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).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 (Gₛ). 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 the complexities in regulation of low levels of β₂AR overexpression or inhibition of βAR kinase (βARK) in improving cardiac contractile function without obvious cardiotoxicity (see review3). However, only recently have investigators explored the possibility that AC also could serve as a therapeutic target.

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 Gₛ; however, different ACs have varying affinities for Gₛ—a finding that may explain the variations in tissue response to a given adrenergic receptor agonist.8 Similarly, inhibition of AC by Gᵢₐ-coupled receptors and activation by βγ subunits and calcium are also isozyme 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 Gᵢᵢₐ-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 intracytoplasmic trafficking and compartmentalization of cAMP. AC also may be activated by G-protein–mediated βₐAR 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

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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 cardiogenic 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. Further-
more, Gq can activate mitogen-activated protein kinase. Four-fold overexpression of Gq
affected the development of
myocyte hypertrophy; increases in expression of atrial natri-
uretic 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 Gq and an increase in Gq with alterations in
the expression of βARK. Hemodynamically, Gq over-
expression impaired intrinsic contractility and a blunted con-
tractile response to βAR stimulation; higher levels of Gq
overexpression resulted in frank cardiac decompensation and
the development of biventricular failure, pulmonary conges-
tion, and death.

When transgenic mice with cardiac-directed expression of
AC6 were crossbred with mice with heart failure secondary to
cardiogenic Gq expression, an increase in myocardial AC6 content was associated with restoration of cAMP
-generating capacity, improved cardiac function, and en-
hanced responsiveness to βAR stimulation. That AC over-
expression 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 cross-

ing Gq-overexpressing transgenic mice with mice overex-
pressing 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 improve-
ments in resting ventricular function and expression of atrial
natriuretic factor and α-skeletal actin mRNA could be ef-
fected by low levels of overexpression of the βAR. In
contrast with overexpression of AC, low levels of βAR also
reduced hypertrophy, and the salutary effects were independ-
ent 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 Gq. As
reported in the present issue of Circulation, AC6 overexpres-
sion restored myocyte AC generation, improved heart func-
tion, 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
cautions 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 pheno-
type of such animals, especially if such a normalization
occurred early in the development of disease. Indeed, trans-
genics 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 overexpres-
sion 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 charac-
terized by abnormal sarcoplasmic reticulum calcium han-
dling. Because human heart failure is not associated with a
decline 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 al20 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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