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Cardiac-Directed Expression of Adenylyl Cyclase VI Facilitates Atrioventricular Nodal Conduction

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OBJECTIVES The purpose of this study was to test the hypothesis that cardiac-directed expression of adenylyl cyclase VI (AC_{VI}) facilitates atrioventricular (AV) nodal conduction.

BACKGROUND Cardiac-directed expression of AC_{VI}, unlike other strategies to increase cyclic adenosine monophosphate generation, reduces mortality in murine cardiomyopathy. Recent reports suggest that AC_{VI} expression may also protect against lethal bradycardia.

METHODS We performed immunofluorescence staining for AC_{VI} in the AV node of transgenic mice. We then performed electrophysiologic studies (EPSs) using a 1.7-F octapolar catheter at the AV junction in 11 transgenic AC_{VI} mice and 14 control mice.

RESULTS Immunofluorescence staining revealed increased AC_{VI} expression in the AV node of transgenic mice versus controls. During EPS, AV intervals approximated PR intervals (R^2 = 0.99) and related linearly to atrial-to-His intervals (R^2 = 0.98; both $p < 0.0001$). Thus, we studied AV intervals to avoid electrocardiogram pacing artifacts and inconsistent inscription of His bundle electrograms. At baseline, AC_{VI} mice had shorter AV intervals (47 ± 9 ms) than controls (57 ± 11 ms; $p = 0.02$), despite similar sinus rates. In pacing, AV intervals were shorter in AC_{VI} mice than controls for a wide cycle-length range ($p < 0.01$). The AC_{VI} mice also had shorter AV Wenckebach cycle lengths (AC_{VI}: 114 ± 12 ms; control: 131 ± 28 ms; $p = 0.05$) and ventriculo-atrial effective refractory periods (AC_{VI}: 97 ± 21 ms; control: 127 ± 15 ms; $p = 0.05$). We observed no differences between groups in sinus node function, and ventricular arrhythmias were not inducible.

CONCLUSIONS Cardiac-directed expression of AC_{VI} facilitates AV nodal conduction without altering sinus node function. These results suggest the need to define a role for AC_{VI} gene transfer in treating diseases of AV conduction. (J Am Coll Cardiol 2006;48:559–65) © 2006 by the American College of Cardiology Foundation

Congestive heart failure (CHF) affects more than 5 million people in the U.S. and is a significant cause of morbidity and mortality. Clinically, inhibition of the renin-angiotensin-aldosterone system (1) or antagonism of beta-adrenergic receptors (βARs) (2,3) reduces the mortality and morbidity of CHF. Conversely, pharmacological agents that increase intracellular levels of cyclic adenosine monophosphate (cAMP) typically shorten life (4), despite favorable effects on left ventricular function and symptoms. These observations are mirrored in animal studies, in which cardiac-directed βAR and $G_{\alpha s}$ expression initially increase cardiac contractile function but are ultimately associated with adverse outcomes (5–7).

Cardiac-directed expression of adenylyl cyclase type VI (AC_{VI}), in contrast, is emerging as a useful strategy to increase contractile function while avoiding deleterious effects usually associated with agents that increase intracellular cAMP (8–10). Adenylyl cyclase type VI, a major cardiac isoform of AC, increases cAMP generation after βAR stimulation without increasing basal cAMP. In murine cardiomyopathy (10) and pacing-induced porcine heart failure (11), increased cardiac AC_{VI} improves cardiac function (9–11) and reduces mortality (10). Clinical trials to study the benefits of AC_{VI} gene transfer in patients with severe CHF will commence shortly.

However, the electrophysiologic effects of increased cardiac AC_{VI} expression are unknown. In particular, although AC_{VI} is expressed in the sinus node (12), its expression and functional importance in the atrioventricular (AV) node are unclear. Studies from our laboratory show that AC_{VI} mice are protected against AV nodal block after coronary artery ligation (13), although the mechanism is unknown. In addition, while AC_{VI} expression enhances sinus node chronotropy (8), it is unclear to what extent cardiac AC_{VI} expression, like other adrenergic interventions, pre-disposes to atrial or ventricular arrhythmias.

In the present study, we confirmed the expression of AC_{VI} in the AV node of transgenic mice, and then tested the hypothesis that cardiac-directed AC_{VI} expression facilitates AV nodal conduction. Furthermore, we performed detailed invasive electrophysiologic studies (EPSs) to determine whether increased cardiac AC_{VI} expression increases susceptibility to atrial or ventricular arrhythmias.

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Abbreviations and Acronyms

AC_{VI}	= adenylyl cyclase type VI
AH	= atrial-to-His
ANOVA	= analysis of variance
AV	= atrioventricular
AVERP	= atrioventricular nodal effective refractory period
βAR	= beta-adrenergic receptor
cAMP	= cyclic adenosine monophosphate
CHF	= congestive heart failure
CL	= cycle length
CSNRT	= corrected sinus node recovery time
ECG	= electrocardiogram
EPS	= electrophysiologic study
ERP	= effective refractory period
MHC	= major histocompatibility complex
SNRT	= sinus node recovery time
VA	= ventriculo-atrial

METHODS

Animals. The study protocol was approved by the Animal Care Program of the San Diego Veterans Affairs Medical Center, and we maintained humane treatment of all study subjects. Transgenic mice with cardiac-directed AC_{VI} expression (n = 20; mean age 11 months) were generated using the major histocompatibility complex (MHC) promoter as previously described (8). Transgene-negative siblings (n = 5) and wild-type mice of the same strain (C57BL/6; n = 14) were used as controls (mean age 8 months). We could not obtain data on 14 of these 39 mice: 11 died during surgical preparation before EPS (7 AC_{VI}, 4 control), and we could not obtain suitable catheter position in 3 (2 AC_{VI}, 1 control). This report is based on data from the remaining 25 mice.

Surgery. Mice were anesthetized with intraperitoneal injections of ketamine (100 mg/kg) and xylazine (5 mg/kg) (14) and received 100% oxygen (1 l/min) via nose cone. Mice were placed on their dorsa on a heating pad maintained at 37°C, and surface electrocardiogram (ECG) leads were recorded from electrodes on each limb; ECG and respiratory rate were monitored throughout the procedure.

Transvenous electrode placement. A 10- to 15-mm longitudinal paratracheal incision was made at the level of the larynx. Blunt dissection aided by an operating microscope was used to expose the right external jugular vein. Microscissors were used to create a venotomy, and a 1.7-F octapolar pace/sense catheter (Numed Inc., Hopkinton, New York) was carefully advanced to the AV junction such that right atrial and ventricular electrograms were observed on proximal and distal bipoles, respectively. Figure 1 shows the surgical field, external jugular vein, and catheter. Electrogram recordings were optimized by adjusting the catheter position and signal gain, and then the catheter was sutured in place.

Programmed stimulation. Intracardiac electrograms and the surface ECG were filtered between 5 and 500 Hz and 30 and 100 Hz, respectively, and analyzed at a scale of 200 mm/s from our physiologic recorder (Labsystem Duo, Bard, Massachusetts). Atrial and ventricular pacing were performed using 1-ms pulse widths at twice diastolic threshold, and each maneuver was performed twice.

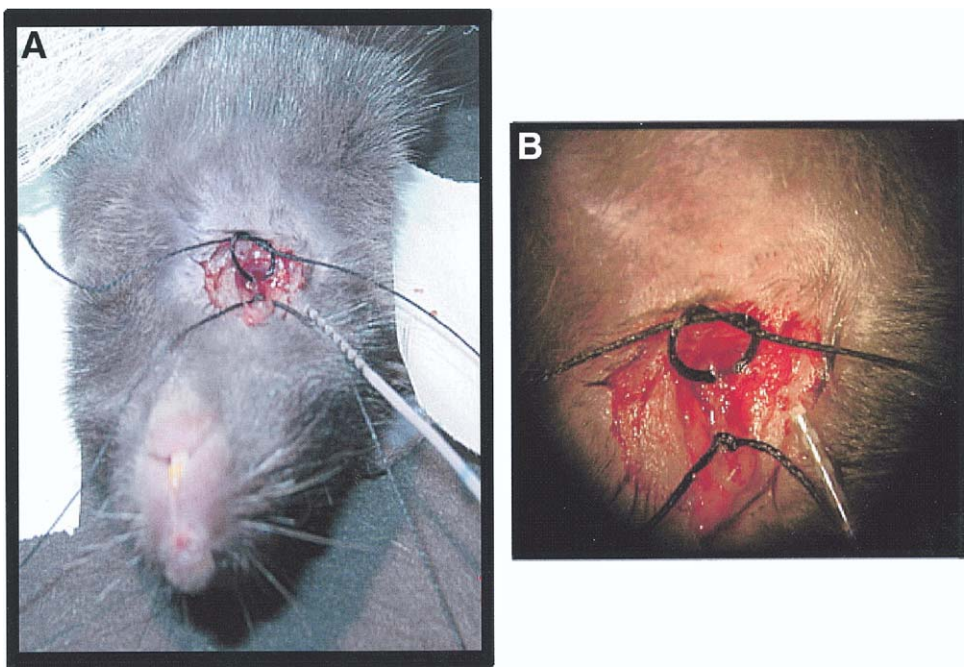

Figure 1. Surgical field and electrophysiology catheter. **(A)** View of right external jugular cutdown and 1.7-F electrophysiology catheter in an anesthetized mouse. **(B)** Close-up of venous cutdown with proximal and distal sutures. The venotomy is made between these sutures, and the catheter is advanced to the atrioventricular junction and then sutured in place.