Cardiac β-Adrenergic Signaling
From Subcellular Microdomains to Heart Failure

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ABSTRACT: β-adrenergic signaling plays a central role in the neurohumoral regulation of the heart and the progression of heart failure. Initially thought to be a simple linear cascade, this complex network is now recognized to utilize cross-talk with numerous other pathways, spatial compartmentation, and feedback control to coordinate cardiac electrophysiology, contractility, and adaptive remodeling. Here, we review recent basic insights and novel quantitative approaches that are leading to a more comprehensive understanding of β-adrenergic signaling and thus motivate new therapeutic strategies for cardiac disease.

KEYWORDS: heart; cell signaling; cyclic AMP; protein kinase A; compartmentation

INTRODUCTION

β-adrenergic signaling transduces sympathetic stimulation into a cascade of biochemical reactions that coordinate cellular responses. This process is initiated by β-adrenergic receptors (β-ARs), members of a large gene family of seven transmembrane receptors, which activate upon binding the sympathetic neurotransmitter norepinephrine or the hormone epinephrine. β-ARs are expressed in a variety of tissues and are responsible for diverse physiologic regulation, such as relaxation of blood vessels, increases in liver metabolism, and relaxation of the bronchioles. In the heart, β-adrenergic signaling increases heart rate and contractility, key components of the fight-or-flight response. β-AR signaling also plays a fundamental role in heart failure and other cardiac diseases. While β-AR signaling is one of the most well-understood cell signaling networks, recent advances are demonstrating that this network is much...
more complex than previously believed. Here, we briefly review conventional cardiac β-AR signaling and then expand upon growing research areas that are casting a new light on β-AR signaling and its consequences for the treatment of heart failure.

**CONVENTIONAL β-ADRENERGIC SIGNALING IN THE HEART**

Cardiac myocytes express two main β-AR isoforms, β₁-AR and β₂-AR (75%;25%). Norepinephrine or epinephrine-bound β-ARs activate the heterotrimeric G protein G₃, promoting dissociation of its α and βγ subunits. Gₛ-GTP then binds the membrane-bound effector protein adenylyl cyclase (AC), stimulating synthesis of cyclic AMP (cAMP) from ATP. cAMP, a ubiquitous second messenger, causes the dissociation of regulatory and catalytic subunits of protein kinase A (PKA), allowing PKA’s catalytic subunits to phosphorylate numerous substrate proteins including ion channels, myofilament proteins, and metabolic enzymes. PKA-dependent phosphorylation mediates many of the physiologic consequences of β-AR signaling. These events are eventually reversed as phosphodiesterases (PDEs) degrade the cAMP and protein phosphatases dephosphorylate PKA substrates.

β-AR signaling has been thought to have four main functional roles in the heart: to increase the heart beating rate (chronotropy), contractility (inotropy), relaxation rate (lusitropy), and to modulate metabolism as required by those increased energetic demands. Heart rate is primarily controlled by the sino-atrial (SA) node, which contains the most effective pacemaker cells. Sympathetic nervous system (SNS) activation increases the pacemaker rate (chronotropy) via voltage-dependent increases in If, the pacemaker current, secondary to direct binding of cAMP. Inotropic responses are primarily attributed to PKA-mediated phosphorylation of the L-type calcium channel (I_{Ca}) and phospholamban. β-AR signaling increases I_{Ca}, bringing additional calcium into myocytes for larger contractions. Phosphorylation of phospholamban releases its tonic inhibition of the sarcoplasmic reticulum (SR) Ca²⁺ pump, sequestering more Ca²⁺ into the SR for larger subsequent contractions. Phospholamban phosphorylation also contributes to lusitropy, as the increase in Ca²⁺ flux from cytosol to SR accelerates relaxation of the myofilaments. Troponin I is also phosphorylated by PKA and has been thought to increase lusitropy by reducing the Ca²⁺ affinity of its partner protein troponin C. The inotropic response, by increasing myofilament work and ATP-dependent SR Ca²⁺ pump rate, expends additional energy in order to generate stronger contractions. To keep up with these demands, PKA also activates phosphorylase kinase, a metabolic enzyme that increases rates of glycogen breakdown, thereby providing additional glucose and increasing cellular ATP.

Congestive heart failure (CHF), while resulting from a number of causes, involves reduced cardiac output and contractility. Historical and conventional
treatments for CHF have aimed at restoring cardiac contractility either indirectly through the use of cardiac glycosides (e.g., digoxin) or more directly by using inotropic agents that act through β-ARs (e.g., dobutamine) or PDEs (e.g., milrinone). While potent inotropic agents have been shown to restore short-term contractility, many have been associated clinically with increased mortality in CHF. In contrast, β-AR blockers are associated with reduced mortality and improved cardiac function in CHF. Such counterintuitive results are forcing a reevaluation of the β-AR signaling network once thought to be so well understood. Does β-AR signaling mediate additional, important cellular functions? Does increased β-AR signaling contribute to the pathophysiology of heart failure, or is it a protective response? Is restoration of cardiac contractility the most appropriate goal in the treatment of heart failure? New findings are revealing surprising answers to some of these questions, with great promise toward future improvements in the treatment of heart failure.

COMPARTMENTATION OF cAMP AND PKA SIGNALING

Initial descriptions of cAMP signaling assumed a linear relationship from hormone-stimulated activation of AC through cAMP, PKA, and functional consequences such as inotropy. However, even 25 years ago, some studies were inconsistent with such a simple relationship, finding that as opposed to β-AR stimulation, activation of prostaglandin E1 (PGE1) receptors increased cAMP but did not increase particulate PKA activity or lead to inotropy. These findings led to the hypothesis of cAMP compartmentation, that the cell could contain distinct pools of cAMP with varying ability to activate PKA and produce functional responses. However, the cAMP compartmentation hypothesis was unconvincing for many as its molecular basis was unknown and cAMP compartmentation could not be directly measured.

In the last several years, a number of candidate molecular mechanisms for cAMP and PKA compartmentation have emerged. The plasma membrane is now known to be a heterogeneous environment, with lipid rafts and nonhomogeneous distribution of membrane-bound proteins among such microdomains. Caveolae, or “little caves,” form membrane invaginations roughly 60 nm in diameter that are thought to play a role in endocytosis in endothelial cells and the formation of T tubules in cardiac myocytes. Caveolae also appear to concentrate signaling proteins involved in β-AR signaling and other cascades. While the precise distribution of components is still unclear, caveolae appear to concentrate β-ARs, ACs, and PKA in both neonatal and adult cardiac myocytes. At the same time, a number of receptors that act through cAMP but are not functionally well coupled to PKA and inotropy appear excluded from caveolae, including PGE2 receptors. Due to the ambiguities involved in the sucrose density fractionation often used to study caveolae,
immunofluorescence microscopy and electron microscopy have provided vital information on caveolae organization from intact isolated adult myocytes and myocardium. Functional evidence for the role of caveolae in cAMP/PKA compartmentation is just beginning to emerge. Filipin, a sterol-binding agent that disrupts lipid rafts, prevented β2- but not β1-AR increases in the spontaneous beating rate of neonatal cardiac myocytes. While L-type calcium channels have been shown to colocalize with caveolae in skeletal muscle and neonatal cardiac myocytes, regulation of these channels by β1-AR- or β2-AR-specific agonists has not been tested with caveolae disruption. Further work is necessary to characterize the extent to which caveolar localization is required for specific β1-AR or β2-AR regulation of L-type calcium currents and inotropic responses in adult cardiac myocytes.

Molecular evidence is also growing for the role of protein complexes in the compartmentation of cell signaling. Numerous A-kinase-anchoring proteins (AKAPs) tether the regulatory subunit of PKA, RII, or RI to individual PKA substrates or microdomains. In the context of cAMP/PKA compartmentation, AKAPs may localize PKA near individual pools of cAMP, ensuring that certain PKA substrates are only regulated in response to a subset of cAMP signals. Some AKAPs recruit many other signaling proteins to these scaffolds as well, possibly forming functional signaling modules. In the heart, AKAPs have been shown to target PKA to the L-type calcium channel, the ryanodine receptor complex, the nuclear membrane, β2-AR, the KNCQ1/KCNE1 K+ channel, mitochondria, and additional sites. While there is insufficient PKA (<1 μmol/L cytosol) to stoichiometrically target high-abundance substrates such as phospholamban (~100 μmol/L cytosol), AKAPs may serve to maintain a stoichiometric balance between PKA and low abundance substrates to ensure their proper regulation.

Functional roles of AKAPs have been studied using AKAP mutagenesis in cell expression systems and Ht31 peptides, which competitively inhibit PKA’s binding site for AKAPs. Ht31 peptides were shown to disrupt β-adrenergic regulation of the L-type calcium channel current in adult mouse cardiac myocytes, providing a clear indication of the functional importance for AKAPs. Unexpectedly, Ht31 peptides expressed in adult rat cardiac myocytes enhanced β-AR-induced increases in contractility and myocyte relaxation rate but not calcium transients. This result may be due to an unidentified myofilament-bound AKAP that facilitates increases in Ca2+ sensitivity. However, methodological differences may have also played a role. In the study by Gao et al., Ht31 peptides were delivered acutely through a patch pipette to freshly isolated mouse cardiac myocytes, while in the study by Fink et al., Ht31 peptides were expressed using an adenovirus in cultured rat cardiac myocytes. In neonatal cardiac myocytes, mAKAP has been shown to integrate cAMP pathway components and many other proteins at the nuclear membrane, playing a role in both cytokine and β-AR-induced hypertrophy.
Caveolae and AKAPs provide intriguing candidate molecular mechanisms to support the cAMP/PKA compartmentation hypothesis. Yet a significant gap has remained between these possible molecular mechanisms and functional or in vitro data that appear difficult to explain without a compartmentation hypothesis. Recently developed experimental approaches are now providing much more solid evidence of cAMP/PKA compartmentation in intact cardiac myocytes. A number of these studies have involved creative use of cellular electrophysiology, which has a tradition of quantitative studies. By providing separate perfusion to opposite ends of a single frog ventricular myocyte, Jurevicius and Fischmeister were able to measure both local and distant changes in \( I_{Ca} \) in response to locally applied stimuli. Through this technique, they demonstrated that isoproterenol but not forskolin-induced signaling was compartmented, and that PDEs played a direct role in limiting cAMP diffusion. Cyclic nucleotide gated (CNG) channels have been genetically modified and expressed in simple cells, providing real-time measurements of sub-membrane cAMP concentrations through the whole-cell patch clamp. Even in simple cells, these CNG channels demonstrated much higher and transient cAMP concentrations at the membrane than seen with average global measurements of cAMP. These constructs have been recently expressed in adult rat ventricular myocytes, allowing characterization of negative feedback involved in PKA-mediated activation of PDEs.

Another exciting innovation in experimental approaches has been the advent of green fluorescent protein (GFP)-based imaging. Numerous recombinant protein biosensors have been designed based on fluorescence resonance energy transfer (FRET) between the cyan (CFP) and yellow (YFP) mutants of GFP, either on different proteins (intermolecular FRET), or in a single protein (intramolecular FRET). These fluorescent indicators have allowed real-time imaging of Ca\(^{2+}\), cAMP, PKA, protein kinase C (PKC), and many other second messengers and kinase activities in live cells. Particularly notable has been the development of cAMP sensors based on dissociation of PKA’s regulatory and catalytic subunits. Expression of a GFP-based cAMP sensor in neonatal cardiac myocytes allowed the first direct visualization of cAMP compartmentation in response to \( \beta \)-AR signaling. While these gradients of cAMP aligned with 2 \( \mu \)m periodicity suggestive of T-tubule membranes, cAMP diffusion is likely even more locally restricted given the above-mentioned differential functional responses to specific \( \beta \)-AR agonists, forskolin, and other G-protein coupled receptor agonists. Further work is required to achieve even greater resolution with genetically targeted sensors and to quantitatively characterize the connections between molecular mechanisms, cAMP compartmentation, and its functional consequences. For example, this GFP-based cAMP sensor was recently used to demonstrate a role for \( \beta_3 \)-ARs in PDE2 activation, which restricts cAMP diffusion during \( \beta_1 \)-AR stimulation with norepinephrine. Appreciation of cAMP/PKA compartmentation and its functional consequences
will help provide a more refined understanding of how β-AR signaling coordinates diverse functions in health and disease.

**LONG-TERM β-ADRENERGIC SIGNALING**

There is increasing evidence that β-AR signaling cross-talks with other signaling pathways in addition to the conventional steps of β-AR, Gs, AC, cAMP, PKA, and substrates (Fig. 1). These “new” forms of signaling trigger long-term consequences (hours to days) of β-AR action such as cardiac hypertrophy and apoptosis. While organized into classical signaling downstream of PKA and nonclassical (cAMP/PKA-independent) β-AR signaling, numerous cross-talk and feedback mechanisms blur this distinction in some situations.

**Classical Long-Term β-Adrenergic Signaling**

As discussed above, β-AR signaling acts through PKA-mediated phosphorylation to increase cardiac myocyte calcium and contractility. Increases in cellular calcium due to β-AR signaling can trigger a number of calcium-dependent signaling pathways, one of which is Ca$^{2+}$-calmodulin-dependent

![FIGURE 1. Functional consequences of cardiac β-AR signaling. Historically, cardiac β-AR signaling was known only to act through the β$_1$-AR–AC–cAMP–PKA signaling axis (bold arrows) to acutely regulate heart rate (chronotropy), inotropy (contractility), and lusitropy (relaxation rate). Recent evidence additionally demonstrates an important role for β$_2$-AR signaling and long-term cardiac remodeling including regulation of hypertrophy and apoptosis.](image-url)
kinase II (CaMKII). CaMKII appears to act as a frequency-dependent positive feedback loop. Increased heart rates and calcium levels from β-AR signaling stimulate CaMKII, which further increases calcium influx through $I_{Ca}$ and SR Ca$^{2+}$ pump activity through phosphorylation of phospholamban. Thus some portion of the functional response to β-AR signaling may be attributed to CaMKII-dependent effects.

CaMKII signaling also appears to mediate some of the long-term consequences of β-AR signaling. The appreciation of a role for cardiac myocyte apoptosis in heart failure has been fairly recent. Long-term β$_1$-AR signaling has been shown to induce apoptosis in cardiac myocytes, which appeared dependent upon PKA and increases in $I_{Ca}$. This led to the finding that CaMKII, activated by increases in calcium influx, was an important intermediate in β$_1$-AR-mediated apoptosis. Unexpectedly, Zhu et al. demonstrated that such signaling may be PKA-independent through the use of multiple PKA inhibitors. H89, used by Communal et al. to inhibit PKA, may not be specific as it has been shown to also act as a β-AR blocker. A subsequent study found that long-term (hours) β-adrenergic responses in excitation–contraction (E–C) coupling may also be due to PKA-independent activation of CaMKII, while short-term (minutes) β-adrenergic regulation of E–C coupling were found to be PKA-mediated and independent of CaMKII.

Together, these studies suggest that β$_1$-AR signaling, long thought to act only through cAMP and PKA, undergoes a functional shift from PKA to CaMKII signaling with long-term stimulation. Responsible mechanisms for PKA-independent activation of CaMKII are unclear. There appear to be some inconsistencies between initial PKA-dependent CaMKII activation, which occurs rather quickly, and PKA-independent CaMKII activation, which occurs over 2 h. This apparent discrepancy may turn out to be due to compartmentation of CaMKII signaling. One intriguing possible mechanism is direct stimulation of $I_{Ca}$ by β-AR-activated G$_s$, shown to contribute only a small portion of the total β-AR response yet may explain a slowly increasing activation of CaMKII.

Regardless of whether or not β-AR activation of CaMKII is downstream of PKA, inhibition of CaMKII may serve as an attractive therapeutic target for heart failure. Such a therapy could prevent CaMKII-dependent apoptosis while retaining responsiveness to β-AR stimuli. This hypothesis was tested in vivo with a transgenic mouse expressing the CaMKII inhibitor peptide AC3-I. Remarkably, CaMKII inhibition in this mouse prevented maladaptive remodeling in response to excessive β-AR stimuli and myocardial infarction while retaining responsiveness to physiologic β-AR stimuli.

Some long-term responses to β$_1$-AR stimulation may be the result of more direct transcriptional regulation. Increases in cAMP allow PKA-mediated phosphorylation of cAMP response element (CRE)-binding (CREB) transcription factors that bind to the CRE in promotor regions of several genes. While CRE-induced transcription contributes to a cardiac hypertrophic response, it
also increases expression of inducible cAMP early repressor (ICER), an inducible repressor of CRE transcription and an inhibitor of the anti-apoptotic protein Bel-2. Through these mechanisms, excessive β-AR stimulation may trigger apoptosis through ICER. Recently, Ding et al. demonstrated that chronic PDE3 but not PDE4 inhibition triggered apoptosis through an ICER-dependent pathway, while constitutive expression of PDE3 A with an adenovirus prevented isoproterenol-induced apoptosis. Differences between the PDE3 and PDE4 responses suggest that ICER-mediated apoptosis may rely on cAMP compartmentation to respond in a context-dependent manner. Thus PKA-independent CaMKII signaling and PKA-dependent ICER signaling represent two pathways that may underlie β1-AR-induced cardiac apoptosis. Future studies will be required to determine the relative roles of these pathways, particularly in animal models relevant to human heart failure.

**Nonclassical β-Adrenergic Signaling**

Unlike traditional β1-AR signaling, which acts through Gs, β2-ARs undergo a transition from Gs to Gt signaling mediated by PKA phosphorylation of β2-AR. This transition has been implicated in enhanced β2-AR compartmentation of cAMP, allowing functional coupling to Ica but not to phospholamban or other cytosolic substrates of PKA. β2-AR stimulation of Gt has also been shown to prevent cardiac apoptosis through a pathway involving PI3 K and Akt. Thus there appears to be a balance between pro-apoptotic β1-AR signaling and anti-apoptotic β2-AR signaling. Such duality is reminiscent of the β-adrenergic inotropy/lusitropy duality, for which Arnold Katz described β-AR signaling as “a man who blows hot and cold air with one breath,” a reference to Aesop’s fable, “The Man and the Satyr.”

Stimulated β-ARs are well recognized to desensitize to prolonged stimulation. Desensitization of β1-ARs occurs via two pathways: phosphorylation of the activated receptor by G-protein receptor kinase 2 (GRK2) or PKA. β2-ARs desensitize via the GRK2 pathway, initializing receptor internalization through clathrin-coated pits. PI3 K has been shown to form a complex with GRK2, contributing to downregulation of β-ARs via desensitization but also initiating a hypertrophic response. Inhibiting GRK2 or disrupting PI3 K/GRK2 interaction has prevented β-AR downregulation and prolonged survival in several heart failure models. Relative roles for β-AR down-regulation and consequent reduction in PI3 K-dependent inhibition of apoptosis in the transition to heart failure remain to be determined.

Recent evidence has shown that in addition to its role in GRK-mediated receptor desensitization, β-arrestin serves as a scaffold for many signaling proteins, bringing them to the membrane upon receptor activation. β-arrestin acts as a scaffold for the MAPK cascade, enabling ERK phosphorylation through Gi signaling in response to isoproterenol in cardiac myocytes. PDE4D is bound to β-arrestin as well, causing an increase in membrane PDE4D activity.
with stimulation of β2-ARs. PDE recruitment by β-arrestin was shown to form a negative feedback loop by decreasing PKA activity at the membrane, which placed a restraint on the transition from Gs to Gi signaling and downstream ERK phosphorylation. Long-term β2-AR activation can also recruit β-arrestin in a G-protein-independent manner leading to prolonged ERK activation. ERK phosphorylation is a key component of hypertrophy signaling, raising the intriguing possibility that long-term β2-AR signaling may lead to cardiac hypertrophy. This would serve as a natural balance to the pro-apoptotic signaling due to β1-AR stimulation.

Much of nonclassical signaling through β-ARs has been discovered in just the last several years. Over 20 proteins have been shown to be targeted to β-arrestins, with β-arrestin 1 and 2 showing selectivity for binding partners and receptors. It is likely that many more binding proteins will be found. Given the large number of binding partners for β-arrestins, there exists a possibility for further functional differentiation and specificity in receptor targeting based on the set of binding proteins on a given β-arrestin scaffold. While further work is required on the mechanisms of nonclassical signaling by β-arrestins and their functional consequences, it is clear that β-arrestins exemplify an emerging theme of compartmentation by signaling complexes.

CONCLUSIONS AND FUTURE DIRECTIONS

The historical view of β-AR signaling as a linear pathway with a single functional role led to the use of inotropic agents in the treatment of heart failure. Recent advances in our knowledge of β-AR signaling have revealed a much more complex signaling network than previously believed. Live-cell imaging techniques are providing direct evidence of cAMP/PKA compartmentation and numerous molecular mechanisms are being revealed, including caveolae, AKAPs, and β-arrestins. Compartmentation underlies important aspects of signaling specificity, and while only short-term consequences have been studied to date, compartmentation is likely to be crucial in the ordered transitions from “short-term” to “long-term” term signaling in the β-AR network. Further work will be required to quantitatively characterize how molecular mechanisms contribute to cAMP compartmentation in response to both short- and long-term stimuli. Long-term β-AR stimuli are now recognized to facilitate coupling to many additional pathways including CaMKII, PI3 K, and MAP kinases. These pathways contribute to pro-apoptotic, anti-apoptotic, and hypertrophic responses. Further work will be required to understand the relative roles of β-adrenergic regulation of cardiac contractility and these additional pathways in the development and progression of human heart failure.

An appreciation for the roles of compartmentation, dynamics, and combinatorial complexity of signaling networks suggests that we should not expect to reduce the pathophysiology of heart failure to a single underlying mechanism.
or treat it with a single therapy. Rather, multiple therapeutics may be required simultaneously to prevent maladaptive responses. One approach may, for example, attempt to prevent cardiac apoptosis while restoring β-AR responsiveness. Rational design of therapies will also require a greater basic understanding of the mechanisms of compartmentation and dynamics in signaling to achieve the desired specificity.

Given the complexity of these processes, this will require quantitative experimental approaches such as live-cell imaging, high-throughput genomics and proteomics, and integrative animal studies. Systems approaches and computational models such as those proposed by the Alliance for Cellular Signaling will also be required to integrate and distill understanding from such heterogeneous data. Mechanistic and experimentally validated computational models of cardiac β₁-AR signaling have already been used to predict the relative efficacy of proposed gene therapies and molecular mechanisms of cardiac myocyte inotropy. Integrated models of β₁-AR signaling and ventricular electrophysiology predicted a mechanistic link between a gene mutation that disrupts AKAP interactions and a form of long-QT syndrome. Similar approaches may be valuable for exploring which molecular mechanisms can quantitatively explain cAMP/PKA compartmentation or how multiple pathways interact to produce coordinated long-term adaptations to adrenergic stimuli. Despite the challenges ahead, a more mechanistic and comprehensive understanding of cardiac β-AR signaling will have a great impact on our understanding and treatment of heart failure and other cardiac diseases.

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