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PRE-CLINICAL RESEARCH

Adenylyl Cyclase 6 Improves Calcium Uptake and Left Ventricular Function in Aged Hearts

Tong Tang, PhD,*† H. Kirk Hammond, MD,*† Amy Firth, PhD,† Yuan Yang, MD,† Mei Hua Gao, PhD,*† Jason X.-J. Yuan, MD, PhD,† N. Chin Lai, PhD*†

San Diego and La Jolla, California

Objectives This study tested the hypothesis that activation of adenylyl cyclase 6 (AC6) expression in cardiac myocytes improves calcium uptake and left ventricular (LV) function in aging mice.

Background Aging hearts exhibit impaired β-adrenergic receptor signaling and LV dysfunction.

Methods Twenty-month-old mice with cardiac-directed and regulated AC6 expression were randomized into 2 groups, and AC6 expression was activated in 1 group (AC6-On) but not the other (AC6-Off). One month later, LV function and sarcoplasmic reticulum calcium uptake were assessed.

Results AC6 expression was associated with increased LV contractility, as reflected by ejection fraction ($p = 0.02$), rate of pressure development ($p = 0.002$), and slope of the LV end-systolic pressure-volume relationship ($p = 0.04$). No changes in LV weight to tibial length ratio, LV fibrosis, and expression of fetal genes (atrial natriuretic factor, α-skeletal muscle actin, and β-myosin heavy chain) and collagens were observed between AC6-On and AC6-Off groups. However, LV samples from AC6-On mice showed increases in: isoproterenol-stimulated cAMP production ($p = 0.04$), cAMP-dependent protein kinase activity ($p < 0.0004$), phosphorylation of phospholamban (at Ser16 site; $p = 0.04$) and cardiac troponin I (at Ser23/24 sites; $p = 0.01$), velocity of sarcoplasmic reticulum calcium uptake ($p < 0.0001$), and sarcoplasmic reticulum calcium-ATPase2a (SERCA2a) affinity for calcium ($p < 0.0001$). Finally, we found that AC6 expression increased sarcoplasmic reticulum calcium storage in cardiac myocytes isolated from 23-month-old rats. In contrast, AC6 expression in 7-month-old mice did not change LV function and calcium uptake.

Conclusions These results indicate that activation of cardiac AC6 expression improves impaired function of aged hearts through improved calcium uptake. (J Am Coll Cardiol 2011;57:1846–55) © 2011 by the American College of Cardiology Foundation

Cardiac senescence is associated with reduced left ventricular (LV) function (1–4) and impaired cardiac β-adrenergic receptor (βAR) responsiveness (5). In humans, LV diastolic and systolic functions in response to βAR stimulation progressively decrease after the age of 20 years. At age 80 years, LV contractile reserve is less than one-half of what it was at age 20 years (6). In addition, congestive heart failure (CHF) is a common disease of the elderly (7–9), and older patients with CHF have a particularly poor prognosis (8).

Adenylyl cyclase (AC) is the effector molecule for βAR signaling (10), playing a pivotal role in contractile responsiveness, cardiac relaxation, and LV diastolic function (11). AC catalyzes ATP to generate cAMP, a second messenger that is required for many intracellular events (12). Reduced βAR responsiveness in aging hearts occurs in the presence of increased plasma catecholamine levels (13), underscoring the abnormality in AC signaling (5,14). Indeed, impaired LV cAMP production is associated with decreased cardiac AC content in hearts from animals of advanced age (15,16). However, the precise mechanism by which AC regulates cardiac function in aging hearts is not known and is the focus of the current investigation.

Cardiac-directed expression of AC type 6 (AC6) increases LV function in CHF (17,18), in which impaired βAR-AC signaling and impaired calcium uptake are prominent (19). Increased cardiac cAMP production and improved sarcoplasmic reticulum (SR) calcium uptake are of mechanistic importance for the beneficial effect of AC6 on failing hearts (18,20). In this study, we used a transgenic line with cardiac-directed tetracycline-regulated

From the *VA San Diego Healthcare System, San Diego, California; and the †Department of Medicine, University of California San Diego, La Jolla, California. This work was supported by Grants-in-Aid from the American Heart Association Western States Affiliate, NIH grants (5P01HL066941, HL081741, HL088426), and a VA Merit Review Award. Dr. Hammond is a founder, consultant, and equity holder for Renova Therapeutics. All other authors have reported that they have no relationships to disclose. Douglas Mann, MD, acted as Guest Editor for this article.

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AC6 expression (21,22) to test the hypothesis that activation of cardiac AC6 expression improves LV function by increasing cardiac cAMP production and correcting calcium uptake impairment in hearts from older mice.

Methods

Animals. The Animal Use and Care Committee of the VA San Diego Healthcare System, in accordance with National Institutes of Health and Association for Assessment and Accreditation of Laboratory Animal Care guidelines, approved this study. Twenty-month-old transgenic mice with cardiac myocyte–specific tetracycline-regulated (tet-off) AC6 expression (22) were used for echocardiography, in vivo physiology, and biochemistry studies. Adenylyl cyclase 6 transgene expression is completely suppressed until tetracycline is removed from the water supply, and chronic tetracycline treatment did not affect cardiac function. Mice were randomized into 2 groups. AC6 expression was activated (by removing tetracycline suppression) in one group (AC6-On) and was continuously suppressed by tetracycline in the other group (AC6-Off). These mice were studied 1 month after activation (or continued inactivation) of AC6 transgene expression. For comparison, echocardiography, in vivo physiology, and biochemistry studies were also performed on 7-month-old tetracycline-regulated AC6 mice. For calcium transients study, cardiac myocytes were isolated from 23-month-old Sprague-Dawley rats (Harlan Laboratories, Indianapolis, Indiana).

Echocardiography. Echocardiography was performed under light anesthesia as previously reported (23). Data were acquired and analyzed without knowledge of group identity.

LV in vivo physiologic studies. A 1.4-F conductance-micromanometer catheter was used to measure LV pressure and volume to assess the LV end-systolic pressure-volume relationship (ESPVR) as previously reported (18). Data were acquired and analyzed without knowledge of group identity.

Necropsy and LV fibrosis assessment. Body and LV weights (including septum) and tibial lengths were recorded. A short-axis midwall LV ring was formalin fixed and paraffin embedded. The LV sections were stained with picrosirius red, and collagen fractional area was quantified using NIH ImageJ software (24).

Biochemistry studies. Total RNA extraction and quantitative reverse transcriptase-polymerase chain reaction were performed as previously reported (24). LV samples were homogenized and used for Western blotting as described previously (23). AC activity, cAMP-dependent protein kinase (PKA) activity, and caspase 3/7 activity in LV samples were measured as reported (18).

Calcium uptake. LV tissues were homogenized, and the ATP-dependent initial rate of SR calcium uptake was measured by a modified Millipore filtration technique as reported (20).

Calcium transients. Cardiac myocytes were isolated from adult rats as previously described (25), plated on laminin-coated 25-mm glass coverslips, and infected with adenovirus encoding green fluorescent protein or murine AC6 (400 viral particles/cell). Forty hours after infection, cells were loaded with the calcium-sensitive fluorescent indicator Fura-2 AM (3 μM), and the intracellular calcium concentration was monitored using a digital fluorescence imaging system (Intracellular Imaging, Cincinnati, Ohio), as described previously (23). To assess SR calcium load, caffeine-induced calcium release was initiated by addition of 10 mM caffeine to Tyrode's solution. The peak amplitude of calcium transients was calculated from the baseline and the transient rise after caffeine treatment. Data were acquired and analyzed without knowledge of group identity.

Abbreviations and Acronyms

- **AC** = adenylyl cyclase
- **βAR** = β-adrenergic receptor
- **CHF** = congestive heart failure
- **cTnI** = cardiac troponin I
- **ESPVR** = end-systolic pressure-volume relationship
- **LV** = left ventricular
- **LV/TL** = LV weight to tibial length ratio
- **MMP** = matrix metalloproteinase
- **PKA** = cAMP-dependent protein kinase
- **PLN** = phospholamban
- **SERCA2a** = sarcoplasmic reticulum calcium-ATPase2a
- **SR** = sarcoplasmic reticulum

Statistical analysis. Results are shown as mean ± SE. Group differences were compared using unpaired, 2-tailed Student *t* test. The null hypothesis was rejected when $p < 0.05$.

Results

AC activity in aging hearts. To confirm that aging is associated with decreased AC activity in the heart (5,14,16,26), we measured LV cAMP production in 7- and 20-month-old mice. There were a 43% reduction in basal ($p = 0.0001$), a 56% reduction in isoproterenol-stimulated ($p = 0.04$), and a 58% reduction in NKH477-stimulated LV cAMP production ($p = 0.0001$) in 20- versus 7-month-old mice (Fig. 1A). We also found a 59% reduction of mRNA expression of AC6, a major cardiac AC isoform, in LV samples from 20- versus 7-month-old mouse hearts (Fig. 1B).

Echocardiography. Aging was associated with a decline in LV ejection fraction (7-month-old [n = 13]: 80 ± 3%; 20-month-old [n = 17]: 57 ± 10%; $p < 0.0001$). Both 7- and 20-month-old mice with cardiac-directed and regulated AC6 expression were randomized, and AC6 expression was activated in the AC6-On but not the AC6-Off group. There were no group differences (AC6-On vs. AC6-Off group) in any of the echocardiographic measures before activation of AC6 expression. However, in 20-month-old mice, activation of AC6 expression increased LV ejection fraction (Table 1). These mice also showed reduced LV end-systolic dimension after AC6 expression was activated