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.

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 Gₛ/cAMP pathway also is their primary mode of signaling (i.e., 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). In stark contrast to the relatively wide therapeutic window for β₁-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. 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 al² 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.,³ 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. The initial attempts from the Colucci laboratory⁶ to delve into the mechanisms through which chronic β-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 antia apoptotic actions of β₂-ARs is uncertain. Two aspects of β₂-AR signaling to AC are predicted to influence this process and must be considered. Do β₂-ARs display a generalized action to stimulate AC in all cardiomyocyte preparations? Does the β₂-AR/Gₛ pathway inhibit cAMP accumulation by β₁-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 al⁷ have put forth the model that β₁- and β₂-ARs both activate AC but in different cellular compartments. According to this model, β₁-ARs (acting through Gₛ 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 G 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/Gₛ 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 G proteins, the consensus of several recent studies is that the β₂-AR/Gₛ,

See related article, pages 1172–1179
pathway does not inhibit AC. Because other results establish the integrity of the G-‐dependent pathway for muscarinic cholinergic receptor (mACHr) inhibition of AC in the same cells, these studies argue for specificity in G protein signaling. It suggests that β2-ARS and mACHRs couple to distinct species or pools of G proteins and that the use of mACHR agonists as a strategy to obtain independent confirmation of the G-‐dependent actions of β2-ARS (an approach adopted by Chesley et al in the present study) may not be optimal.6,9,10

Other laboratories identify an alternative mechanism for the distinct signaling properties of β1- and β2-ARS. Here, experimental results demonstrate that β2-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 β1-ARS).11-13 These age- and species-‐dependent differences in β2-AR linkage to cAMP provide a plausible explanation for the differential functional effects of β1- and β2-ARS (including on cell survival). For example, β2-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 β-AR subtypes on apoptosis in neonatal rat cardiomyocyte cultures. To date, β2-AR-‐dependent protection from β2-AR-‐induced apoptosis has been reported only in adult rat cardiomyocytes.6 Traditional random collision-‐coupling models for receptor action do not provide an obvious mechanism for selective activation of AC by cell surface β2-ARS but not by β1-ARS (coexpressed on the cell surface at levels sufficient to provide functional inotropic support11). Rather, compartmentation of components of the receptor complex to membrane subdomains (caveolae), with distinct submembrane distributions for β1- and β2-ARS, allows for specificity in β-AR-‐subtype activation of AC.14

Chesley et al5 build on recent efforts to identify the cAMP-‐independent pathways recruited by agonist-‐activated β-AR subtypes. Nonconventional pathways for β-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 βγ dimers/Src/Ras/Raf/extracellular signal-‐regulated kinase (ERK) were implicated in the anabolic response to β-ARS.15 Two studies place ERK activation downstream from β1- and β2-AR subtypes; there is agreement that ERK activation by β2-AR is the quantitatively more robust response, but the data regarding the role of PTX-‐sensitive G proteins are less consistent.5,12 The ERK cascade generally is credited with conferring protection from proapoptotic stimuli. However, Chesley et al5 provide evidence that ERK activation is not required for β2-AR protection from hypoxia-‐induced apoptosis. Rather, these investigators place the phosphoinositide 3′-kinase (PI3K)/Akt pathway (a survival signal previously implicated in β-AR-‐dependent induction of atrial natriuretic factor expression16) downstream from the PTX-‐sensitive β2-AR subtype and demonstrate that this pathway figures critically in β2-AR protection from apoptosis induced by hypoxia or H2O2.3 Noticeably absent from the study by Chesley et al5 is any consideration of p38-‐mitogen-‐activated protein kinase (MAPK), another MAPK family member that variably has been placed downstream from PI3K17,18 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 al12 indicate that p38-MAPK is activated largely by the β1-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 al10 identify p38-‐MAPK activation by β1- and β2-AR and argue that p38-‐MAPK figures importantly in the antiapoptotic G2-‐dependent pathway for β2-ARS in adult rat ventricular myocytes. However, these conclusions regarding the role of p38-MAKP in antiapoptotic signaling by β2-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 1.19 Hence, the most parsimonious interpretation of available literature is that PI3K/AKT is the prosurvival signal activated by β2-ARS.

With present enthusiasm for β-AR antagonists as therapeutic agents for heart failure, studies to decipher the signaling properties of individual cardiomyocyte β2-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 β1- and β2-ARS (to cAMP and nontraditional signaling pathways)? Recent literature identifies considerable heretofore-‐unrecognized complexity for β2-AR-‐subtype signaling. Differences in β2 subtype/G-protein linkage, β2-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 β2-ARS promote apoptosis? Studies in cardiomyocytes implicate a G/cAMP/PKA pathway and calcium entry via voltage-‐dependent calcium channels.20 A recent study identifies the calcium-‐dependent target of the proapoptotic β2-AR as calcineurin (possibly acting through the dephosphorylation of the protein Bad21) and comes as a surprise; calcineurin has attracted considerable attention as a mediator of cardiac hypertrophy and prevented apoptosis in a previous study.22 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 Go2 pathway leading to apoptosis in thymocytes.23 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 protein? Present research implicating G proteins in β2-AR signaling comes from studies with PTX, which cannot distinguish individual G protein family members (and could be confounded by direct cellular actions of the PTX cell surface—
binding B-oligomer\(^{24}\)). 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 \(\beta_2\)-ARs.

What is the biological significance of signals recruited by the minor \(\beta_2\)-AR subtype typically only at high-agonist concentrations? One of the more unnoticed features of \(\beta_2\)-AR signaling to growth regulatory pathways is the concentration-response relationship for agonist activation. \(\beta_2\)-AR activation of AC is maximal at 0.1 \(\mu\)mol/L, but studies by Chesley et al\(^{5}\) and others\(^{12}\) typically rely on 100-fold higher agonist concentrations to optimally engage cAMP-independent growth regulatory pathways. Recent experiments with \(\beta_2\)-AR G\(\alpha\)-subunit fusion proteins demonstrate that the pharmacologic profile of the \(\beta_2\)-AR can be influenced by the identity of the G-protein \(\alpha\) subunit to which it binds.\(^{25}\) Because \(\beta_2\)-ARs adopt a conformation that displays higher affinity for ligand when coupled with G\(\alpha_i\) than with G\(\alpha_i\), these observations are compatible with a Gi-independent pathway for growth regulation by \(\beta_2\)-ARs.

Which is the optimal system to study the cellular actions of \(\beta_2\)-ARs in cardiomyocytes? The discrepancy between the studies describing the actions of \(\beta_2\)-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 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 \(\beta_2\)-AR signaling in human cardiomyocytes also must be taken into consideration, given the evidence for distinct modes for \(\beta_2\)-AR coupling to cAMP accumulation and activation of nontraditional cAMP-independent growth regulatory pathways between neonatal and adult cardiomyocytes. Although knowledge of \(\beta_2\)-AR signaling in human cardiac tissue is still limited, the preponderance of available evidence identifies a robust cAMP-dependent pathway for \(\beta_2\)-ARs in human ventricular myocardium.\(^{7}\) This pathway is more similar to the mode for \(\beta_2\)-AR signaling in neonatal (rather than adult) cardiomyocytes and suggests that neonatal cardiomyocytes also may be the preferred cell type for studies of \(\beta_2\)-AR actions.

Does the polymorphic variation of the \(\beta_2\)-AR with impaired G\(_i\) coupling and AC activation confer protection from apoptosis? Recent studies identify polymorphisms of both \(\beta_1\) and \(\beta_2\)-ARs, with patients harboring a hypofunctional \(\beta_2\)-AR variant (using coupling to G\(_i\), as the endpoint) at increased risk for heart failure progression.\(^{26,27}\) The signaling properties of structurally distinct \(\beta_2\)-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 difference in heart failure progression and sympathetic responsiveness.

Ultimately, studies directed at these and other issues will refine prevailing models for \(\beta_2\)-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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