The Cellular Actions of β-Adrenergic Receptor Agonists
Looking Beyond cAMP

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General concepts regarding the role of the sympathetic nervous system in the pathogenesis of heart failure (and as a site for therapeutic intervention) have undergone a remarkable transition in the last few years. When β-adrenergic receptor (β-AR) blockers were first introduced into clinical practice more than 30 years ago, they were viewed as contraindicated in heart failure. Conventional wisdom held that patients with impaired ventricular function rely on increased sympathetic drive as a mechanism to maintain mechanical performance and would clinically deteriorate if exposed to the negative inotropic actions of β-AR antagonists. However, clinical practice demonstrated that although positive inotropic agents and vasodilators (agents that directly or indirectly activate neurohormonal pathways) induce short-term hemodynamic improvement, this is offset by long-term adverse effects to accelerate the natural history of heart failure. In contrast, β-AR–blocking drugs prevent or reverse many of the structural and functional changes that develop during the progression of heart failure and prolong life in experimental animal models.1

The mechanisms whereby long-term β-AR activation leads to abnormalities in cardiomyocyte growth, energy use, calcium regulation, and a progressively dysfunctional and mechanically inefficient heart have become an important focus of recent research. The cellular actions of catecholamines generally are attributed to the predominant β1-AR subtype that couples to the stimulatory GTP regulatory protein (Gs), activation of adenylyl cyclase (AC), and accumulation of cAMP. Although cardiomyocytes also express pharmacologically distinct β2-ARs and these assume increasing importance in heart failure syndromes (where β1-ARs are downregulated), the traditional teaching holds that the G/cAMP pathway also is their primary mode of signaling (ie, β-AR subtypes are functionally redundant). However, recent studies in transgenic mice challenge this concept. Cardiac-specific overexpression of β2-ARs at relatively high levels leads to increased basal AC activity and elevated contractile function without obvious cardiotoxicity (unless receptor overexpression is maintained at very high levels or for protracted intervals).2,3 In stark contrast to the relatively wide therapeutic window for β2-ARs, even low levels of transgenic β1-AR overexpression lead to rapidly progressive cardiac deterioration with prominent histological evidence of fibrosis and cardiomyocyte apoptosis and hypertrophy.4 The distinct biological consequences of β1- and β2-AR overexpression argue for their distinct roles in transmembrane signaling in the heart.

In a study in this issue of Circulation Research, Chesley et al5 use cultured neonatal rat cardiomyocytes to decipher the distinct molecular mechanisms activated by cardiomyocyte β1- and β2-ARs. These studies follow on earlier research from Communal et al,6 demonstrating that β1-ARs promote apoptosis and that the proapoptotic actions of β1-ARs are countered by β2-ARs in adult rat ventricular myocytes. In contrast, Chesley et al5 focus on β2-AR protection from apoptosis induced by hypoxia and H2O2, because the effects of β2-AR subtypes on basal apoptosis were not reproduced in neonatal rat ventricular myocyte cultures.5 The initial attempts from the Colucci laboratory6 to delve into the mechanisms through which chronic β2-AR stimulation alters cardiomyocyte survival focused on cAMP, demonstrating that the proapoptotic actions of β1-ARs are mediated by a cAMP-dependent mechanism, whereas the opposing effects of β2-ARs could be attributed to a mechanism activated by a pertussis toxin (PTX)-sensitive G protein. Although an obvious potential target of the cardiomyocyte (PTX-linked) β2-AR is the AC enzyme, the precise role of cAMP in the antiapoptotic actions of β2-ARs is uncertain. Two aspects of β2-AR signaling to AC are predicted to influence this process and must be considered.1 Do β2-ARS display a generalized action to stimulate AC in all cardiomyocyte preparations? Does the β2-AR/Gi pathway inhibit cAMP accumulation by β2-ARS?

The notion that cAMP is an obligate downstream effector of β2-ARs in all cardiomyocyte preparations remains the focus of lingering controversy. Xiao et al8 have put forth the model that β1- and β2-ARs both activate AC but in different cellular compartments. According to this model, β2-ARs (acting through Gi proteins) generate a cAMP signal that is broadcast throughout the cell. In contrast, β2-ARs promote cAMP accumulation, but the actions of cAMP are confined to effectors at the sarcolemma as a result of simultaneous β2-AR activation of a PTX-sensitive Gi protein with opposing function. This model is based upon experiments demonstrating that PTX functionally enhances β2-AR (but not β1-AR) signaling (with the target of the β2-AR/Gi pathway identified as an intracellular phosphatase that counters the stimulatory effects of protein kinase A [PKA] at intracellular sites such as phospholamban). Although inhibitory regulation of AC is a more traditional target for PTX-sensitive Gi proteins, the consensus of several recent studies is that the β2-AR/Gi,

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pathway does not inhibit AC. Because other results establish the integrity of the $G_i$-dependent pathway for muscarinic cholinergic receptor (mACHr) inhibition of AC in the same cells, these studies argue for specificity in $G_i$-protein signaling. It suggests that $\beta_2$-ARs and mACHRs couple to distinct species or pools of $G_i$ proteins and that the use of mACHr agonists as a strategy to obtain independent confirmation of the $G_i$-dependent actions of $\beta_2$-ARs (an approach adopted by Chesley et al in the present study) may not be optimal.

Other laboratories identify an alternative mechanism for the distinct signaling properties of $\beta_1$- and $\beta_2$-ARs. Here, experimental results demonstrate that $\beta_2$-ARs elevate cAMP levels in cultured neonatal rat cardiomyocytes but not in adult rat and embryonic mouse cardiomyocytes (where control experiments identify pronounced elevations of cAMP by $\beta_1$-ARs). These age-and species-dependent differences in $\beta_2$-AR linkage to cAMP provide a plausible explanation for the differential functional effects of $\beta_1$- and $\beta_2$-ARs (including on cell survival). For example, $\beta_2$-AR coupling to the proapoptotic cAMP signal in neonatal, but not adult, cells could (at least in part) explain the failure of Chesley et al to reproduce the reciprocal actions of $\beta_2$-AR subtypes on apoptosis in neonatal rat cardiomyocyte cultures. To date, $\beta_2$-AR-dependent protection from $\beta_1$-AR-induced apoptosis has been reported only in adult rat cardiomyocytes. Traditional random collision-coupling models for receptor action do not provide an obvious mechanism for selective activation of AC by cell surface $\beta_2$-ARs but not by $\beta_1$-ARs (coexpressed on the cell surface at levels sufficient to provide functional inotropic support). Rather, compartmentation of components of the receptor complex to membrane subdomains (caveolae), with distinct submembrane distributions for $\beta_1$- and $\beta_2$-ARs, allows for specificity in $\beta_1$-AR-subtype activation of AC.

Chesley et al build on recent efforts to identify the cAMP-independent pathways recruited by agonist-activated $\beta_2$-AR subtypes. Nonconventional pathways for $\beta_2$-ARs initially came under scrutiny in the context of efforts to identify catecholamine-dependent hypertrophic signaling mechanisms. Here, bifurcating pathways via G/cAMP/PKA and G protein $\beta Y$ dimers/Src/Ras/Raf/extracellular signal–regulated kinase (ERK) were implicated in the anabolic response to $\beta_2$-ARs. Two studies place ERK activation downstream from $\beta_1$- and $\beta_2$-AR subtypes; there is agreement that ERK activation by $\beta_2$-AR is the quantitatively more robust response, but the data regarding the role of PTX-sensitive G proteins are less consistent. The ERK cascade generally is credited with conferring protection from proapoptotic stimuli. However, Chesley et al provide evidence that ERK activation is not required for $\beta_2$-AR protection from hypoxia-induced apoptosis. Rather, these investigators place the phosphoinositide 3'-kinase (PI3K)/Akt pathway (a survival signal previously implicated in $\beta_2$-AR dependence) downstream of the PTX-sensitive $\beta_2$-AR subtype and demonstrate that this pathway figures critically in $\beta_2$-AR protection from apoptosis induced by hypoxia or $\text{H}_2\text{O}_2$. Noticeably absent from the study by Chesley et al is any consideration of p38–mitogen-activated protein kinase (MAPK), another MAPK family member that variably has been placed downstream from PI3K and has been the focus of considerable attention (and confusion) as an intermediate in signaling pathways leading to cardiac hypertrophy and apoptosis. Recent studies by Sabri et al indicate that p38-MAPK is activated largely by the $\beta_1$-AR subtype (and a PTX-insensitive pathway) in embryonic mouse cardiomyocytes and (in the absence of ERK activation) is not sufficient to induce cardiomyocyte hypertrophy. Other studies from Communal et al identify p38-MAPK activation by $\beta_1$- and $\beta_2$-AR and argue that p38-MAPK figures importantly in the ant apoptotic $G_i$-dependent pathway for $\beta_2$-ARs in adult rat ventricular myocytes. However, these conclusions regarding the role of p38-MAPK in ant apoptotic signaling by $\beta_2$-ARs are entirely on the basis of results of experiments with high concentrations of the inhibitor compound SB203580 and may be open to question. Recent studies indicate that micromolar SB203580 blocks Akt phosphorylation by phosphoinositide-dependent protein kinase 1.

With present enthusiasm for $\beta_2$-AR antagonists as therapeutic agents for heart failure, studies to decipher the signaling properties of individual cardiomyocyte $\beta_2$-AR subtypes and distinguish their roles on cardiac muscle biology become critical. Key unresolved issues include the following.

What is the structural basis for the distinct signaling properties of $\beta_1$- and $\beta_2$-ARs (to cAMP and nontraditional signaling pathways)? Recent literature identifies considerable heretofore-unrecognized complexity for $\beta_2$-AR-type signaling. Differences in $\beta_2$-subtype/G-protein linkage, $\beta_2$-AR association with scaffolding proteins that assemble second messenger–regulated signaling enzymes, and compartmentalization to membrane subdomains are among the mechanisms that can impart diversity in signaling that require additional study.

How do $\beta_2$-ARs promote apoptosis? Studies in cardiomyocytes implicate a $G_i$/cAMP/PKA pathway and calcium entry via voltage-dependent calcium channels. A recent study identifies the calcium-dependent target of the proapoptotic $\beta_2$-AR as calcineurin (possibly acting through the dephosphorylation of the protein Bad) and comes as a surprise; calcineurin has attracted considerable attention as a mediator of cardiac hypertrophy and prevented apoptosis in a previous study. However, given the broad range of targets for calcineurin in the physiological context (including to effectors that suppress and induce apoptosis), its influence on the decision to hypertrophy versus commit to the apoptosis program is likely to be defined by the identity and magnitude of associated receptor-activated signals. In this context, recent studies identify Src family tyrosine kinases as alternate effectors for the $G_\alpha_i$ pathway leading to apoptosis in thymocytes. Because signaling pathways frequently are very context-dependent, direct examination of this process in cardiomyocytes is warranted in future studies.

Is cardiac protection mediated by a specific PTX-sensitive $G_i$ protein? Present research implicating $G_i$ proteins in $\beta_2$-AR signaling comes from studies with PTX, which cannot distinguish individual $G_i$ protein family members (and could be confounded by direct cellular actions of the PTX cell surface-
binding B-oligomer"). Ultimately, molecular (rather than pharmacological) strategies to ablate Gi proteins are required to validate these conclusions and identify the pertinent Gi proteins. On the basis of their distinct actions to inhibit AC, this approach also is predicted to distinguish Gi-dependent pathways for mACHR and β2-ARs.

What is the biological significance of signals recruited by the minor β2-AR subtype typically only at high-agonist concentrations? One of the more unnoticed features of β2-AR signaling to growth regulatory pathways is the concentration-response relationship for agonist activation. β2-AR activation of AC is maximal at 0.1 μmol/L, but studies by Chesley et al and others typically rely on 100-fold higher agonist concentrations to optimally engage cAMP-independent growth regulatory pathways. Recent experiments with β2-AR Gα-subunit fusion proteins demonstrate that the pharmacologic profile of the β2-AR can be influenced by the identity of the G-protein α subunit to which it binds. Because β2-ARs adopt a conformation that displays higher affinity for ligand when coupled with Gi than with Gs, these observations are compatible with a Gi-independent pathway for growth regulation by β2-ARs.

Which is the optimal system to study the cellular actions of β-ARs in cardiomyocytes? The discrepancy between the studies describing the actions of β-ARs on basal apoptosis in adult and neonatal cardiomyocytes serves to emphasize the uncertainties regarding the optimal model for investigations of catecholamine action in the heart. Because experience maintaining adult ventricular myocytes cultures that retain a highly differentiated phenotype has become more widespread, there has been a growing temptation to dismiss research in neonatal rat ventricle cultures as irrelevant. However, such categorical conclusions may be premature, because the preferred assay system may differ depending on the nature of the stimulus, response, or cardiomyocyte (normal or diseased) under study. For example, certain components of growth regulatory pathways are more abundant in neonatal than in normal adult cardiomyocytes. This could undermine the validity of extrapolating results obtained in neonatal cardiomyocytes to the normal adult ventricle. However, several examples of disease-associated functional increases in regulatory kinases in adult cardiomyocytes might suggest that neonatal cardiomyocytes are a valid model for the diseased adult heart. The cell type that provides the best surrogate for β2-AR signaling in human cardiomyocytes also must be taken into consideration, given the evidence for distinct modes for β2-AR coupling to cAMP accumulation and activation of nontraditional cAMP-independent growth regulatory pathways between neonatal and adult cardiomyocytes. Although knowledge of β-AR signaling in human cardiac tissue is still limited, the preponderance of available evidence identifies a robust cAMP-dependent pathway for β2-ARs in human ventricular myocardium. This pathway is more similar to the mode for β2-AR signaling in neonatal (rather than adult) cardiomyocytes and suggests that neonatal cardiomyocytes also may be the preferred cell type for studies of β2-AR actions.

Does the polymorphic variation of the β2-AR with impaired Gi coupling and AC activation confer protection from apoptosis? Recent studies identify polymorphisms of both β1- and β2-ARs, with patients harboring a hypofunctional β2-AR variant (using coupling to Gi as the endpoint) at increased risk for heart failure progression. The signaling properties of structurally distinct β-ARs to cAMP-independent pathways have not been examined (and are not necessarily predictable). This line of study could reveal additional mechanisms whereby genetic variations contribute to interindividual differences in heart failure progression and sympathetic responsiveness.

Ultimately, studies directed at these and other issues will refine prevailing models for β-AR action in the heart and provide a framework to develop newer strategies targeted to the sympathetic nervous system to optimize heart failure management.

References


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