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Mechanisms of cAMP Compartmentation in Cardiac Myocytes: Experimental and Computational Approaches to Understanding

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Abstract

The small diffusible second messenger 3',5'-cyclic adenosine monophosphate (cAMP) is found in virtually every cell in our bodies, where it mediates responses to a variety of different G protein coupled receptors (GPCRs). In the heart, cAMP plays a critical role in regulating many different aspects of cardiac myocyte function, including gene transcription, cell metabolism, and excitation-contraction coupling. Yet, not all GPCRs that stimulate cAMP production elicit the same responses. Subcellular compartmentation of cAMP is essential to explain how different receptors can utilize the same diffusible second messenger to elicit unique functional responses. However the mechanisms contributing to this behavior and its significance in producing physiological and pathological responses are incompletely understood. Mathematical modeling has played an essential role in gaining insight into these questions. This review discusses what we currently know about cAMP compartmentation in cardiac myocytes and questions that are yet to be answered.

Graphical Abstract

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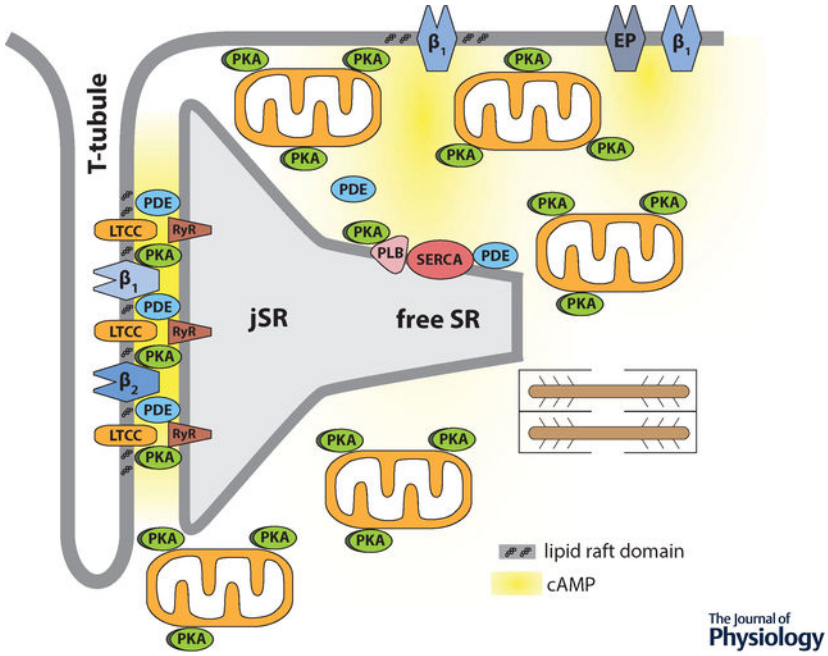
AUTHOR CONTRIBUTIONS

Both authors contributed to the writing of this review. In addition, both authors approved the final version of the manuscript, both authors qualify for authorship, and all those who qualify for authorship are listed.

COMPETING INTERESTS

The authors have no conflicts of interests to declare.



Compartmentation of receptor dependent cAMP signaling in adult cardiac ventricular myocytes depends on many factors, including localized production by different G-protein coupled receptors, localized degradation by phosphodiesterases, buffering by protein kinase A, and restricted diffusion involving tight spaces such as the dyadic cleft.

INTRODUCTION

The autonomic nervous system plays a critical role in regulating cardiac function in response to stress and exercise. Sympathetic stimulation enhances cardiac output by increasing both heart rate and contractility as part of the classic “fight or flight” response. These effects are mediated primarily through neurotransmitter stimulation of β-adrenergic receptors (βARs) and subsequent production of the small diffusible second messenger 3’,5’-cyclic adenosine monophosphate (cAMP).

In the heart, cAMP actually plays a critical role in regulating many different aspects of cardiac myocyte function, including gene transcription and cell metabolism, in addition to electrical and mechanical activity. Ironically, there are many different G-protein coupled receptors (GPCRs) capable of stimulating cAMP production in the heart, yet they do not all elicit the same responses. The classic example of this behavior was first reported more than forty years ago (Brunton *et al.*, 1979; Hayes *et al.*, 1979). βARs were found to stimulate cAMP production and activate protein kinase A (PKA), resulting in phosphorylation-dependent changes in contraction. On the other hand, E-type prostaglandin receptors (EPRs) were also found to increase cAMP production, but there were no concomitant functional changes. This led to the original hypothesis that cAMP signaling must be compartmentalized. In other words, this cytosolic second messenger, often thought of as being freely diffusible, must be spatially restricted in order to elicit unique cellular responses.

Compartmentalized production of cAMP is also believed to contribute to functional responses involving βARs. Cardiac myocytes express both β_1 and β_2AR subtypes. However, while β_1ARs produce a global increase in cAMP, affecting many different processes, β_2ARs produce a more localized cAMP response that selectively regulates L-type Ca^{2+} channel (LTCC) activity (Chen-Izu *et al.*, 2000). The specificity of the β_2AR response is attributed at least in part to the formation of a signaling complex that links β_2ARs and LTCCs (Balijepalli *et al.*, 2006). This difference in cAMP production may also contribute to the fact that β_1ARs produce a strong positive inotropic response (increase in the rate and force of contraction) as well as a positive lusitropic response (increase in the rate of relaxation), while in many species, β_2ARs produce a more modest increase in contraction without affecting the rate of relaxation (Xiao *et al.*, 1994). Production of cAMP by both β_1 and β_2ARs regulate LTCCs associated with the plasma membrane of the transverse (t) tubules, which contributes to the increase in contractility (figure 1A). However, only β_1AR production of cAMP leads to phosphorylation of phospholamban (PLN), which is found in non-junctional regions of the sarcoplasmic reticulum (SR). This later effect results in an increase in sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) activity, which not only contributes to the increase in contraction by increasing SR Ca^{2+} content, it also contributes to an increase in the rate of relaxation.

It should be noted that compartmentation of cAMP signaling is not unique to cardiac myocytes. Evidence for this type of behavior has been found in virtually every cell type in which it has been examined. Despite the ubiquitous nature of this phenomenon, our knowledge of the underlying mechanisms is incomplete. So how does a cell “decide” whether or not to produce a cAMP-dependent response following stimulation of any given receptor? One important means of ensuring the fidelity of cAMP-mediated signaling is through the formation of signaling complexes with A kinase anchoring proteins (AKAPs) (Scott *et al.*, 2013). Disrupting AKAP interactions has been shown in numerous studies to alter cAMP responses by preventing PKA binding to and subsequent phosphorylation of specific target proteins. However, the anchoring of PKA by AKAPs alone is not sufficient to explain compartmentation. If cAMP were able to move freely throughout the cell, the same response would be expected regardless of the receptor that produced it. Therefore, there must also be mechanisms for creating discrete, localized pools of cAMP.

Over the past two decades, a number of new approaches have been developed, which have greatly impacted the study of cAMP signaling mechanisms. One of the first major advances was the generation of genetically encoded biosensors that can be used to measure cAMP activity in intact cells (Dikolayev *et al.*, 2019). Another has been the implementation of computational modeling (Saucerman *et al.*, 2014). This review will highlight some of the experimental evidence identifying factors contributing to cAMP compartmentation as it occurs in cardiac myocytes. We will also discuss the critical role that computational modeling has played by pointing us to new mechanisms contributing to this behavior and its significance in generating complex cellular responses. Table 1 lists those studies and indicates the aspects of cAMP compartmentation that are discussed in this review.

LOCALIZED PRODUCTION

Perhaps the easiest way to explain the ability of different receptors to produce unique cAMP responses is if they are found in discrete locations throughout the cell. One way in which receptors are segregated is by their inclusion or exclusion from caveolar signaling complexes (Brown, 2006; Allen *et al.*, 2007). Caveolae are a subset of cholesterol-rich lipid raft domains of the plasma membrane that contain the multi-functional protein caveolin. There are three caveolin isoforms, with caveolin type 3 (Cav3) being the predominant version expressed in cardiac myocytes. Caveolins contain a caveolin scaffolding domain (CSD), which is believed to be involved in interactions with other signaling proteins, including certain GPCRs along with other components of the cAMP signaling cascade (Harvey & Calaghan, 2012).

β_1-Adrenergic Receptors

β_1ARs make up ~80% of the total βAR population in the hearts of most species (Steinberg, 1999). Furthermore, these receptors have been found in caveolar as well as non-caveolar fractions of the plasma membrane (Rybin *et al.*, 2000; Ostrom *et al.*, 2004; Balijepalli *et al.*, 2006; Nichols *et al.*, 2010; Agarwal *et al.*, 2011). The effects of β_1AR stimulation involve the activation of adenylyl cyclase (AC) by the stimulatory G protein G_s. Cardiac myocytes actually express multiple AC isoforms. The most abundant are AC5 and AC6, which are also found in caveolar membrane fractions (Rybin *et al.*, 2000; Head *et al.*, 2005; Balijepalli *et al.*, 2006). This appears to involve direct interactions of AC with Cav3 (Rybin *et al.*, 2000; Head *et al.*, 2005; Balijepalli *et al.*, 2006), although at least one study has suggested that interactions between Cav3 and AC involve an AKAP (Nichols *et al.*, 2010).

The wide distribution of β_1ARs is often associated with the production of global rather than compartmentalized cAMP responses in cardiac myocytes (Nikolaev *et al.*, 2006). However, the effects that disrupting caveolae have on cAMP activity measured in different subcellular locations indicate that it may not be that simple. Depleting membrane cholesterol with agents such as methyl-β-cyclodextrin (MβCD) disrupts the inhibitory effect that Cav3 has on AC activity in caveolae. Agarwal et al. (2011) found that this selectively enhanced β_1AR stimulation of cAMP activity detected by a FRET-based biosensor targeted to type II PKA signaling domains in adult cardiac myocytes. However, it had no effect on global changes in cAMP activity detected by a probe expressed throughout the cytosolic compartment. The increase in sensitivity of cAMP responses detected by the type II PKA probe correlated with an increase in the sensitivity of contractile and LTCC responses (Agarwal *et al.*, 2011). These results suggest that a subpopulation of β_1ARs associated with caveolae regulate target proteins involved in EC coupling, while β_1ARs found elsewhere contribute to more global changes, which may be involved in regulating other responses.

β_2-Adrenergic Receptors

β_2ARs make up the bulk of the remaining βAR population in cardiac myocytes, and while they are capable of simulating cAMP production, the effects they produce differ from β_1ARs. Some of those differences have been attributed to the ability of β_2ARs to couple to the inhibitory G protein, G_i, in addition to G_s. although the G_i signaling

mechanism involved is still unresolved (Xiao *et al.*, 1995; Kuschel *et al.*, 1999a; Xiao *et al.*, 1999). Another difference between the sub-types of βARs in the heart is that β_2ARs are believed exist exclusively in caveolar membrane domains (Ostrom *et al.*, 2000; Rybin *et al.*, 2000; Ostrom *et al.*, 2001; Xiang *et al.*, 2002; Ostrom *et al.*, 2004; Head *et al.*, 2005; Balijepalli *et al.*, 2006; Head *et al.*, 2006). Furthermore, there are distinct differences in the precise location of βAR sub-types within the 3D architecture of the cell. Adult ventricular myocytes possess an extensive t-tubule network that allows the plasma membrane to reach throughout the cell interior (see figure 1A), facilitating EC coupling. Nikolaev et al. (2010) used scanning ion conductance microscopy in combination with FRET-based biosensors to elegantly demonstrate that while β_1AR stimulation produces cAMP responses associated with all areas of the plasma membrane, β_2AR production of cAMP is confined to regions of the cell associated specifically with t-tubules. However, in heart failure, compartmentalized production of cAMP by β_2ARs is lost, suggesting that these receptors are redistributed to the peripheral sarcolemma (see figure 1B).

βAR production of cAMP associated with t-tubules most likely occurs in dyadic clefts, which are junctional membrane complexes where LTCCs in the plasma membrane come in close proximity to ryanodine receptors (RyRs) in the junctional SR (Scriven *et al.*, 2000) (see figure 1A). This is supported by the fact that βAR regulation of LTCCs occurs primarily in the t-tubules (Orchard & Brette, 2008), where LTCCs form signaling complexes with Cav3 that include both β_1 and β_2ARs (Balijepalli *et al.*, 2006; Nichols *et al.*, 2010).

The formation of signaling complexes that include G_i may explain how β_2ARs selectively regulate LTCC function, since blocking this signaling pathway with pertussis toxin allows these receptors to produce a more global response, which includes PKA-dependent phosphorylation of PLN in the non-junctional SR, resulting in a positive lusitropic effect (Xiao *et al.*, 1995; Kuschel *et al.*, 1999b). It has been suggested that G_i signaling regulates PLN through a mechanism that involves changes in phosphatase activity (Kuschel *et al.*, 1999a; Macdougall *et al.*, 2012). However, there is evidence that β_2ARs can also recruit phosphodiesterase (PDE) activity (Perry *et al.*, 2002; Baillie *et al.*, 2003), which breaks down cAMP, limiting its diffusion to more distant targets. Consistent with this idea, it has recently been shown that β_1, but not β_2AR stimulation is able to produce a cAMP response that can be detected by a FRET-based biosensor targeted to the free or non-junctional SR where PLN and SERCA are located. However, β_2AR stimulation was able to produce a cAMP response detected at that more distant location after selective inhibition of PDE2 or PDE3 activity (Rudokas *et al.*, 2021). It has also been shown that PDE4 activity prevents β_2AR production of cAMP from reaching RyRs, which are presumably found just a few nanometers away, on the opposite side of the dyadic cleft. The loss of PDE4 activity can then explain how β_2AR stimulation results in hyperphosphorylation of RyRs, leading to spontaneous release of Ca^{2+} from the SR and generation of ventricular arrhythmias in cardiac hypertrophy and heart failure (Berisha *et al.*, 2021) (see figure 1B).

Prostaglandin Receptors

One of the first studies directly demonstrating compartmentation of cAMP produced by EPRs in adult cardiac myocytes compared responses detected by a FRET based biosensors

targeted to type II PKA signaling domains and a globally expressed cytosolic biosensor in guinea pig ventricular myocytes (Warrier *et al.*, 2007). EPR activation by PGE1 did not produce a cAMP response that could be detected by the PKA-targeted probe, which correlated with the inability of PGE1 to regulate LTCC activity. However, PGE1 was able to produce cAMP responses detected by the globally expressed biosensor. This demonstrated that EPRs are unable to stimulate cAMP production in subcellular locations where type II PKA regulation of functional responses normally occur.

Interestingly, EPR stimulation did produce a cAMP response that could be detected by the biosensor targeted to type II PKA signaling domains in adult rat ventricular myocytes. Yet, there was still no effect on myocyte contraction or LTCC activity (Agarwal *et al.*, 2011). Furthermore, disrupting caveolae by cholesterol depletion, which enhanced β_1AR responses, had no effect on the EPR mediated responses detected by the PKA targeted probe. This is consistent with the fact that the EP_2 and EP_4 receptor subtypes expressed in cardiac myocytes, both of which couple to G_s and stimulate cAMP production, are excluded from caveolar membrane fractions (Ostrom *et al.*, 2001; Ostrom *et al.*, 2004; Agarwal *et al.*, 2011). In fact, PGE1 stimulation was found to produce changes in cAMP activity that were more readily detected by a biosensor targeted specifically to non-raft regions of the plasma (Agarwal *et al.*, 2018). These results indicate that EPR stimulation produces a compartmentalized cAMP response associated with non-caveolar membrane domains that may be able to activate type II PKA in some species, but it is not associated with the regulation of EC coupling. The physical location of EPRs in cardiac myocytes is yet to be determined, but they are able to produce a cAMP response associated with protection from ischemia/reperfusion injury (Xiao *et al.*, 2004; Pang *et al.*, 2016).

Muscarinic Receptors

Cardiac myocytes also express M_2 muscarinic receptors (M_2Rs), which are involved in mediating parasympathetic responses. Many of these effects, especially in ventricular myocytes, involve G_i-dependent regulation of AC and changes in cAMP activity (Harvey & Belevych, 2003). The dominant effect is inhibitory, and this can be explained by direct inhibition of AC5/6 by the α subunit of G_i. However, M_2Rs can also stimulate cAMP production (Warrier *et al.*, 2005; Iancu *et al.*, 2008). The consequence is a complex biphasic response, where upon exposure to ACh there is a rapid inhibition of cAMP that is then followed by a rebound increase upon washout of the agonist (Zakharov & Harvey, 1997; Belevych *et al.*, 2001) (see figure 2A). In the presence of agonist, the inhibitory effect is dominant. However, upon termination of receptor activation, the inhibitory effect turns off rapidly, revealing the stimulatory response, which turns off more slowly. It has been suggested that this type of mechanism can trigger arrhythmogenic responses during transient activation of M_2Rs (Song *et al.*, 1998).

This complex behavior can be explained by the ability of G_i signaling to stimulate AC4 and/or AC7, which are also present in cardiac myocytes. (Ishikawa & Homcy, 1997; Defer *et al.*, 2000). Thus, M_2Rs can inhibit cAMP production by AC5/6, while at the same time stimulating cAMP production by AC4/7 (Belevych *et al.*, 2001; Harvey & Belevych, 2003; Warrier *et al.*, 2005). It is hypothesized that the resulting biphasic response is due to the

fact that the different AC isoforms are located in different microdomains of the plasma membrane (Iancu *et al.*, 2007). AC5/6 are found in caveolae, while AC4/7 are excluded from those membrane domains (Ostrom & Insel, 2004; Willoughby & Cooper, 2007). The feasibility of this hypothesis was tested by developing a computational model that includes βAR and M_2R signaling mechanisms affecting cAMP production in caveolar and extra-caveolar signaling domains (figure 2B). The results predict that the complex changes in cAMP activity reflect the ability of M_2Rs to inhibit and stimulate cAMP production in different subcellular locations (figure 2C), with functional responses correlating most closely with changes occurring in caveolar membrane domains (Iancu *et al.*, 2007; Iancu *et al.*, 2008).

LOCALIZED DEGRADATION

Although localized production of cAMP is an intuitive factor to consider when explaining mechanisms contributing to compartmentation, computational analysis suggests that biochemically measured rates of AC activity are not high enough to generate significant cAMP gradients on their own (Rich *et al.*, 2000). In addition to production of cAMP in discrete subcellular locations, the specificity of cAMP responses can only be maintained if the movement of this diffusible second messenger is somehow restricted. A number factors have been postulated to contribute to this behavior. The one that has received the most attention is localized degradation by PDE activity.

The primary mechanism for reversing the effects of cAMP production is via hydrolysis of the cyclic nucleotide by a PDE (Francis *et al.*, 2001; Conti & Beavo, 2007), which may also serve as a means of generating cAMP gradients within a cell. PDEs are often depicted as creating functional barriers, which limit the movement of cAMP from one location to another, a concept that garners support from the fact that some PDE subtypes are known to be targeted to specific structures within the cell. In many cases this involves interactions with AKAPs (McConnachie *et al.*, 2006).

One of the most striking examples of the role that PDE activity plays in limiting the movement of cAMP in cardiac myocytes was illustrated by the work of Jurevicius et al. (1996). Application of the βAR agonist isoproterenol to one-half of a frog ventricular myocyte caused half-maximal enhancement of the whole-cell, L-type Ca^{2+} current recorded using patch clamp techniques, suggesting that the only channels affected were those located in the region of the cell exposed to drug. However, when the non-selective PDE inhibitor 3-isobutyl-1-methylxanthine (IBMX) was applied together with isoproterenol, the Ca^{2+} current was maximally enhanced, indicating that PDE activity had been responsible for preventing cAMP at the site of production from regulating channels in more distal portions of the cell.

Subsequent development of genetically encoded biosensors has made it possible to directly monitor cAMP activity and how its movement is affected by PDE activity in live cells. One of the first studies using this approach involved a biosensor constructed using type II PKA (Zaccolo & Pozzan, 2002). Because of its interactions with AKAPs, the probe exhibits a distinct expression pattern in cardiac myocytes that corresponds with the Z line of the sarcomere. Furthermore, βAR activation produced changes in cAMP activity that remained

localized to these striations. However, application of IBMX resulted in a uniform increase in cAMP, supporting the idea that movement of the second messenger produced following receptor activation had been limited by PDE activity.

There are 4 primary PDE isozymes involved in cAMP degradation in the heart – PDE1, PDE2, PDE3, and PDE4 (Osadchii, 2007). While PDE1 and PDE2 can hydrolyze both cAMP and cGMP, PDE3 preferentially hydrolyzes cAMP, and PDE4 is specific for cAMP. These PDE families also vary in how their ability to metabolize cAMP can be regulated. While PDE1 is activated in a Ca^{2+}/calmodulin-dependent manner, PDE2 can be allosterically stimulated by cGMP, PDE3 can be competitively inhibited by cGMP, and both PDE3 and PDE4 can be activated by PKA-dependent phosphorylation (Conti & Beavo, 2007). The relative contribution of each PDE isozyme varies depending on species. Perhaps most notable is PDE4, which is in greatest abundance in the hearts of mice and rats, where it makes up as much as 60% of all cAMP hydrolytic activity (Mongillo *et al.*, 2004; Leroy *et al.*, 2008; Richter *et al.*, 2011; Mika *et al.*, 2012). However, in humans it represents only 10% of total PDE activity. Despite the striking difference in the relative amount of each PDE isoform expressed in different species, it is how each PDE isozyme is distributed throughout the cell that is likely most relevant. For example, selective inhibition of PDE4 activity in the mouse or rat results in a general increase in all phosphoproteins, whereas in human myocardium it results in phosphorylation of a specific subset of proteins (Richter *et al.*, 2011). The use of selective pharmacologic inhibitors of the different PDE isozymes has provided important insight into the roles that each plays in contributing to cAMP production in different subcellular locations as well as the regulating specific functional responses. The exception to this is PDE1, for which the availability of selective inhibitors is limited (Vandeput *et al.*, 2007; Miller *et al.*, 2009).

PDE2

This PDE isozyme is associated primarily with membrane fractions of cardiac myocytes (Simmons & Hartzell, 1988; Mongillo *et al.*, 2006), where it is believed to play a critical role in regulating sub-sarcolemmal cAMP-dependent responses. Consistent with this idea, PDE2 inhibition produced significantly greater changes in cAMP activity in subcellular locations associated with lipid raft and non-raft domains of the plasma membrane than it did in the cytosolic domain of adult ventricular myocytes. (Agarwal *et al.*, 2018). In addition, PDE2 has been shown to play an important role in regulating the effects of βAR stimulation on LTCC activity (Hartzell & Fischmeister, 1986; Kirstein *et al.*, 1995; Méry *et al.*, 1995; Dittrich *et al.*, 2001). Furthermore, FRET-based biosensors have shown that in neonatal ventricular myocytes, cGMP-signaling specifically decreases cAMP activity stimulated by βAR agonists (Stangherlin *et al.*, 2011). Local regulation of cAMP activity by PDE2 has also been reported to selectively modify PKA-dependent regulation of LTCCs as well as RyRs, where it plays an important role in nitric oxide/cGMP dependent regulation of excitation-contraction coupling (Mohamed *et al.*, 2011). In addition, there is evidence that PDE2 activity is involved in local control of cAMP that affects mitochondrial function (Liu *et al.*, 2019). Additionally, PDE2 activity has been shown to play an important role in regulating cAMP-dependent responses in cardiac hypertrophy (Zoccarato *et al.*, 2015). Those results were consistent with the finding that PDE2 expression is upregulated in

cardiac hypertrophy (Mehel *et al.*, 2013; Bastug-Ozel *et al.*, 2019), and overexpression of PDE2 in normal cardiac myocytes can mitigate norepinephrine induced hypertrophy (Mehel *et al.*, 2013). This suggests that targeting local control of cAMP by PDE2 may provide a therapeutic strategy. Similarly, it has been proposed that inhibition of PDE2 may be a target to selectively regulate PLN/SERCA activity and enhance relaxation in certain forms of heart failure (Rudokas *et al.*, 2021).

PDE3

In general, PDE3 can be found in both membrane and cytosolic fractions of cardiac myocytes, although the relative distribution differs among species (Muller *et al.*, 1992; Maurice *et al.*, 2003; Lugnier, 2006). Consistent with these findings, selective inhibition of PDE3 activity resulted in similar changes in steady-state cAMP levels detected by FRET-based biosensors targeted to different regions of the plasma membrane as well as the bulk cytoplasmic compartment of adult rat ventricular myocytes (Agarwal *et al.*, 2018). Interestingly, responses near the membrane were transient, suggesting that feedback regulation may play an important role in affecting sub-sarcolemmal cAMP activity. In neonatal rat ventricular myocytes, regulation of PDE3 activity by cGMP signaling pathways has been shown to modulate cAMP activity specifically associated with a soluble or type I PKA signaling domain (Stangherlin *et al.*, 2011).

There are two PDE3 subfamilies, PDE3A and PDE3B. PDE3A is the predominant subtype found in cardiac myocytes of most species (Weishaar *et al.*, 1987; Wechsler *et al.*, 2002; Abi-Gerges *et al.*, 2009). In human myocardium, alternative splicing results in at least three PDE3A isoforms. These differ in their N-terminus, which contains sites involved in feedback regulation by PKA and phosphoinositide-3 kinase γ (PI3Kγ) as well as targeting to different subcellular locations (Wechsler *et al.*, 2002; Hambleton *et al.*, 2005).

In the mouse heart it has been shown that knocking out PI3Kγ increases basal cAMP levels, enhancing ventricular contraction (Crackower *et al.*, 2002). Furthermore, it was reported that PI3Kγ binds PDE3B, but not PDE3A, leading to the conclusion that PI3Kγ activates PDE3B (Patrucco *et al.*, 2004; Alloatti *et al.*, 2005). Kerfant et al. (2005; 2007) demonstrated that this effect of PI3Kγ was selectively affecting cAMP in subcellular locations associated regulation of Ca^{2+} transients, but not LTCCs. However, Beca et al. (2013) found that knocking out expression of PDE3B had no effect on ventricular myocyte contractility, while loss of PDE3A enhanced both the SR Ca^{2+} content as well as the amplitude of the intracellular Ca^{2+} transient, without affecting LTCCs. They also demonstrated that PDE3A forms a signaling complex with both SERCA2a and PLN. These results suggest that in the adult mouse heart PDE3A is the primary subtype involved in regulating ventricular myocyte function under basal conditions, and that it does so by selectively regulating cAMP in a microdomain associated with PLN and SERCA in the non-junctional SR.

Despite the evidence that PDE3 selectively regulates cAMP in the vicinity of PLN and SERCA under basal conditions, numerous studies have demonstrated that inhibition of PDE3 activity significantly affects cAMP dependent regulation of LTCC activity in the presence of βAR stimulation in adult ventricular myocytes (Verde *et al.*, 1999). This occurs

despite reports that PDE3 activity does not play a major role in regulating the amplitude and duration of cAMP responses measured globally, as well as in type II PKA signaling domains, using FRET-based biosensors following βAR stimulation (Mongillo *et al.*, 2004; Nikolaev *et al.*, 2006).

PDE4

Selective inhibition of PDE4 activity under baseline conditions typically has little if any effect on cardiac function (Mika *et al.*, 2012). This is despite the fact that changes in cAMP activity have been detected by certain FRET-based biosensors, especially those targeted to the plasma membrane or type II PKA signaling domains (Mongillo *et al.*, 2004; Agarwal *et al.*, 2018). These results reflect the fact the PDE4 activity in general has been shown to be more important in affecting cAMP responses in the presence of βAR activation. Notably, PDE4 activity has been demonstrated to be more important than other PDE isozymes in regulating cAMP production by β_1ARs (Mongillo *et al.*, 2004; Nikolaev *et al.*, 2006).

There are four PDE4 family genes, but only three are commonly expressed in cardiac myocytes: PDE4A, PDE4B, and PDE4D (Mongillo *et al.*, 2004; Mika *et al.*, 2012). Alternative splicing yields an even larger number of PDE4 isoforms. Differences in the N-terminus often determine where each isoform is targeted. PDE4B3, PDE4D3, and PDE4D5 have been reported to represent ~90% of the total PDE4 activity in neonatal rat ventricular myocytes (Mongillo *et al.*, 2004; Mika *et al.*, 2014). Pharmacologic inhibitors of PDE4 activity do not differentiate between the different isoforms. However, their unique functional roles have been elucidated using transgenic mouse, siRNA knockdown, or dominant negative over-expression approaches.

Both PDE4B and PDE4D have been shown to be part of a signaling complex that includes LTCCs in cardiac myocytes and, using transgenic mice, knockout of either subtype resulted in an increase in the Ca^{2+} current as well as the associated Ca^{2+} transient and contraction under basal conditions. However, the LTCC response to βAR stimulation was only enhanced in PDE4B, but not PDE4D, knockout myocytes. Furthermore, PDE4B knockout animals exhibited an increased susceptibility to arrhythmogenesis (Leroy *et al.*, 2011).

It was subsequently shown that genetic ablation of PDE4B selectively alters cAMP responses to β_1AR stimulation measured at the plasma membrane, but not the bulk cytoplasmic compartment of neonatal mouse ventricular myocytes (Mika *et al.*, 2014). However, there was no change in the response to either β_2AR or EPR activation. Furthermore, disrupting PDE4B activity specifically affected PKA-dependent phosphorylation of LTCCs and RyRs, which are in close proximity to one another in the dyadic cleft (see figure 1). PDE4B ablation did not affect phosphorylation of PLN or troponin-I, which are believed to be more distally located.

PDE4D has also been shown to be part of a signaling complex, along with PDE3A, that includes SERCA and PLN. Moreoverr, knocking out expression of PDE4D has been shown to enhance SR Ca^{2+} content as well as the amplitude of the intracellular Ca^{2+} transient without affecting the magnitude of the L-type Ca^{2+} current under basal conditions (Beca *et al.*, 2011). This correlated with changes in PKA-dependent phosphorylation of PLN but not

the RyR. These results indicate that PDE4D selectively regulates local control of cAMP near the non-junctional SR in adult ventricular myocytes.

PDE4D3 has been shown to form a complex with RyRs. Deleting expression of PDE4D in the mouse heart results in development of dilated cardiomyopathy, and despite normal levels of cAMP measured globally, evidence for a local increase in cAMP activity was detected specifically at the Z line of the sarcomere using a FRET based biosensor targeted to type II PKA signaling domains (Lehnart *et al.*, 2005). This correlated with hyperphosphorylation of the RyR, which has been shown to disrupt interactions with calstabin-2, destabilizing its activity and allowing Ca^{2+} to leak from the SR, increasing the incidence of arrhythmias. Interestingly, there is down regulation of PDE4D3 in failing human hearts, which may explain the increased incidence of arrhythmias and sudden cardiac death in these patients. PDE4D3 has also been found to form part of a signaling complex with the slow delayed rectifier K^+ channel (Marx *et al.*, 2002; Terrenoire *et al.*, 2009; Li *et al.*, 2012).

PDE4D5 has been shown to play a specific role in β_2AR signaling in cardiac myocytes. Following G_s-dependent activation of AC and cAMP production, these receptors can undergo desensitization involving various mechanisms (Lefkowitz *et al.*, 1998). One involves phosphorylation at specific sites on the receptor by G-protein coupled receptor kinases resulting in the binding of β-arrestin, which in turn recruits PDE4D5 (Perry *et al.*, 2002; Baillie *et al.*, 2003). Consistent with this observation, inhibition of PDE4D5 activity was shown to enhance PKA phosphorylation of the receptor, facilitating its ability to couple to other pathways, including G_i inhibition of AC as well as activation of ERK1/2 signaling (Daaka *et al.*, 1997). PDE4D5 has also been shown to play an important role in local cAMP dependent regulation of the small heat shock protein 20 (HSP20). This chaperone protein is involved in different cardioprotective signaling mechanisms. Furthermore, this effect is mediated by cAMP/PKA-dependent phosphorylation. It has been shown that PDE4 inhibition has a greater effect on the cAMP activity detected by a FRET-based biosensor tethered to HSP20 than it does on cAMP responses detected using a cytosolic probe (Sin *et al.*, 2011). This localized effect can be explained by the ability of PDE4D5 to interact directly with HSP20.

The concept that PDE activity is a critical factor in creating localized cAMP responses is well established. However, while PDEs may act as “functional barriers” in some circumstances, in other instances they may serve as a “sink”. This is based on predictions that basal cAMP levels in some cytoplasmic domains are quite high and that PDE activity may act to keep concentrations below the level necessary to activate effectors such as PKA under unstimulated conditions (Iancu *et al.*, 2007; Iancu *et al.*, 2008). However, the fact that inhibition of PDE activity can produce localized changes in cAMP activity does not mean that it alone is sufficient to explain compartmentation.

PKA BUFFERING

Computational modeling has been particularly useful in investigating the likelihood that factors other than localized production and/or localized degradation play a role in generating discrete pools of cAMP. One such element that has been implicated is buffering of cAMP

movement, specifically by PKA. There are a limited number of effectors that bind cAMP. In addition to PKA, cAMP can bind the exchange protein activated by cAMP (Epac), cyclic nucleotide gated (CNG) ion channels, and Popeye (POPDC) domain containing proteins (Brand & Schindler, 2017). Of these, PKA is the most abundant and therefore the most likely to affect cAMP diffusion in cardiac myocytes. It has been estimated that the PKA buffering capacity for cAMP in cardiac myocytes is ~1.2 μM (Corbin *et al.*, 1977; Saucerman *et al.*, 2003). Additionally, biochemical studies have indicated that a significant fraction of total cAMP is bound to PKA, even under basal conditions (Beavo *et al.*, 1974; Corbin *et al.*, 1977). In the first computational model of cAMP signaling in cardiac myocytes, Saucerman et al. predicted that buffering could help stabilize cAMP at concentrations near its binding affinity for PKA (Saucerman *et al.*, 2003). Subsequent modeling efforts suggested that buffering might also contribute to gradients between compartments by slowing cAMP diffusion (Saucerman *et al.*, 2006). The idea that interactions with PKA may affect the movement of cAMP are not unexpected given that measurements of the cAMP concentration in cardiac myocytes are in the same range as the predicted buffering capacity of PKA (Iancu *et al.*, 2008; Borner *et al.*, 2011; Agarwal *et al.*, 2018).

Based on size alone, the predicted diffusion coefficient for cAMP in an aqueous solution devoid of any other factors that might affect its movement (free diffusion) is ~300 $\mu m^2/s$ (Neves *et al.*, 2008; Agarwal *et al.*, 2016). Early attempts to measure cAMP diffusion coefficients in various cell types came up with values ranging anywhere from approximately one-half to more than twice the rate of free diffusion (Bacskai *et al.*, 1993; Chen *et al.*, 1999; Nikolaev *et al.*, 2004; Nikolaev *et al.*, 2006; Saucerman *et al.*, 2006). Many of these initial estimates involved the use of various FRET based biosensors to monitor the spread of cAMP within a cell following receptor activation. More recently Agarwal et al. (2016) used the technique of raster image correlation spectroscopy (RICS) to measure the diffusion coefficient of fluorescently labeled cAMP in intact, adult ventricular myocytes. This technique applies the principle of fluorescence correlation spectroscopy to images from a laser scanning confocal microscope on a pixel-by-pixel basis (Rossow *et al.*, 2010). In these experiments, the fluorescent cAMP molecule used was 8-[Pharos-450]-cAMP (φ450-cAMP) (Moll *et al.*, 2008). Attaching the Pharos dye to cAMP did not affect its affinity for PKA binding, but it did render it resistant to PDE hydrolysis (Moll *et al.*, 2008). Although the dye alone has a molecular weight nearly identical to cAMP, estimates using Stokes-Einstein theory indicate that this would not significantly affect diffusion. This made φ450-cAMP an ideal choice to study cAMP diffusion independent of PDE activity. Using this approach, it was found that under basal conditions φ450-cAMP has a diffusion coefficient of 10 $\mu m^2/s$, dramatically slower than previous estimates.

A major factor contributing to the slow rate of cAMP diffusion was revealed in the images of cells loaded with φ450-cAMP. Rather than being uniformly distributed throughout the cell, cAMP co-localized with mitochondria due to interactions with PKA anchored to the outer mitochondrial membrane (Agarwal *et al.*, 2016). This was confirmed by demonstrating that the pattern could be disrupted by blocking interactions between the regulatory subunit of PKA and AKAPs. This maneuver also increased the diffusion coefficient of cAMP. Consistent with the idea that PKA buffering plays an important role in slowing cAMP

diffusion, Nikolaev et al. (2010) demonstrated that disrupting AKAP anchoring allowed cAMP generated by β_2ARs, which is normally not detectable away from the site of production in adult cardiac myocytes, to propagate throughout the entire cell. Mitochondria make up approximately 30% of the intracellular volume of a cardiac myocyte (Schaper *et al.*, 1985; Barth *et al.*, 1992). Therefore, anchoring a buffer to the outer membrane of these organelles would be an effective means of limiting the movement of cAMP as it attempts to diffuse throughout the cell.

Interestingly, the diffusion coefficients of the free pharos dye alone or fluorescein alone, both of, which have molecular weights similar to that of cAMP, were estimated to be ~60 $\mu m^2/s$ (Agarwal *et al.*, 2016). This suggests that even in the absence of PKA binding, the diffusion coefficient of cAMP should still be significantly slower than the rate of free diffusion. This can be attributed to non-binding interactions with other solutes and macromolecules in the cytoplasm, which is referred to as molecular crowding. It is also consistent with the observation that cytoplasmic diffusion of small molecules is typically 3 to 8 times slower than their rate of free diffusion (Dix & Verkman, 2008). Furthermore, the diffusion coefficient of these molecules was found to be virtually the same in adult cardiac myocytes and morphologically simpler HEK293 cells, suggesting that factors slowing the movement of cAMP are similar across cell types. It should be noted, however, that Bock et al. have argued that the slow rate of cAMP diffusion in HEK293 cells may be due to mechanisms other than PKA buffering (Bock *et al.*, 2020).

These results are largely consistent with modeling predictions, supporting the idea that slow diffusion of cAMP is an important factor contributing to cAMP compartmentation, but experimental evidence providing more direct proof of this hypothesis is needed. If substantiated, these initial studies raise some intriguing questions. Yet designing experiments that might provide that kind of proof requires additional information about the buffering effect itself. For example, why does cAMP seem to associate specifically with mitochondria? Is it because PKA is more heavily concentrated in this location? Or could it be that there is something different about the PKA found there? Could it be that there is more type I PKA targeted to that location? Type II PKA is most often thought of as being anchored by AKAPs. However, it is now known that there are type I specific AKAPs as well as dual specific AKAPs that interact with both type I and type II PKA. Furthermore, type I PKA has been reported to have a higher affinity for cAMP, which might then explain the tighter association with mitochondria. Another interesting possibility is that cAMP is interacting with free regulatory subunits of PKA, not the holoenzyme. Normally, we think of PKA as a heterotetrameric complex, with equal numbers of catalytic and regulatory subunits. However, it has been demonstrated that number of regulatory subunits far exceeds that of the catalytic subunits in most cell types. In cardiac myocytes the ratio is 6 to 1 (Walker-Gray *et al.*, 2017). Furthermore, the cAMP affinity of free regulatory subunits is in the nanomolar range, while that of the holoenzyme is in the micromolar range (Dao *et al.*, 2006). This would suggest the intriguing possibility that free regulatory subunits may exist for the purpose of buffering the movement of cAMP. It is also interesting to speculate about the role that changes in cAMP buffering and compartmentation play in the development of heart failure and injury due to myocardial infarction that are associated with the loss of

mitochondrial AKAPs (Marin, 2020) (see figure 1B). These are all ideas that merit further investigation.

RESTRICTED SPACES

Some computational models have suggested that PDE activity alone is sufficient to generate cAMP gradients, even if it is assumed that cAMP can move at rates equal to free diffusion. For example, Oliveira et al. used a stochastic modeling approach to demonstrate the high levels of PDE activity are theoretically sufficient to explain cAMP compartmentation in HEK 293 cells (Oliveira *et al.*, 2010). However, the rates of cAMP synthesis and degradation used in those simulations were significantly higher than experimental values measured in most cells (Saucerman *et al.*, 2014). Other modeling studies have predicted that PDE activity alone is not sufficient to create cAMP gradients associated with compartmentation (Chen *et al.*, 2008; Lohse *et al.*, 2017). Neves et al. (2008) predicted that cAMP gradients can occur in the presence of more realistic levels of PDE activity when the cytosolic space is restricted to the geometry of a neuron, where the surface-to-volume ratio in the dendrites is quite high. Similarly, Feinstein et al. (2012) predicted that while the surface-to-volume ratio alone was not sufficient to explain cAMP compartmentation in endothelial cells, it did affect the potential role of other variables affecting cAMP diffusion. Early computational modeling also pointed to the idea that restriction of cAMP diffusion due to physical barriers between the plasma membrane and the cytosol could contribute to cAMP compartmentation in simple cells or cell free systems, although the exact nature of those barriers was not defined (Rich *et al.*, 2000; Rich *et al.*, 2001). Modeling by Iancu et al. (2007; 2008) also predicted that there must be some factor other than PDE activity that limits the flux of cAMP between compartments in cardiac myocytes.

Yang *et al.* (2016) addressed the question of whether or not PDE activity alone can act as a functional barrier by developing a 3D stochastic model of cAMP diffusion that represented the cytosolic space of an adult ventricular myocyte. The amount of PDE activity calculated to exist within a single myocyte was assumed to be distributed uniformly along a plane 100 nm from the inner surface of the t-tubule membrane, with no other physical barriers to diffusion. In these simulations, realistic estimates of PDE activity alone were not sufficient to prevent uniform distribution of cAMP throughout that space, even if diffusion was slowed to mimic the effects of molecular crowding or PKA buffering (figure 3A). Gradients could be generated by the model, but only when PDE activity was increased to unrealistically high levels. Yet, under those conditions, the total cAMP concentration was predicted to drop below levels necessary to activate known effectors such as PKA.

The authors then evaluated the possibility that a more realistic representation of the space where cAMP signaling occurs might have an effect on the predicted outcome. Cryo-transmission electron microscopy images of adult mouse ventricular myocytes were used to generate a 3D reconstruction of the space surrounding the dyadic cleft. These junctional membrane complexes are already known to be essential for creating localized Ca^{2+} signaling domains (Bers, 2001; Winslow & Greenstein, 2011). Evidence that subpopulations of βARs are components of signaling complexes with LTCCs found at those sites suggests that cAMP signaling is likely to be confined within that space as well (Scriven *et al.*, 2000; Balijepalli

et al., 2006; Nichols *et al.*, 2010). This anatomically restricted space is also surrounded by mitochondria that might further restrict cAMP diffusion. The 3D model of cAMP signaling was then implemented within this framework (Yang *et al.*, 2016) (figure 3B). Adenylyl cyclase production of cAMP was initiated at the plasma membrane in the center of the dyadic cleft, and realistic levels of PDE activity were simulated as a barrier surrounding this space. T-tubules, SR, and mitochondria were treated as impenetrable barriers limiting the movement of cAMP. No significant gradients were predicted when cAMP was assumed to move at rates mimicking free diffusion or even at the slower rates attributed to molecular crowding. However, significant gradients were predicted when the diffusion rate was reduced to levels taking into account the potential effect of PKA buffering. These results demonstrated a plausible explanation for how production of cAMP by receptors within the dyadic cleft could lead to an increase in cAMP that is limited to that restricted space. The same mechanisms are also likely to limit the ability of cAMP produced by receptors located outside the dyadic cleft from entering. These results also suggest that the disruption of dyadic clefts that occurs in diseases states such as heart failure might be expected to affect compartmentalized cAMP responses (Zhang *et al.*, 2013) (see figure 1B). However, these are predictions that still need experimental confirmation.

The dyadic cleft is just one example of spatial restriction contributing to cAMP compartmentation in the heart. Richards et al. (2016) addressed the question of cAMP mobility in adult ventricular myocytes expressing a cytosolic FRET-based biosensor together with a microfluidics system, which made it possible to apply an agonist to one-half of a cell while monitoring changes in cAMP activity in both halves simultaneously. The diffusion coefficient was then estimated by fitting the time course of the responses, taking into account predicted rates of synthesis and degradation. Using this method the authors suggested that the cAMP diffusion coefficient in adult cardiac ventricular myocytes is 35 $\mu m^2/s$. Like the results obtained by Agarwal et al. (2016), this is significantly slower than the expected rate of free diffusion. However, Richards et al. concluded that this was not due to PKA buffering, since the calculated diffusion coefficient was not affected by the presence of a cAMP analog that prevented PKA binding. Switching to a fluorescence recovery after photobleaching (FRAP) approach, the authors determined that the diffusion coefficient of fluorescein, a molecule the same size as cAMP, is faster in neonatal cardiac myocytes than is in adult ventricular myocytes. Because neonatal cells or reported to have a lower density of mitochondria, it was concluded that the slow rate of diffusion in adult cardiac myocytes must be a function of cellular tortuosity due to the presence of mitochondria, although other structural differences were not considered. If the density of mitochondria does limit the movement cAMP in adult myocytes, the loss of mitochondrial organization that occurs in disease states such as heart failure (Miragoli *et al.*, 2016) might be expected to alter compartmentalized responses (see figure 1B).

Predictions based on the studies like those described above support the idea that physically restricted spaces play an essential role in explaining the mechanisms of cAMP compartmentation. Proof of this concept awaits further experimental evidence.

CONCLUSIONS AND FUTURE DIRECTIONS

Our understanding of compartmentalized cAMP signaling in cardiac myocytes and the mechanisms that contribute to this behavior have advanced significantly over last 20 years, largely due the development of new experimental and computational approaches. The evidence is now quite clear that cAMP is not uniformly distributed throughout the cell and that a number of factors contribute to this behavior. Experimental approaches thus far have largely focused on the roles of localized production and localized degradation of cAMP. However, predictions advanced by computational modeling have pointed to other factors affecting cAMP diffusion such as PKA buffering and restricted physical spaces. Early experiments support the idea that these mechanisms contribute to this complex behavior as well, but additional experimental evidence is needed to confirm these predictions. Other factors that should be considered, but were not discussed here include the role of liquid phase separation of the cytosolic space (Zhang *et al.*, 2020) and localized cAMP extrusion by membrane transporters.

The functional role of cAMP compartmentation is perhaps most clearly illustrated by comparing the responses between βARs and EPRs or between βAR subtypes. However, various experimental approaches have highlighted the functional significance that changes in local cAMP activity play in different disease states. Yet there are likely to be even more examples of functional responses that can be attributed to cAMP compartmentation. Again, this is where the power of computational modeling can be leveraged to provide additional insight. One example highlighted here is the prediction that this process may contribute to the complex behavior associated M_2 receptor activation. But there are likely other examples yet to be discovered. Such approaches can provide insight that may be used to design appropriate experiments to test the predictions that arise from the emergent behavior identified by such models.

There has been substantial improvement in understanding mechanisms related to restricted signaling by cAMP. These advances have arisen from multiple new techniques and technologies in the experimental and computational space. As protein structural approaches continue to mature and allow the exploration of protein complexes through visualization, and atomistic scale modeling provides insights into the dynamics of signaling, there is the promise of a complete understanding of signaling via the ubiquitous second messenger cAMP.

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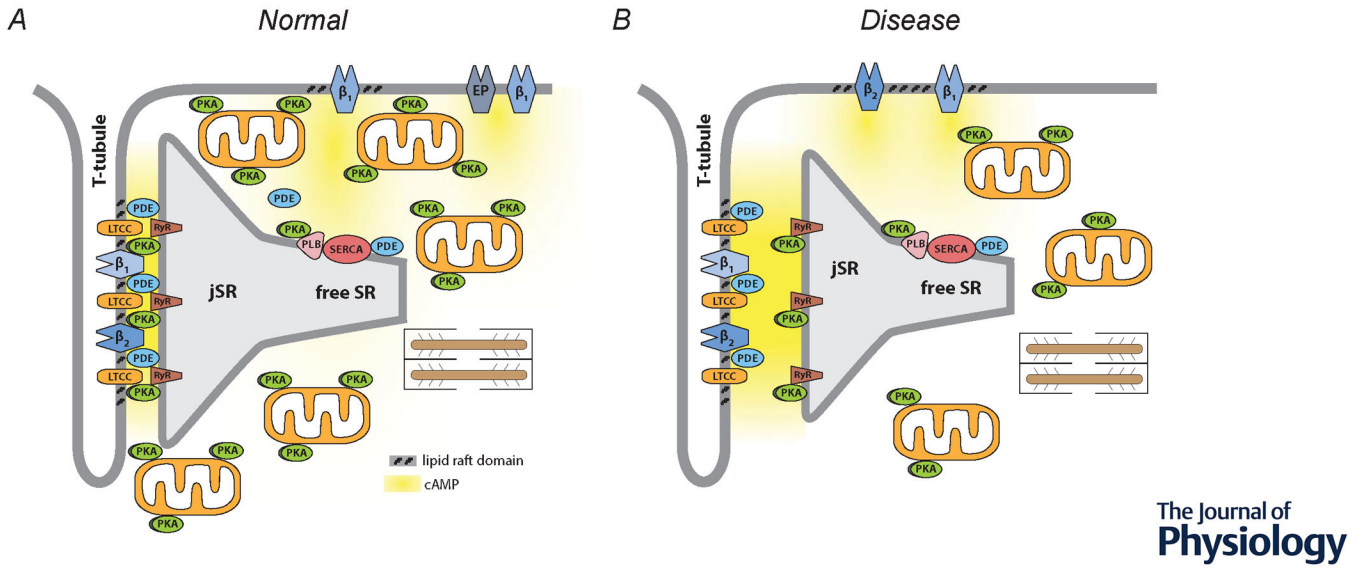

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Figure 1.

Compartmentation of receptor-dependent cAMP signaling in adult ventricular myocytes. *A*, Under normal conditions, dyadic clefts are formed by tight junctions between the plasma membrane of t-tubules and the junctional sarcoplasmic reticulum (jSR). This is where β_1 and β_2ARs found in the plasma membrane of t-tubules are believed to be part of caveolar signaling complexes that include L-type Ca^{2+} channels (LTCCs), which are in close proximity to ryanodine receptors (RyRs) found in the jSR. E-type prostaglandin (EP) receptors, as well as some β_1ARs, are excluded from of caveolar signaling complexes and t-tubule membranes. Phospholamban (PLN) and the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) are found outside of dyadic clefts in the free SR. Under normal conditions, β_1AR production of cAMP leads to protein kinase A (PKA)-dependent phosphorylation of LTCCs, RyRs, and PLN. β_2ARs lead to phosphorylation of LTCCs, but not RyRs or PLN. EP receptors do not regulate any of these effectors. Strategically placed phosphodiesterase (PDE) activity plays a critical role in cAMP compartmentation. Restricted spaces such as those created by dyadic clefts and tight mitochondrial packing as well as buffering by PKA anchored to the outer membrane of mitochondrial and other structures may also contribute to this behavior. *B,* Disease states, such as heart failure, are associated with changes in factors believed to contribute to compartmentalized cAMP responses. These changes include: disruption of dyadic clefts; redistribution of β_2ARs from t-tubules to the peripheral sarcolemma; loss of AKAPs anchoring PKA to mitochondria; disruption of mitochondrial organization; and loss of PDE activity in some locations.

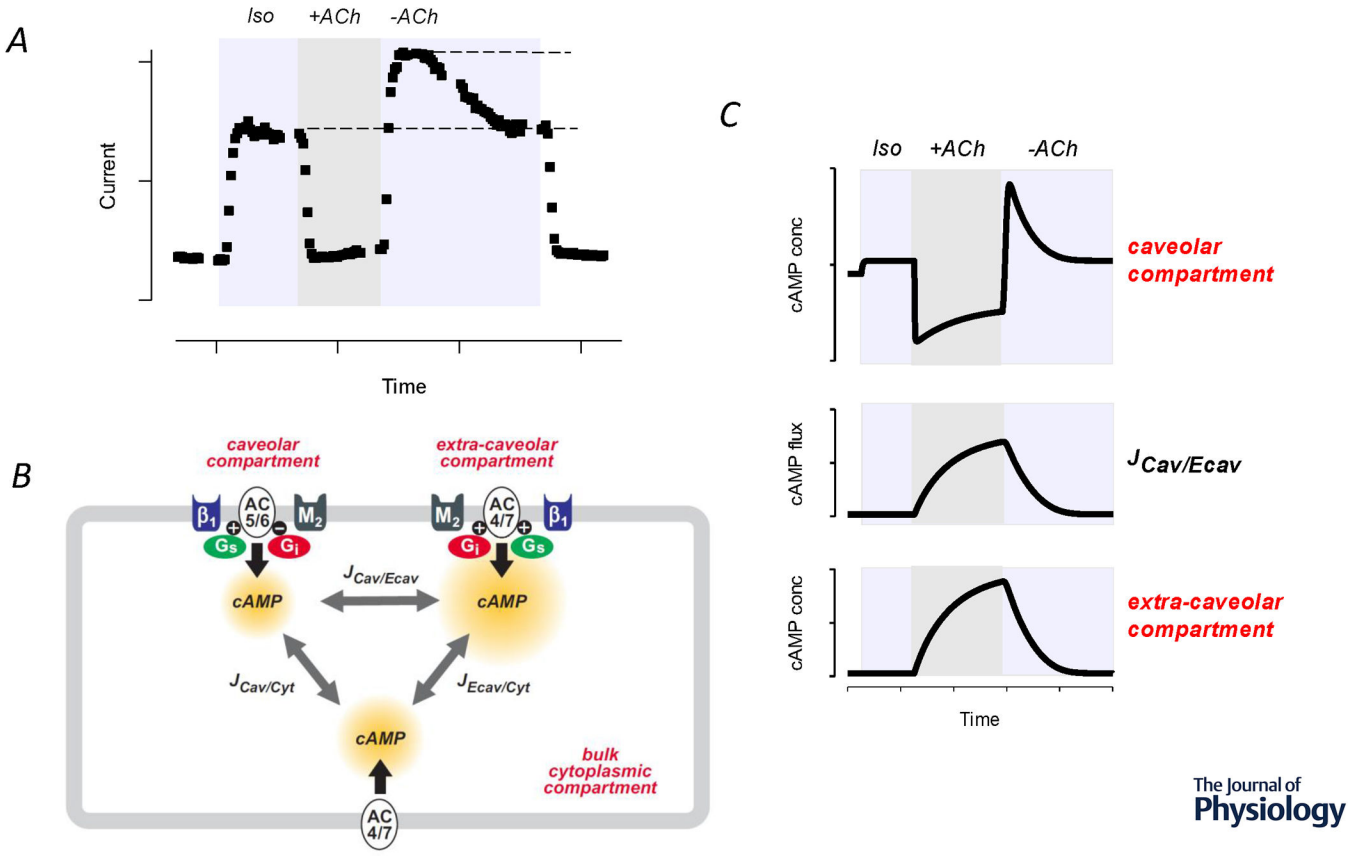

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Figure 2.

M_2 muscarinic receptor (M_2) responses attributed to cAMP compartmentation in a cardiac ventricular myocyte. *A,* Time course of changes in the magnitude of the cAMP-regulated Cl^- current in a guinea pig ventricular myocyte during exposure to a submaximally stimulating concentration of the βAR agonist isoproterenol (Iso), followed by addition of the M_2 receptor agonist acetylcholine (ACh). Note: In the presence of ACh (+ACh), there is rapid inhibition of the current activated by Iso. Upon washout of ACh (-ACh), the inhibitory response is rapidly reversed, revealing a stimulatory effect. Adapted from Zakharov and Harvey, 1997. *B,* Cartoon diagram of computational model used to evaluate role of different adenylyl cyclase (AC) isoforms found in caveolar and extra-caveolar membrane domains. β_1AR activation of the stimulatory G protein (G_s) increases cAMP production by AC5/6 and AC4/7 in caveolar and extra-caveolar domains, respectively. M_2 muscarinic receptor activation of the inhibitory G protein (G_i) inhibits cAMP production by AC5/6 in caveolar domains, but stimulates cAMP production by AC4/7 in extra-caveolar domains. It is hypothesized that cAMP produced in the caveolar compartment is associated with most functional responses and that this can be affected by the flux (J) of cAMP from other compartments. *C,* Model prediction of changes in cAMP concentration in caveolar and extra-caveolar domains in response to submaximal β_1 receptor stimulation by isoproterenol (Iso) followed by transient exposure to the muscarinic receptor agonist acetylcholine (ACh). Note: that exposure to ACh produces a rapid inhibition of cAMP activity in the caveolar domain, while simultaneously producing a slow increase in cAMP in the extra-caveolar

domain. The model predicts that the transient changes observed in the caveolar domain upon termination of M_2 receptor activation can be explained by the flux of cAMP between compartments. Adapted from Iancu et al. (2007).

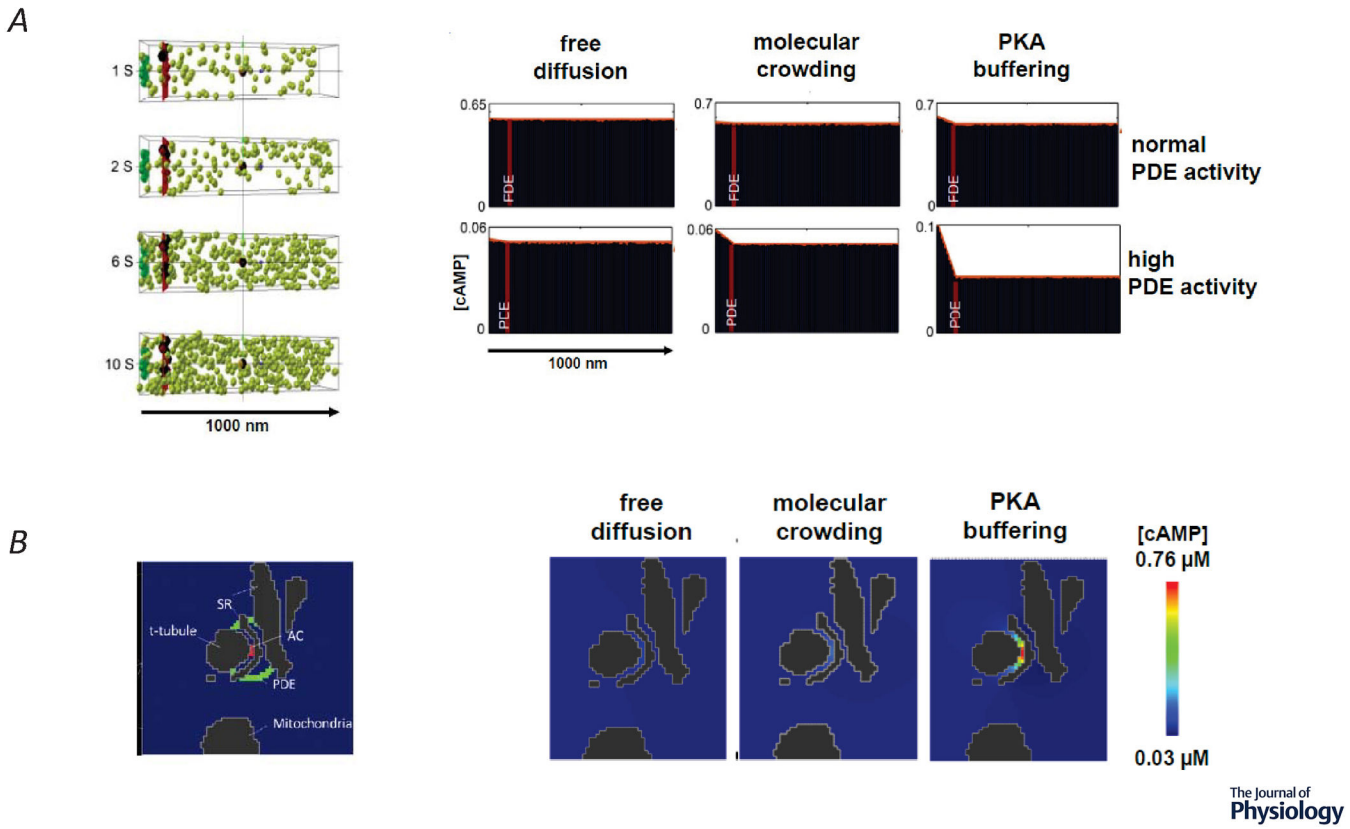

Figure 3.

A, Stochastic 3D simulation of cAMP diffusion. *Left, f*our snapshots in time of the distribution of individual cAMP molecules (green spheres), generated by adenylyl cyclase (AC) activity (green box) in a caveolar membrane domain on the left, with cytosolic space to the right. Phosphodiesterase (PDE) molecules were placed in a plane (red bar) 100 nm from the plasma membrane to simulate a functional barrier. *Right,* average concentration of cAMP at various distances from the site of production when the number of PDE molecules and diffusion coefficient were varied. Normal PDE activity (415 µM), high PDE activity (41.5 mM). Free diffusion (200 $\mu m^2/s$), molecular crowding (60 $\mu m^2/s$), PKA buffering (10 $\mu m^2/s$) *B,* 3D Continuum model of cAMP diffusion in an anatomically restricted space. *Left*, Cross section (1040 × 765 × 415 nm) through a 3D reconstruction of the dyadic space between the sarcoplasmic reticulum (SR) and t-tubule in an adult ventricular myocyte generated by cryo-transmission electron microscopy z-stacks. Location of AC activity is represented in red and PDE activity is represented in green. *Right,* Effect of diffusion coefficient on cAMP concentration in dyadic cleft in presence of normal PDE activity. Adapted from Yang et al. (2016).

Table 1:

Computational models addressing mechanisms of cAMP compartmentation

Study	Localized production	Localized degradation	PKA buffering	Restricted spaces
Rich et al. 2000				X
Rich et al. 2001		X		X
Saucerman et al. 2006	X	X	X	X
Iancu et al. 2007	X	X		X
Neves et al., 2008		X		X
Chen et al., 2008	X	X		
Oliveira et al. 2010		X		
Feinstein et al. 2012	X	X	X	X
Yang et al. 2016	X	X	X	X
Lohse et al. 2017		X		