Adenylyl Cyclase
A New Target for Heart Failure Therapeutics

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In the early 1960s, Earl Sutherland and his colleagues performed a series of experiments that led to the understanding that the positive inotropic effects of β-adrenergic agonists were mediated by the activation of the enzyme adenylyl cyclase (AC) and the subsequent production of cyclic adenosine monophosphate (cAMP). By measuring tissue cAMP levels while simultaneously monitoring the mechanical properties of the heart, these investigators were able to show that cAMP levels increased with extraordinary speed, an increase that correlated directly with increased force of contraction. Subsequent studies demonstrated that AC activity is enhanced through a pathway beginning with ligand binding to β1-adrenergic receptors (β1ARs) and subsequent activation of stimulatory guanine nucleotide–binding signal transduction proteins (G↓). The resultant production of cAMP mediates improvements in both cardiac inotropy and lusitropy via stimulation of cAMP-dependent protein kinase A and the protein kinase A–dependent phosphorylation of key target proteins, including the L-type calcium channel, phospholamban, and troponin I. In the normal heart, activation of the βAR–G protein–AC pathway effects enhanced contractility, which is paramount in facilitating the “flight or fight” response. However, in the failing human heart, profound alterations in multiple components of this βAR–G protein–AC signal transduction cascade reduce cardiac reserve and contribute to decreased exercise response in patients with heart failure. Paradoxically, long-term activation of the neurohormonal pathway accelerates the natural history of heart failure.

Thus, a therapeutic conundrum has arisen: How can one enhance myocardial contractility while at the same time protecting the heart from the untoward consequences of heightened and continuous adrenergic activation? With the use of transgenic overexpression and gene-targeted knockouts, investigators have demonstrated the potential utility of cardiac Adenylyl Cyclase
Isoforms
Cardiac Adenylyl Cyclase

At least 9 closely related isoforms of AC (AC1 through AC9) have been cloned and characterized in mammals, each encoded by a distinct gene (see reviews). The expression of the various isoforms is tissue dependent, with AC5 and AC6 being the most abundant isoforms in the heart. Although these 2 isoforms are equivalent at birth, AC5 mRNA becomes predominant in the adult rat heart. Furthermore, with aging, there is an increase in the AC5 isoform and a decrease in the AC6 isoform, an isoform shift that may influence cardiac function. Both isoforms can be phosphorylated and inhibited by protein kinase A, thus providing feedback regulation in the transduction cascade. The ability of cardiac ACs to be modified by protein kinase C–dependent phosphorylation remains more controversial. The various AC isoforms are all activated by G↓; however, different ACs have varying affinities for G↓—a finding that may explain the variations in tissue response to a given adrenergic receptor agonist. Similarly, inhibition of AC by G↓-coupled receptors and activation by β1 subunits and calcium are also isozyme specific.

Although AC is activated by nonselective agonists such as isoproterenol, recent data suggest that the various AC isoforms may be differentially activated by specific membrane receptors. For example, purinergic receptors activate AC5 but not AC6. In addition, purinergic stimulation of AC5 does not seem to be susceptible to inhibitory modulation, whereas βAR activation of both AC5 and AC6 is inhibited by G↓,-associated receptor-effector activation. Thus, individual AC isoforms may effect selective functions in cardiomyocytes. Furthermore, the complexities in regulation of βAR–AC coupling and selectivity of cAMP downstream effects suggest the presence of intramembrane compartmentalization of specific βARs with AC isoforms or intracytoplasmic trafficking and compartmentalization of cAMP. AC also may be activated by G-protein–mediated βAR signaling; however, this possibility remains controversial. It is interesting to note that AC may be activated independent of receptor activation, inasmuch as β-adrenergic receptors may exist in an activated state even in the absence of agonist.

Crystallography and site-directed mutagenesis studies have identified the 3-dimensional structure of the enzyme. However, this information has not led to the development of selective small molecule agonists. The chromosomal localization of each of the AC isoforms has also been identified, although little is known about the promoter and regulatory regions of each of the genes. Indeed, the presence of multiple...
AC isoforms in most cells and the heterogeneity of most tissues, including heart tissue, preclude expeditious identification of the tissue-specific regulatory control of any AC isoform.

**Adenyllyl Cyclase as a Therapeutic Target**

Important information about the role of AC in the heart has come from studies that use transgenic and gene transfer technology. For example, transgenic mice with cardiac-directed expression of AC6 showed increased transgene AC expression, no change in myocardial AR number or G-protein content, normal levels of myocardial cAMP, and normal cardiac function. However, when hearts were exposed to an adrenergic agonist, there was an increase in both function and myocardial cAMP levels in the transgenic hearts when compared with wild-type controls. Similarly, the intracoronary injection of a recombinant adenovirus encoding AC6 effectively improved agonist-stimulated cAMP production and cardiac hemodynamics but had no effect on basal heart rate, blood pressure, or left ventricular contractility. That these effects were isofrom specific was demonstrated by the finding that overexpression of the Ca^{2+}/calmodulin-activatable isoform AC8 led to a higher basal intrinsic contractility that was unresponsive to further adrenergic stimulation.

To test the hypothesis that AC could serve as a therapeutic target, several groups of investigators have evaluated the effects of AC overexpression on the pathophysiology of mice with hypertrophy and heart failure secondary to overexpression of the signal transduction protein G_s. G_q mediates the effects of a variety of neurohormones by activating phosphatidylinositol-specific phospholipase C, resulting in inositol triphosphate–mediated calcium release and diacylglycerol-mediated activation of protein kinase C. Furthermore, G_s can activate mitogen-activated protein kinase. Four-fold overexpression of G_s,4,5,11,12,13,14,15 overexpression of G_s, effeted the development of myocyte hypertrophy; increases in expression of atrial natriuretic factor, β-myosin heavy chain, and α-skeletal actin; and a 3-fold decrease in βAR-stimulated AC activity in the absence of a decrease in receptor number. Furthermore, G_q overexpression also has been associated with uncoupling of the βAR from G_s and an increase in G_q, without alterations in the expression of βARK. Hemodynamically, G_s overexpression impaired intrinsic contractility and a blunted contractile response to βAR stimulation; higher levels of G_q overexpression resulted in frank cardiac decompensation and the development of biventricular failure, pulmonary congestion, and death.

When transgenic mice with cardiac-directed expression of AC6 were crossbred with mice with heart failure secondary to cardiac-directed G_q expression, an increase in myocardial AC6 content was associated with restoration of cAMP-generating capacity, improved cardiac function, and enhanced responsiveness to βAR stimulation. That AC overexpression provided increased “recruitable” cAMP responsiveness without sustained adrenergic activation was thought to provide an important advantage in the setting of heart failure and a possible explanation for the marked differences in phenotype when compared with βAR or G_q overexpression. Transgenic replacement of AC5 by crossing G_q-overexpressing transgenic mice with mice overexpressing AC5 also restored levels of forskolin-stimulated AC activity, normalized basal cardiac AC activity, and improved cardiac contractility and fractional shortening. However, the G_q/AC5 mice had persistent hypertrophy. Similar improvements in resting ventricular function and expression of atrial natriuretic factor and α-skeletal actin mRNA could be effected by low levels of overexpression of the β_s,AR. In contrast with overexpression of AC, low levels of βAR also reduced hypertrophy, and the salutary effects were independent of enhanced activity of AC. Higher expression of βARs effectively improved AC activity, but hypertrophy and ventricular function were either unchanged or worsened.

The salutary benefits of AC overexpression were viewed with caution because prolonged overexpression of other members of the βAR–G–protein–AC cascade effected the development of cardiac dilatation and failure. To address these concerns, Roth and colleagues undertook an elegant study in which they assessed the long-term effects of AC overexpression on survival in mice overexpressing G_q. As reported in the present issue of Circulation, AC6 overexpression restored myocyte AC generation, improved heart function, abrogated myocardial hypertrophy, and improved survival.

**Interpreting Studies in Transgenic Heart Failure Models**

Although intriguing, results from transgenic models in which AC has effected salutary benefits must be viewed with some caution before translating the basic research results to the clinical arena. For example, it is important to understand whether a therapeutic approach that is effective in an animal model with a relatively homogenous defect will be equally beneficial in animals or humans with more heterogenous defects. For example, overexpression of G_q effects a marked decrease in both basal and forskolin-stimulated AC activity. Thus, normalization of AC activity and of isoproterenol-stimulated AC activity would logically improve the phenotype of such animals, especially if such a normalization occurred early in the development of disease. Indeed, transgenic technology has been highly successful in correcting focal defects in signal transduction pathways with resultant normalization of the muscle phenotype. For example, mice overexpressing a βARK inhibitor (βARKct) have rescued the myopathic phenotype when crossbred with myopathic strains of mice with marked increases in βARK. In each of these models, crossbreeding with βARKct mice effectively improved cardiac function. However, βARKct overexpression failed to rescue cardiac function in mouse models of heart failure that did not demonstrate elevation of βARK. Similarly, phospholamban ablation or sarcoplasmic reticulum ATPase overexpression was most likely to improve cardiac contractility and survival in animal models that were characterized by abnormal sarcoplasmic reticulum calcium handling. Because human heart failure is not associated with a decrease in AC activity, it will be important to define the therapeutic benefits of AC overexpression in mice that do not...
demonstrate marked abnormalities in AC activity or in humans with heart failure.

Crossbreeding experiments also must be interpreted with care because of the fact that the “rescue” of the abnormal phenotype is temporally related to the change in expression of the target protein. Thus, treatment of animals or humans with mature disease might not be as beneficial. This possibility could be excluded by crossbreeding a myopathic transgenic with a conditional transgenic, thereby allowing the rescue protein to be produced at later time points in the development of the heart failure phenotype. As with pharmacological therapy, it also will be important to ascertain the effects of dose on the biology of AC overexpression. To date, studies have increased cAMP levels ~2-fold. It would be useful to know the effectiveness of higher levels of cAMP. High levels of exogenous AC could result in overcrowding, and thus, alterations in the structure of the sarcolemmal membrane or high levels of cAMP could have ambiguous effects. Alternatively, long-term overexpression of one AC isoform might alter its endogenous expression or expression of other AC isoforms.

Summary

In conclusion, the recent studies of AC gene transfer by either adenoviral mediated gene transfer or crossbreeding mice overexpressing selective AC isoforms with myopathic transgenic mice have provided strong support for the hypothesis that AC gene transfer can effectively improve cardiac performance and rescue contractile function in the failing myocardium. The studies by Roth et al.20 provide substantive data, inasmuch as they demonstrate that long-term exposure to AC overexpression provides salutary benefits without evidence of cardiotoxicity. Although exciting, these studies raise important questions about the applicability of studies in transgenic mouse models to the human condition and the intricacies of the βAR-mediated signal transduction pathways. However, these studies also inspire optimism with regard to the ability of AC agonists or virally mediated AC gene transfer to improve exercise performance and possibly survival in other heart failure models. In addition, they provide a strong rationale for investigating the efficacy of increasing AC activity in patients with heart failure.

References

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