Editorial

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 adenyl cyclase (AC) and the subsequent production of cyclic adenosine monophosphate (cAMP).1 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 (Gα1). 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.2 Paradoxically, long-term activation of the neurohormonal pathway accelerates the natural history of heart failure.

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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 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 (Gα1). 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.2 Paradoxically, long-term activation of the neurohormonal pathway accelerates the natural history of heart failure.

The opinions expressed in this editorial are not necessarily those of the editors or of the American Heart Association.

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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 reviews4,5). 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,6 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.7 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 Gαq; however, different ACs have varying affinities for Gαq—a finding that may explain the variations in tissue response to a given adrenergic receptor agonist.8 Similarly, inhibition of AC by Gβγ-coupled receptors and activation by βγ subunits and calcium are also isoform specific.9

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 AC6.10 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 Gβγ-associated receptor-effector activation.10 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 intracyttoplasmic trafficking and compartmentalization of cAMP. AC also may be activated by G-protein–mediated βAR signaling; however, this possibility remains controversial.11 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.

Adenylyl 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 isoform specific was demonstrated by the finding that overexpression of the Ca2+/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 Gq. Gq 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, Gq can activate mitogen-activated protein kinase.

Four-fold overexpression of Gqq effected 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, Gq overexpression also has been associated with uncoupling of the βAR from Gi and an increase in Go, without alterations in the expression of βARK. Hemodynamically, Gq overexpression impaired intrinsic contractility and a blunted contractile response to βAR stimulation; higher levels of Gq 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 Gq 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 Gq overexpression. Transgenic replacement of AC5 by crossing Gqq-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 Gq/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 β3AR. 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 β3ARs 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 Gqq. As reported in the present issue of Circulation, AC6 overexpression restored myocyte AC generation, improved heart function, aborted 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 Gq 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 defects. For example, overexpression of Gq 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.
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 al20 provide substantive data, looking beyond cAMP. Circ Res. 2000;87:1079–1082.


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