β-adrenergic receptor (β-AR) signaling in cardiac myocytes influences contractile and relaxation states in the heart. Classically, following hormone activation, β-AR preferentially couples with Gs, which in turn activates adenylyl cyclase and cAMP production. The predominant effector of cAMP, cAMP-dependent protein kinase (PKA), then phosphorylates many proteins important for cardiac function such as L-type calcium channels and phospholamban in the sarcoplasmic reticulum membrane. However, studies in myocytes have shown not all receptors that transduce signals via cAMP generate the same functional effects.1 These observations have led to the concept of cAMP compartmentation, which attributes the functional specificity and differential regulation to intricate spatial and temporal control of signaling molecules in the cAMP pathway. In this issue of Circulation Research, Nikolaev et al2 take another look at the differentially regulated β1 and β2-adrenergic signaling by using FRET-based cAMP imaging in adult cardiomyocytes, a method that is well suited for revealing the spatiotemporal complexity in cAMP signaling.

Fluorescence ratio imaging of cAMP in living cells was first introduced 15 years ago with the development of a bimolecular indicator using fluorescent dye-tagged regulatory and catalytic subunits of PKA.3 This class of cAMP indicators has evolved to become genetically encodable with the use of green fluorescent protein (GFP) variants4,5 and in recent years, unimolecular with the use of single cAMP binding domain-containing proteins and protein fragments.6–8 Nikolaev et al add to our molecular toolbox with the development of yet another FRET-based cAMP sensor with new characteristics. This sensor uses the cAMP binding domain of the hyperpolarization-activated cyclic nucleotide gated channel 2 (HCN2) as opposed to the binding domains from PKA or exchange proteins directly activated by cAMP (Epac), used in other FRET sensors. The resulting biosensor, HCN2-camps, maintains a high sensitivity for cAMP but does not appear to saturate at physiological cAMP concentrations in adult cardiomyocytes, thus being able to report agonist-induced changes in cAMP. Another important characteristic of the HCN2-camps is its uniform distribution in cardiomyocytes that allows for the measurement of cAMP dynamics throughout the entire cell without bias from sensor localization.

A significant point illustrated by design and application of this new cAMP biosensor is the importance of being able to use a tailored cAMP biosensor that is most suited for a particular experiment, cell system, or functional study. Several key characteristics are worth considering when designing or choosing a cAMP sensor. First, the binding property of the sensor, such as binding affinity for cAMP, is among the most important characteristics. Different cell types have varying basal levels of cAMP and different capacity of cAMP production and degradation, thus requiring use of biosensors that have appropriate detection ranges that match concentrations of endogenous cAMP. Secondly, expression levels and localization patterns vary between different sensors. For example, a PKA-based cAMP sensor showed distinct subcellular localization within cardiomyocytes because of interaction with A kinase anchoring proteins (AKAPs).5 Furthermore, biosensors can be targeted to various subcellular sites in the cell for examining specific pools of cAMP and signaling microdomains,7 whereas a diffusible indicator is more suited for visualizing global cAMP changes. Thirdly, the dynamic range of a sensor refers to the difference in fluorescence signals, usually reported as emission ratios for this class of sensors, between cAMP-bound and unbound states. A large dynamic range is always a desirable property for achieving high signal to noise ratios, particularly in the case of detecting subtle and suboptimal changes. Other parameters, such as fluorescent properties of the FRET pair, could also be tailor-designed to accommodate specific application needs. Fortunately, an array of cAMP biosensors is already available that expresses a combination of characteristics, which are often complementary to one another. It can be expected that new variants with improved or novel properties will be further developed to provide revealing windows into the complex cAMP regulation in various live systems.

The live systems suitable for fluorescence imaging range from cells to tissues and animals. Although a popular choice and good model systems in many cases, cultured cell lines are usually far removed from their tissue origin and may exhibit an altered signaling environment such as altered surface expression of receptors and remodeling of innate cellular architecture. Primary cells, which may be more physiologically relevant, are often difficult to transfect. The introduction of viral vectors for gene transduction has pushed research past this obstacle. However, the expression of fluorescent protein-based probes in primary cells usually requires longer culturing to insure a highly expressed sensor with matured...
fluorophores, although in some cases fluorescence can be detected in several hours following transduction. In this study, Nikolaev et al describe the creation of transgenic mice expressing HCN2-camps specifically in cardiac myocytes, which enables the authors to perform FRET imaging experiments with freshly isolated adult myocytes, avoiding any potential adverse effects associated with long-term culturing. This approach can be extended to sensor expression in other tissues and use of reporters of other cellular processes. Furthermore, with the development of suitable platforms, for instance involving multiphoton imaging, transgenic animals expressing these fluorescent reporters may be used in tissue imaging and in vivo imaging, allowing for analyzing signaling processes in these live systems to be correlated with functional studies.

In this study, using single adult cardiomyocytes isolated from the transgenic mice, Nikolaev et al reveal differential signaling of β-AR subtypes directly at the level of cAMP dynamics, extending this FRET-based imaging approach to a physiological model system to generate new insights into cAMP compartmentation. They show that selective stimulation of β2-AR leads to robust cAMP accumulation and propagation throughout the entire cell even with a reduced amount of active receptors, whereas selective stimulation of β1-AR results in a smaller localized increase in cAMP that does not propagate even in the presence of phosphodiesterase (PDE) inhibitors. Therefore, some other mechanism besides local degradation of cAMP by PDEs is responsible for the different signaling profiles of β-AR subtypes.

The data presented by the authors coincides very nicely with results from previous studies on the downstream signaling and functional effects of these receptor subtypes. β1, the predominant subtype in myocytes, robustly activates AC and cAMP production throughout the myocyte, increasing contraction. However, specific activation of β2-AR causes a less positive inotropic effect. Differences in signaling profiles among the β-AR subtypes were also observed in downstream signaling events. β1-AR activation leads to phosphorylation of many proteins to elicit positive inotropic and lusitropic effects whereas β2-AR activation appears to be confined to the phosphorylation of L-type calcium channels, although some difference among species was observed. It has been suggested that cAMP levels produced by β2-AR could be restricted to microdomains near calcium channels.

Such localized cAMP/PKA signaling was initially observed in cardiac myocytes more than twenty years ago and led to the proposal of compartmentation of cAMP signaling. Buxton and Brunton observed isoproterenol increased cAMP levels and subsequent PKA activity in both soluble and particulate fractions of cell homogenates, whereas PGE1 only activated PKA in the soluble fraction of cardiac myocytes. It was hypothesized that although activation of different receptors types can lead to increased levels of cAMP, strict spatial and temporal interaction among components of the signaling cascade are responsible for the specific functional response. Jurevicus and Fischmeister further contributed to this idea of subcellular compartmentation by demonstrating β-adrenergic receptor activation led to localized activation of L-type calcium channels whereas stimulation of adenylyl cyclase also activated more distal channels.

The mechanisms that create highly specialized cAMP signaling compartments can be defined in terms of spatial compartmentation and temporal coordination of signaling molecules. Spatial microdomains within the plasma membrane are thought to contribute to the observed differences in β-adrenergic receptor subtype signaling. Caveolae, cholesterol-rich invaginations in the plasma membrane, have been implicated as regulators in GPCR signaling as they create a local environment that can bring receptors, G proteins, and AC in close proximity. Both β1- and β2-AR subtypes are found in caveolae; however, whereas β2-AR is distributed throughout the plasma membrane including caveolae, β1-AR is exclusively found in caveolae in cardiomyocytes. Interestingly, following agonist-stimulation, β2-AR translocates out of caveolae presumably to undergo clathrin-mediated endocytosis, whereas β1-AR distribution remains unchanged.

This change in distribution on receptor activation may affect how signals propagate within myocytes. On a smaller scale, β-AR subtypes may form different signaling complexes, in a sense, “nanodomains” of cAMP signaling, which can evoke specific functional responses. For example, it is suggested that β-AR subtypes have differential coupling to adenylyl cyclases in cardiac myocytes, at least partially mediated by colocalization in caveolae. Furthermore, a kinase anchoring proteins (AKAPs) can assemble signaling complexes including PKA and PDE at the site of receptor activation and cAMP production. The functional effects induced by β1-AR and β2-AR activation also showed differential sensitivity to phosphatase inhibition.

Temporal regulation of signaling, which is tightly coupled with the spatial compartmentation, is also important for mediating differential signaling between different β-AR subtypes. Following activation, β1-AR, more so than β2-AR, is localized at the plasma membrane which restricts the receptor from engaging in further cAMP signaling. Furthermore, β2-AR can undergo switching from Gs to Gi coupling which also turns off cAMP production. In fact, the coupling of β1-AR to Gi, has been suggested to contribute to the localized cAMP signaling. Of note, Nikolaev et al demonstrate inhibition of Gi by pertussis toxin did not change the restricted cAMP accumulation on β1-AR stimulation. Further investigation is therefore needed to evaluate the contributions of the various spatial and temporal controls to the observed differential cAMP responses elicited by β-AR subtypes.

As exemplified by Nikolaev et al, FRET-based biosensors promise to be powerful tools to illuminate intricate regulatory mechanisms of cAMP signaling that govern normal cardiac function. A better understanding of differential β-adrenergic signaling in the heart could lead to more effective therapeutics for cardiac failure.
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References


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