seem to be due to concomitant inhibition of AC since PTX treatment has no effect on β₂-AR evoked cAMP accumulation or PKA activation [36,38,39]. The mechanisms acting downstream of G_i are not fully elucidated. In rat ventricular myocytes, G_i signaling probably involves activation of phosphatidylinositol-3 kinases (PI3K) and phosphatases, since inhibition of these enzymes reproduce the effects of PTX [36,40]. An alternative signalling pathway, previously shown in chick embryonic myocytes, involves G_i activation of cytosolic phospholipase A2 (cPLA2) [37]. Similarly to PI3K inhibition, cPLA2 inhibition enhances β₂-AR evoked Ca²⁺ transients and uncovers PLB phosphorylation by PKA. This signaling cascade is recruited to caveolae upon β₂-AR stimulation [31]. Together, these data are consistent with the hypothesis that spatial restriction of β₂-AR cAMP signalling by G_i occurs within the context of caveolae [32].

Receptor coupling to different adenylyl cyclase isoforms

The heart expresses several AC isoforms (AC4-6, AC9), among which AC5 and AC6 represent the major forms [41]. AC5 and AC6 share strong similarities in sequence and functional characteristics: both are activated by G_s subunits and forskolin and inhibited by G_{α_i} and $G_{\beta\gamma}$ subunits [42,43], Ca^{2+} ions [44,45], and PKA phosphorylation [41,42,46,47]. There are very few evidences that AC5 and AC6 mediate distinct hormonal responses in the heart. However, over-expression of AC6 in neonatal cardiomyocytes selectively enhances β -AR cAMP accumulation vs. EP-R, histamine H2 receptors, Glu-R or adenosine A2-R [23]. A subsequent study in adult cardiomyocytes further demonstrated that AC6 over-expression selectively increased β_1 -AR contractile response but not that of β_2 -AR [48]. In contrast, purinergic receptors seem to couple selectively to AC5 in cardiomyocytes [49].

Restriction of cAMP diffusion

Spatial restriction of cAMP in cardiomyocytes was shown directly by electrophysiological and imaging techniques. Patch clamp recordings of $I_{Ca,L}$ showed that β_2 -ARs are unable to regulate remote LTCC, implying that cAMP diffusion can be strongly restricted [50,51]. The use of fluorescent resonance energy transfer (FRET)-based sensors [39,52] and cyclic nucleotide-gated (CNG) channels [53] to measure directly cAMP in living cells confirmed spatial confinement of the second messenger. Combination of both techniques revealed distinct temporal characteristics of β -AR signals at the membrane and in the cytosol, thus suggesting the existence of cAMP gradients in cardiomyocytes [54]. At least four different mechanisms may explain why cAMP does not diffuse: cAMP buffering, physical barriers, cAMP degradation and cAMP extrusion.

Early biochemical studies and recent models indicate that PKA regulatory subunits can effectively buffer cAMP, and thus likely participate in cAMP compartmentation [55,56]. CNG channel measurements revealed that upon hormonal stimulation cAMP raises to higher level at the plasma membrane than in the bulk cytosol, implying the existence of physical barriers, possibly formed by caveolae and endoplasmic reticulum juxtaposition [57]. Emerging evidence supports the notion that cAMP export, in particular through multidrug resistance-associated proteins 4, may participate in the functional compartmentation of cAMP in certain tissues [58,59]. However, the most documented mechanism for preventing cAMP diffusion in cardiac myocytes is cAMP degradation by cyclic nucleotide phosphodiesterases (PDEs). At present, at least eleven PDE families have been identified, among which PDE1, PDE2, PDE3, and PDE4 are responsible for cAMP degradation in heart [60-62]. Among these, PDE1-3 can hydrolyze both cAMP and cGMP, whereas PDE4 hydrolyzes exclusively cAMP. PDE1 is stimulated by Ca^{2+} -calmodulin; PDE2 is stimulated by cGMP; PDE3 is inhibited by cGMP; and PDE4 is activated by PKA. The PDE1 family is encoded by three genes (PDE1A-C), PDE2 is encoded by one gene (PDE2A), the PDE3 family is encoded by two genes (PDE3A and PDE3B) and PDE4 is encoded by four genes (PDE4A-D). Cardiac myocytes express two forms of PDE2A [63], three forms of PDE3A [64] and >5 forms of PDE4 derived from PDE4A, B and D genes [65,66]. Whether cardiac PDE1 is expressed predominantly in fibroblasts or cardiomyocytes is unknown and might depend on the species considered [67,68]. In addition, the lack of selective inhibitors largely precludes investigation of PDE1 function in cardiomyocytes. In contrast, selective inhibitors of PDE2 (EHNA), PDE3 (cilostamide) and PDE4 (rolipram or Ro 201724) allows dissection of the respective role of these PDEs in cardiac function. Although basal PDE2 activity is much smaller than that of PDE3 and PDE4 in cardiomyocytes, it can effectively suppress β -

AR responses when cGMP levels are elevated [61,69]. However, the main control over ECC is provided by the dominant PDE3 and PDE4 subtypes. PDE3 is about 10 times more sensitive to cAMP than PDE4, which may explain why PDE3 inhibitors exert direct positive inotropic effects especially in large mammals [70], whereas PDE4 exert positive inotropic effects only in the presence of elevated cAMP [62,71].

Role of phosphodiesterases in cardiac cAMP compartmentation

Early evidence for a contribution of PDEs to cAMP compartmentation comes from the differential effect of ISO and PDE inhibitors such as IBMX (a broad spectrum PDE inhibitor) or milrinone (a selective PDE3 inhibitor) on PLB and TnI phosphorylation in guinea pig hearts, [72] which were attributed to different expression of PDEs at the membrane and in the cytosol [70]. In a subsequent study, the fact that PDE inhibition decreased the proportion of particulate vs. cytosolic cAMP suggested that these enzymes limit the amount of cAMP diffusing from the membrane to the cytosol upon β -AR stimulation [73]. Similar conclusions were drawn from $I_{Ca,L}$ or direct cAMP measurements in intact cells with CNG channels or FRET-based biosensors [39,50,52,53]. A number of subsequent studies showed that PDE2, PDE3 and mainly PDE4 shape β -AR cAMP signals in cardiomyocytes [7,54,74-78]. The property of PDE4 long forms to be phosphorylated and activated by PKA [79] and to bind to AKAPs [80] allows efficient suppression of β -AR cAMP by these enzymes (Figure 2) [53,54]. Interestingly, a distinct pattern of PDEs regulate cAMP generated by different G_s PCRs. Indeed, cAMP generated by glucagon receptors is specifically controlled by PDE4, whereas both PDE3 and PDE4 regulate cAMP elicited by β_1 -ARs and β_2 -ARs and both PDE3, PDE4 plus other PDEs regulate EP-R cAMP in adult cardiac myocytes [7]. Studies in mice deficient for the PDE4 genes, and biochemical approaches suggest that products of the PDE4D gene are the dominant PDE4 involved in β -AR

signaling. Indeed, β -AR stimulation of beating frequency is significantly prolonged in neonatal cardiomyocytes lacking the PDE4D gene [81]. In addition, distinct variants of the PDE4D gene were shown to be transiently associated to β_1 -ARs and β_2 -ARs [82,83]. However, the use of dominant negatives approaches rather points to a role of PDE4B2 in the termination of β -AR cAMP signals [76]. This example shows that, although obvious progress were made since the initial proposition that cAMP is compartmentalized, much work remains to be done to provide an exhaustive molecular description of the cAMP signaling pathways in cardiac cells. Understanding these details may not only satisfy academic curiosity, but might also prove useful to envisage new therapeutic strategies in pathological hypertrophy and heart failure. This is suggested by two recent studies in which disruption of a single isoform of PDE3 or PDE4 was shown to favor the development of cardiac dysfunction [84,85].

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Figure legends

Figure 1. Autonomic regulation of cardiac excitation-contraction coupling. Noradrenaline liberated by sympathetic nerves binds to β -ARs (the most classical case of β_1 -ARs is represented) at the surface of cardiac myocytes. β_1 -ARs stimulate cAMP synthesis by adenylyl cyclases (AC) through coupling to Gs proteins. The second messenger in turn activates cAMP-dependent protein kinase (PKA), which phosphorylates a large number of target proteins in cardiomyocytes. Among these, the concerted phosphorylation of L-type Ca^{2+} channels (LTCC), ryanodine receptor subtype 2 (RyR2), phospholamban (PLB) and troponin I (TnI) are responsible for the positive inotropic effect of β -AR stimulation.

Figure 2. Control of β -AR cAMP signals by PDE4 in cardiac myocytes. Upon β -AR stimulation, elevation of cAMP activates PKA (1), which in turn phosphorylates and activates PDE4 (2) to limit cAMP accumulation (3). This negative feedback loop is facilitated by the close proximity of PKA and PDE4 through their binding to the same A-kinase anchoring protein (AKAP).

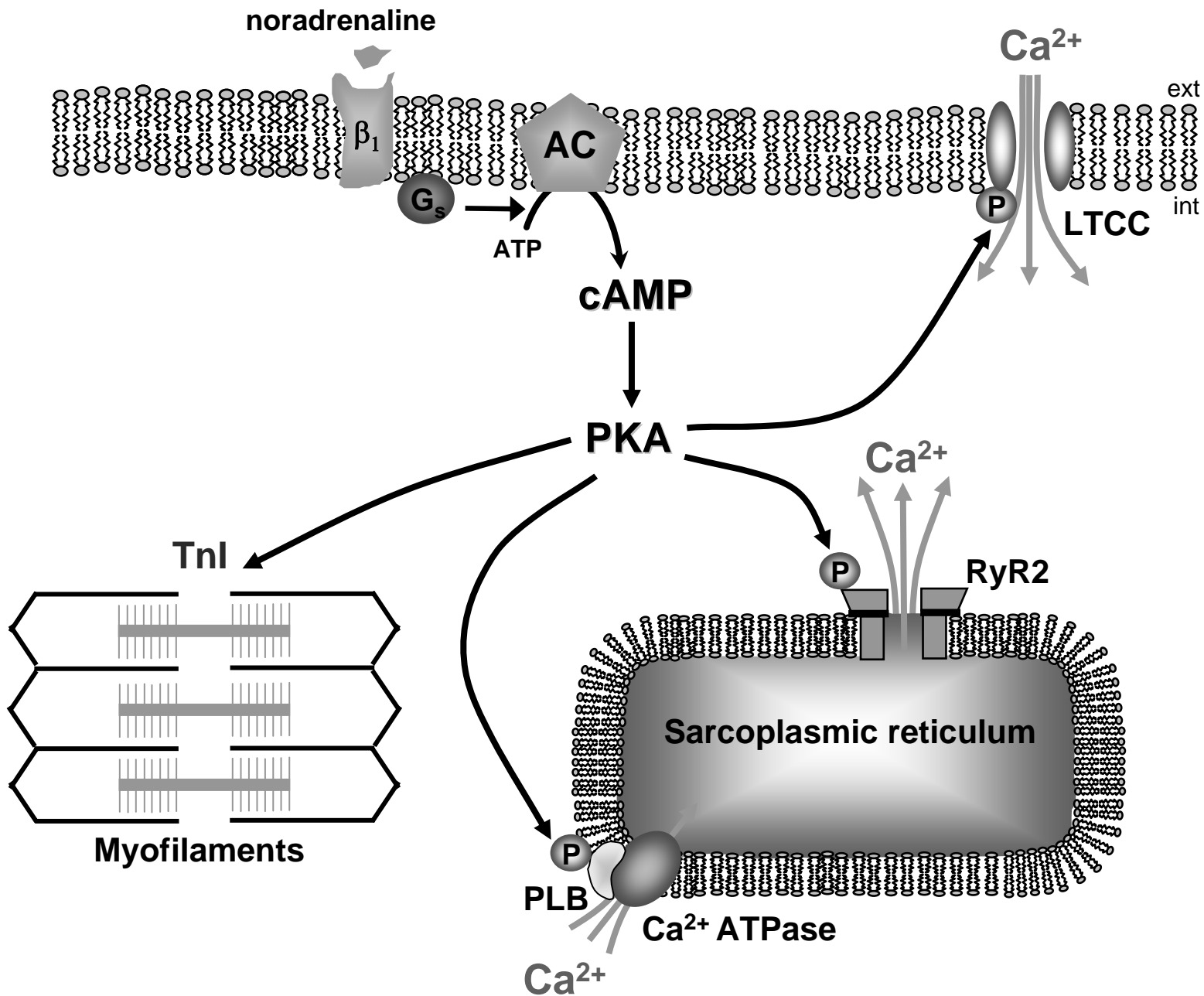


Figure 1

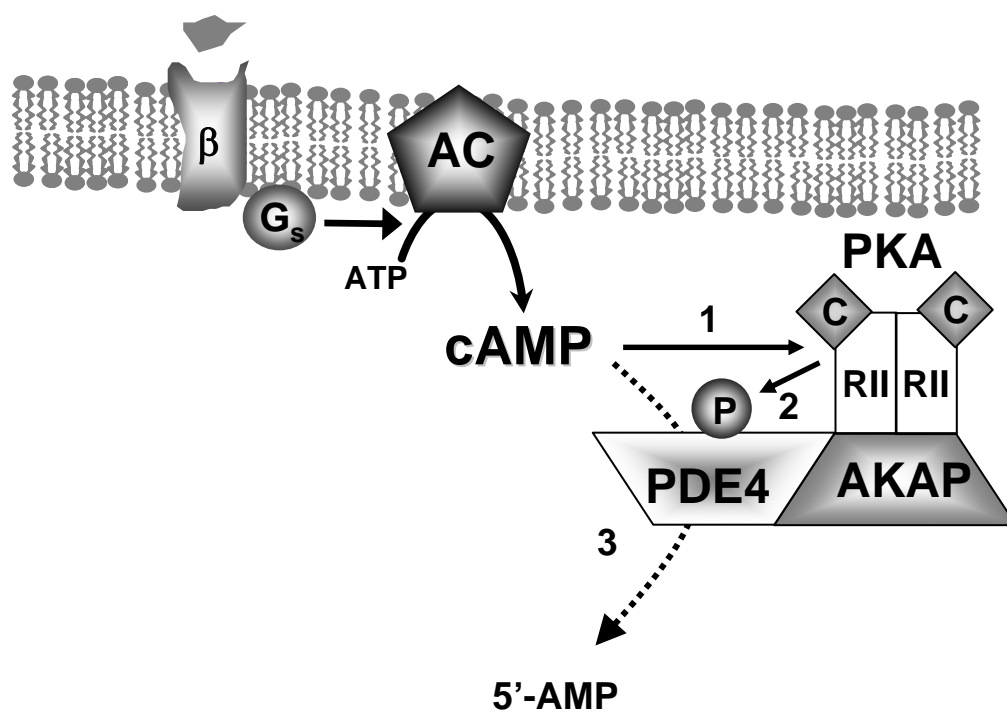


Figure 2

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