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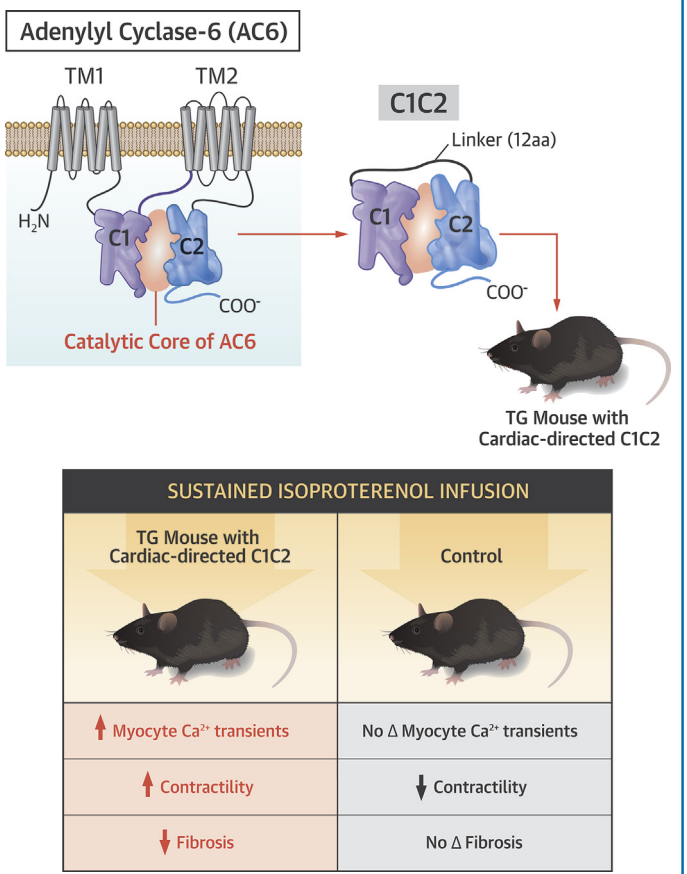
PRECLINICAL RESEARCH

Cardiac-Directed Expression of Adenylyl Cyclase Catalytic Domain Reverses Cardiac Dysfunction Caused by Sustained Beta-Adrenergic Receptor Stimulation

Mei Hua Gao, PhD, N. Chin Lai, PhD, Dimosthenis Giamouridis, MSc, Young Chul Kim, PhD, Zhen Tan, PhD, Tracy Guo, BS, Wolfgang H. Dillmann, MD, Jorge Suarez, PhD, H. Kirk Hammond, MD

VISUAL ABSTRACT



Gao, M.H. et al. J Am Coll Cardiol Basic Trans Science. 2016;1(7):617–29.

HIGHLIGHTS

- **Cardiac-targeted expression of C1C2 reduces cAMP production yet mice maintain normal cardiac function through increased Ca^{2+} handling.**
- **Sustained isoproterenol infusion reduces heart function in normal mice, but improves heart function in mice with increased cardiac C1C2 expression.**
- **Reduced cardiac cAMP generation and resistance to catecholamine cardiomyopathy are attractive features of this potential heart failure therapeutic.**
- **Removing the large transmembrane domains of AC6 and fusing the two intracellular domains provides a small molecule, C1C2, that replicates many of the beneficial effects of AC6, but is sufficiently small to be expressed in an AAV vector for gene transfer.**

From the Veterans Affairs San Diego Healthcare System and Department of Medicine, University of California-San Diego, San Diego, California. This work was supported by U.S. National Institutes of Health grants P01 HL66941 and HL088426, and U.S. Department of Veteran Affairs Merit grants 1101BX001515 and 5101BX001121. Dr. Hammond is a co-founder, board member, and unpaid consultant for Renova Therapeutics, which had no involvement or licenses related to this work and played no role in the studies. The other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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ABBREVIATIONS AND ACRONYMS

AAV = adeno-associated virus

AC = adenylyl cyclase

AC6mut = AC6 mutant, contains an amino acid substitution that reduces its catalytic activity

Ad5 = adenovirus-5

AKAP = A-kinase-anchoring protein

ATP = adenosine triphosphate

AV = atrioventricular

βAR = β-adrenergic receptor

C1C2 = a fusion of the C1 and C2 cytoplasmic domains of AC

cAMP = 3′,5′-cyclic adenosine monophosphate

CREB = cAMP response element binding protein

CryAB = αB-crystallin

HF = heart failure

Iso = isoproterenol

LV = left ventricle, left ventricular

MDM2 = murine double mutant 2

PHLPP2 = PH domain leucine-rich protein phosphatase 2

PI3K = phosphatidylinositide 3-kinase

PLB = phospholamban

P-Rex2 = phosphatidylinositol-3,4,5-trisphosphate-dependent Rac exchange factor 2

RGAS = regulator of G protein signaling

SERCA2a = sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase

SUMMARY

Transgenic mice with cardiac-directed C1C2, a fusion protein of the intracellular C1 and C2 segments of adenylyl cyclase type 6, had normal left ventricular (LV) function, but diminished cAMP generation. Cardiac myocytes from C1C2 mice showed increased Ca^{2+} release. Mice underwent continuous isoproterenol infusion to stress the heart. In C1C2 mice, sustained isoproterenol infusion increased rather than decreased LV function. LV SERCA2a and Ca^{2+} release were increased. Reduced cAMP generation and resistance to catecholamine cardiomyopathy are attractive features of this potential heart failure therapeutic. (J Am Coll Cardiol Basic Trans Science 2016;1:617–29)

Adenylyl cyclase (AC) is a transmembrane protein in cardiac myocytes and other cells, the effector molecule for β-adrenergic receptor (βAR) and other G protein-coupled receptors, which regulates the conversion of adenosine triphosphate (ATP) to 3′,5′-cyclic adenosine monophosphate (cAMP) and thereby initiates a variety of intracellular signaling cascades that influence heart function and additional physiological events.

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There are 9 membrane-bound isoforms of mammalian ACs, each consisting of 2 transmembrane domains and 2 cytoplasmic domains (C1 and C2). The C1 and C2 domains form the catalytic core of AC (**Figure 1A**). When expressed as a fusion protein, C1C2 is soluble and retains forskolin-stimulated catalytic activity (1). C1C2 contains binding sites for Gαs, Gαi, forskolin, ATP, Mg^{2+}, the regulator of G protein signaling (RGAS2), protein associated with Myc (PAM), Snapin, Ric8a, A-kinase-anchoring protein (AKAP79), protein kinase C, PH domain leucine-rich protein phosphatase 2 (PHLPP2) and phosphorylation and dephosphorylation sites for protein kinase A. Interactions of these factors alters the conformation of C1C2 and regulates AC activity (2).

We have published a series of papers indicating that increased cardiac expression of AC type 6 (AC6), a dominant AC isoform expressed in mammalian cardiac myocytes (3), has protean beneficial effects on the failing left ventricle (LV). These effects include: 1) increased survival in genetically-induced cardiomyopathy (4) and in acute myocardial infarction (5); 2) reduced action potential duration (6), facilitated atrioventricular (AV) conduction (7), and reduced AV block (5); 3) reductions in both LV dilation and pathological hypertrophy (4,8); 4) beneficial effects on Ca^{2+} handling via altered activity of SERCA2a and phospholamban (PLB) (9,10); and 5) increased cardiac troponin I phosphorylation (11).

These beneficial effects, consistent in several species and models, appear in large part to not depend upon increased cAMP generation. A phase 2 randomized clinical trial in patients with symptomatic heart failure (HF) and reduced ejection fractions showed that intracoronary AC6 gene transfer appears to be safe and potentially effective, and not associated with increased cardiac arrhythmias (12). Even so, there may be advantages in selecting a transgene that attenuates βAR responsiveness when treating HF.

We subsequently generated a catalytically inactive AC6 mutant (AC6mut) molecule by replacing Ala with Asp at position 426 in AC6's catalytic core. This AC6mut is catalytically inactive (does not generate cAMP) but retains the cellular distribution pattern and favorable signaling effects associated with normal AC6, thereby providing compelling evidence that the beneficial effects of AC6 do not require increased cAMP generation (13). AC6mut seemed an ideal candidate for the treatment of HF, retaining the beneficial effects of the parent AC6 while circumventing the potential deleterious effects of sustained cAMP generation. However, a shortcoming of both AC6mut and AC6 is that the molecules are too large to insert into an adeno-associated virus (AAV) with regulated expression, therefore only constitutive expression would be possible, a potential limitation.

Eliminating the amino terminus and the 2 transmembrane domains of AC6 and subsequently fusing the 2 cytoplasmic domains (C1 and C2) with a 12-amino acid linker yields a C1C2 protein (**Figure 1A**). C1C2 has an intact catalytic domain but is disengaged from membrane-associated βARs and is therefore less responsive to βAR stimulation in intact cells. C1C2 is sufficiently small to be inserted in an AAV vector with