

When Is cAMP Not cAMP? Effects of Compartmentalization

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Many important cellular processes are controlled via stimulation (or inhibition) of signal transduction systems, among which heptahelical G protein–coupled receptors (GPCRs) figure prominently. A classical example in cardiac myocytes is the β -adrenergic receptor (β -AR) cascade (see Figure, panel A), which leads to positive inotropic and lusitropic effects.¹ Occupation of the β -ARs by an agonist activates a GTP binding protein (G_s), such that the α subunit dissociates and activates adenylyl cyclase (AC), thereby producing cAMP. The increase in cAMP leads to the dissociation of the regulatory and catalytic subunits of protein kinase A (PKA). PKA can be tethered near its substrates by an A-kinase anchoring protein (AKAP). The PKA catalytic subunit phosphorylates several key myocyte proteins involved in excitation-contraction (E-C) coupling, including the L-type Ca^{2+} channel, phospholamban (PLB), ryanodine receptor (RyR), myosin binding protein C, and troponin I (TnI). These effects produce PKA-dependent increases in Ca^{2+} current (I_{Ca}), sarcoplasmic reticulum (SR) Ca^{2+} uptake and release, as well as a desensitization of the myofilaments to Ca^{2+} . The net result is the characteristic positive inotropic and lusitropic effects of β -AR activation in cardiac myocytes.

The stimulatory effects of GPCR activation can be inhibited at several levels. The receptor can be desensitized by G protein receptor kinases (eg, β -ARK) and arrestins.² The activation of AC by G_{sa} can be antagonized by an inhibitory G protein (G_i), which can be activated by muscarinic receptors (and may also be coactivated during β_2 -AR activation).^{3–5} The effects of cAMP can also be limited by cAMP hydrolysis by phosphodiesterases (PDEs). The PKA phosphorylation target can also be dephosphorylated by phosphatases.

Thus, there are many points where regulation can occur, and all of these proteins can occur in different isoforms creating a rich montage of PKA-dependent regulation of cardiac myocyte function. Indeed, the proximity to PKA targets, local amounts of regulatory proteins, and different isoforms can create highly specialized local signaling between a given hormone and its cellular targets, even when the

major players are the same. Moreover, it is simple to envisage variations that could cause either rapid and highly transient target phosphorylation (eg, with rapid dephosphorylation) or more gradual, integrated phosphorylation and dephosphorylation, using almost the same molecular players.

β_1 -AR activation in ventricular myocytes produces robust inotropic and lusitropic effects that are paralleled (and explained) by increases in cAMP and phosphorylation of Ca^{2+} channels, PLB, and TnI. However, β_2 -AR activation can be more restricted to I_{Ca} enhancement, with less particulate PKA activity (although enough to phosphorylate L-type Ca^{2+} channels).⁶ Other GPCRs, which can stimulate cAMP production (eg, prostaglandin E and histamine), do not produce the robust inotropic effects that β_1 -AR activation does.⁷ Similarly, a report⁸ in this issue of *Circulation Research* shows that glucagon-like peptide-1 (GLP-1) produces comparable cAMP levels as does isoproterenol (a β -AR activator), but GLP-1 produces a modest negative inotropy and no lusitropic effect (in sharp contrast to isoproterenol). Thus, not all hormones that lead to increased cAMP levels result in the classic β -AR effect. This is consistent with compartmentalization where different pools of cAMP and cascade elements lead to differing effects. GLP-1 may be linked more centrally to a pathway that alters glucose utilization, rather than Ca^{2+} transport per se. Moreover, this raises a question as to whether total cellular [cAMP] is really a central modulator or a ubiquitous epiphenomenon of local cAMP-mediated signal transduction systems. That is, perhaps all of the important (and targeted) control occurs in local domains where higher [cAMP] and [PKA] may be closer to critical targets. So not all cAMP molecules are equal. Furthermore there is increasing evidence that many of the key regulatory proteins are tightly colocalized. For example, the L-type Ca^{2+} channel appears to coassemble with β_2 -ARs, G_s , AC, PKA, and phosphatase 2A (PP2A).⁹ The RyR (or SR Ca^{2+} release channel) serves both as a PKA target and as a scaffolding protein, where PKA and phosphatases 1 and 2A are all bound to the RyR via anchoring proteins.¹⁰

Compartmentalization in the cAMP-PKA Cascade

Let us consider a tightly coupled cascade from β -ARs to cAMP to PKA-dependent regulation of cardiac I_{Ca} , PLB, and RyR as a model system (whether truly accurate or not). There are many ways this cascade can be modified (eg, different receptors, G proteins, AC isoforms, AKAPs, local PKA targets, local PDEs, and local phosphatases) resulting in different phenotypes, and many permutations have been reported.

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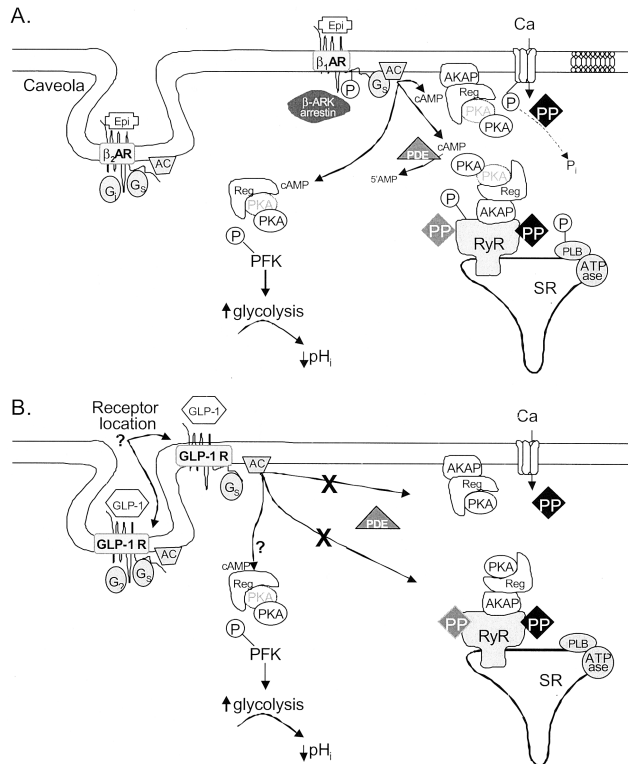
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A, Local β -AR signaling cascade in cardiac myocytes. B, GLP-1 signaling cascade. In this pathway, cAMP may activate glycolysis but cannot activate I_{Ca} , PLB, or TnI phosphorylation. Epi indicates epinephrine; PFK, phosphofruktokinase; and ATPase, SR Ca^{2+} -ATPase (see text for other abbreviations).

For example, β_1 -AR activation phosphorylates L-type Ca^{2+} channel, PLB, RyR, TnI, and C-protein and causes the characteristic positive inotropic and lusitropic effects, whereas β_2 -AR activation can selectively stimulate I_{Ca} causing a lesser positive inotropic effect and no lusitropic effect.⁶ The cAMP levels produced by β_2 -ARs were apparently restricted to microdomains near Ca^{2+} channels by coupling to not only G_s but also to G_i .^{4,5} Thus, interaction of receptor isoforms with different G proteins plays a role in compartmentalization of cAMP. Phosphatase inhibition also increased the inotropic effect of β_2 -AR activation but not β_1 -ARs.⁴ Thus, phosphatases may be involved in restricting functional domains in PKA signaling.

PDEs may also be spatially localized to discrete regions.^{11–13} A patch-clamp study of I_{Ca} tested the effect of local AC activation in part of a single cell.¹⁴ Forskolin (a direct AC activator), applied locally, increased I_{Ca} throughout the myocyte. In contrast, local isoproterenol application increased I_{Ca} only locally, but PDE inhibition allowed local isoproterenol to activate I_{Ca} throughout the cell. It was speculated that forskolin caused widespread AC activation, whereas isoproterenol only activated AC that was coupled to β -ARs and thus had only a local effect. However, when cAMP breakdown was inhibited, the cAMP could spill over and exert more global effects. Thus, PDE may also play a role in limiting the spatial spread of PKA activation.

Another component of compartmentalization is receptor location. L-type Ca^{2+} channels and RyRs are colocalized to

SR sarcolemmal junctions, and the strong regulation of I_{Ca} via β -ARs may indicate close physical proximity of PKA signaling molecules with the E-C coupling complex. However, some signaling complexes are targeted to sarcolemmal invaginations called caveolae (eg, endothelin receptors, nitric oxide synthase, and β_2 -ARs). Rybin et al¹⁵ showed that most β_1 -ARs are located in noncaveolar regions, whereas β_2 -ARs are almost exclusively located in caveolae (ie, distant from junctional SR). β_2 -ARs couple to AC more efficiently than β_1 -ARs,¹⁶ which could be due in part to the colocalization of AC (types V and VI) in caveolae.¹⁵ Other receptor locations can also regulate signaling cascades. For example, activation of M_2 muscarinic receptors has different effects on cAMP levels produced by β_1 -ARs (decrease) versus β_2 -ARs (no change).¹⁷ This may be explained by the relative exclusion of M_2 muscarinic receptors from caveolar regions. Thus, the location of the receptor and its signaling cascade components (caveolae vs noncaveolae) can also play a role in determining the functional compartmentalization of cAMP.

Anchoring proteins for PKA and phosphatases can also be important components of regulatory complexes.^{10,18} AKAPs localize the PKA subunits near phosphorylation targets. Thus, while PKA can phosphorylate many substrates in vitro, PKA in vivo may preferentially target those sites that are near AKAPs. Likewise, locally anchored phosphatases^{9,10} may allow for shorter-acting phosphorylation effects.

Hohl and Li¹⁹ found a closer correlation between particulate (versus total) cAMP levels and the amplitude of myocyte shortening and Ca^{2+} transients in response to various agents. These data agree with a large inotropic effect and high-particulate cAMP with β_1 -ARs versus no increase in particulate PKA activity or inotropy with β_2 -ARs.⁶ This distinction may also explain why prostaglandin E is not inotropic despite increased total cAMP.²⁰ Thus, there are extensive data suggesting subcellular compartmentalization in cAMP/PKA regulation in cardiac myocytes.

cAMP Compartmentalization by GLP-1

Vila Petroff et al⁸ show that GLP-1 has effects similar to prostaglandin E. GLP-1 causes an increase in cAMP levels (comparable to that with isoproterenol) without causing any inotropic or lusitropic effect. This indicates that cAMP production via GLP-1 is compartmentalized and unable to increase I_{Ca} or SR Ca^{2+} transport. These investigators explored cAMP compartmentalization but were unable to determine a clear mechanism. Neither pertussis toxin (to block G_i), PDE inhibition, nor phosphatase inhibition could unmask a positive inotropic effect of GLP-1, despite a further increase of cAMP levels with PDE inhibition. Thus, even large increases in total cAMP (mediated by GLP-1) appeared unable to stimulate either I_{Ca} or SR Ca^{2+} transport. This implies that cAMP produced by GLP-1 has especially poor access to the junctions where I_{Ca} and SR proteins exist (even compared with β_2 -ARs, which are in caveolae). However, the effect of PKA activation to produce a modest acidosis (presumed to be due to stimulation of glycolysis) was similar between isoproterenol and GLP-1 (see Figure, panel B). It would be helpful to determine whether the GLP-1-induced rise in cAMP is in the particulate fraction or is spatially

uniform in the myocyte (versus isoproterenol), and the cAMP indicator that uses fluorescence energy transfer²¹ is hopeful in this regard. It would also be helpful to know where the GLP-1 receptor is located (eg, caveolae, nonjunctional sarcolemma).

An implication of these results (not mentioned) is that global cAMP is irrelevant to the inotropic effects of isoproterenol. In this sense, the global cAMP level might be considered an epiphenomenon to the signaling cascades (ie, a spillover of cAMP involved in local control of signal transduction). We speculate that the global [cAMP] might be sufficient to stimulate glycolysis and cause similar acidosis with isoproterenol or GLP-1, but that activation of E-C coupling proteins might require higher local [cAMP] (eg, near Ca²⁺ channels). A problem with this idea is that most of the PLB and TnI sites that are readily phosphorylated in the β -AR response are nonjunctional. The usual assumption is that global cAMP and PKA are involved at these sites (and low concentration of membrane-permeant cAMP analogues can induce the lusitropic effects), but functional PKA targeting to PLB and TnI cannot be ruled out. Such targeting to PLB and TnI would be costly in energetic terms because of the large numbers of targeting proteins and cAMP molecules required (eg, PLB and TnI are present at levels of 50 to 70 μ mol/L in the cell) and the broad spatial distribution of PLB and TnI versus Ca²⁺ channels. Although this may be a provocative interpretation of these results, it could explain why blocking PDE (which raised cAMP) or phosphatases still could not unmask any positive inotropic effect of GLP-1. So we may further speculate that the location of GLP-1 receptors is not located near E-C coupling proteins (see Figure, panel B). It is unclear how functionally important the cardiac effects of GLP-1 used in diabetic therapy may be. However, while isoproterenol is still inotropic with GLP-1, GLP-1 may increase basal energy consumption without increasing contractility, a potential concern with respect to cardiac energy supply/demand issues. On a more general note, additional insightful work on compartmentalization of cAMP signaling is needed.

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