Overexpression of *SCN5A* in mouse heart mimics human syndrome of enhanced atrioventricular nodal conduction

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BACKGROUND In enhanced atrioventricular (A-V) nodal conduction (EAVNC) syndrome, patients have short A-V conduction times. Multiple mechanisms have been proposed to explain EAVNC; however, the electrophysiological or molecular substrate responsible for it remains unclear.

OBJECTIVE The purpose of this study was to test the hypothesis that overexpression of *SCN5A* in the mouse heart may provide an animal model mimicking EAVNC.

METHODS Electrocardiogram, atrial, His bundle, and ventricular electrograms were recorded from wild-type (WT) and transgenic (TG) mice overexpressing human *SCN5A*. The sodium current and $Na_V1.5$ expression were measured using patch-clamp and immunohistochemistry techniques.

RESULTS The P-R interval in TG mice (13.6 ± 1.2 ms) was much shorter than that in WT mice (40.2 ± 0.59 ms). In TG isolated hearts, the A-V conduction time (14.4 ± 0.81 ms) during right atrial pacing was also shorter than that in WT hearts (39.5 ± 0.62 ms). Records of His bundle electrograms revealed that atrial-to-His and His-to-ventricular intervals were shorter in TG than in WT hearts. In addition, TG hearts had a shorter Wenckebach cycle length and A-V effective refractory period. The sodium current was 2-fold greater in TG ventricular myocytes than in WT ventricular myocytes. Flecainide prolonged the A-V conduction time in TG hearts to a value close to that in WT hearts. Nifedipine prolonged the atrial-to-His interval in WT hearts but not in TG hearts. Immunohistochemistry studies revealed increased $Na_V1.5$ labeling in TG atrial and ventricular tissues, and $Na_V1.5$ expression in A-V junction and A-V ring regions in TG hearts.

CONCLUSION Enhanced A-V conduction in mice overexpressing *SCN5A* in the heart mimics the human syndrome of EAVNC. Thus, variants in sodium channel expression in the A-V nodal region may be an electrophysiological substrate responsible for EAVNC.

KEYWORDS Enhanced atrioventricular nodal conduction; *SCN5A*; Animal model

ABBREVIATIONS **A-H** = atrial-to-His; **A-V** = atrioventricular; **AV-ERP** = atrioventricular effective refractory period; **CL** = cycle length; **EAVNC** = enhanced atrioventricular nodal conduction; **ECG** = electrocardiogram; **H-V** = His-to-ventricular; **I_{Na}** = sodium current; **LGL** = Lown-Ganong-Levine; **MAP** = monophasic action potential; **PFA** = paraformaldehyde; **TG** = transgenic; **WT** = wild-type

Introduction

A large and rapid influx of sodium through voltage-gated sodium channels during the upstroke of the cardiac action potential initiates myocyte depolarization and propagation of the electrical impulse throughout the cardiac conduction system and myocardium. The amplitude of the peak sodium current (I_{Na}) determines the rate of rise of the action potential upstroke and therefore the conduction velocity of an electrical impulse in the heart.[1] Enhanced atrioventricular (A-V) nodal conduction (EAVNC) syndrome describes a population of patients with a short A-V conduction time who are capable of 1:1 A-V conduction at rapid atrial pacing rates. In EAVNC, the P-R interval is shortened and A-V conduction time is decreased.[2–6] Several mechanisms have been proposed to explain short P-R intervals in EAVNC, including a partial bypass of the A-V node, an underdeveloped or anatomically small A-V node, and an anatomically normal A-V node that has rapid conduction properties either intrinsically or as a result of alterations in autonomic tone,[6–12] but the subject has not been resolved. A transgenic (TG) mouse with cardiac-specific overexpression of *SCN5A* (which encodes the cardiac sodium channel $Na_V1.5$) was recently developed and studied by Zhang et al.[13] In this mouse model, prominent functional manifestations of overexpression of *SCN5A* included shortening of the P wave and the P-R interval on the surface

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electrocardiogram (ECG).[13] On the basis of these results, we hypothesize that overexpression of human *SCN5A* may affect A-V nodal conduction mimicking EAVNC. Accordingly, in this study we further determined the effect of overexpression of *SCN5A* on A-V conduction in the TG mouse model.

Methods

Mouse model of cardiac-specific overexpression of *SCN5A*

The use of animals in this investigation conformed to the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication No. 85-23, revised 1996) and was approved by the Institutional Animal Care and Use Committee of Gilead Sciences (Fremont, CA). Breeding pairs of TG mice with cardiac-specific overexpression of human *SCN5A* (under the control of the mouse α-myosin heavy chain promoter) were obtained from Dr Wang at the Cleveland Clinic Foundation.[13] A breeding colony of these mice at Gilead Sciences served as the source of the TG mice used in this study. Non-TG wild-type (WT) littermate mice were used as controls. Animals of either sex and 4–8 months of age were used for experiments.

Recording of the surface ECG in vivo and the cardiac electrogram ex vivo

For recording of the surface ECG, mice were anesthetized using an intraperitoneal injection of Avertin (12.5 mg/mL, 0.02 mL/g; Sigma Chemical, St Louis, MO) with supplemental dosing (0.1–0.2 mL) as needed. Then, a surface 6-lead ECG was recorded by subcutaneous placement of 24-G needles in each limb. After an ECG recording, each mouse was heparinized (0.2 mL, 1000 U/mL), and the chest was opened to excise the heart. The isolated heart was immediately mounted on a Langendorff apparatus for retrograde perfusion via the aorta with oxygenated (95% O_2, 5% CO_2) Krebs-Henseleit buffer at a rate of 2.5 mL/min. The buffer solution contained 118 mM NaCl, 4.0 mM KCl, 2.3 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2 mM sodium pyruvate, 7.5 mM glucose, 0.5 mM Na_2EDTA (Ethylenediaminetetraacetic acid), and 25 mM $NaHCO_3$. Hearts were allowed to beat spontaneously or were paced with an EP-4 stimulator (St Jude Medical Inc, Austin, TX) and a right atrial bipolar electrode. Additional unipolar electrodes were placed on the surface of the heart for simultaneous recording of left and right atrial electrograms and the left ventricular electrogram and monophasic action potential (MAP). His bundle potentials were recorded with an intracardiac electrode that was placed in the heart just above the tricuspid valve.

Patch-clamp electrophysiology

Adult atrial and ventricular myocytes were prepared from hearts of WT and TG mice through enzymatic dissociation, as described previously.[14] Borosilicate glass patch pipettes had a resistance of 1.4–1.8 MΩ when filled with the internal solution containing 110 mM CsCl, 10 mM NaCl, 10 mM TEA-Cl (Tetraethlyammonium), 5 mM Mg-ATP (Adenosine 5′-triphosphate magnesium salt), 5 mM EGTA (Ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid), 5 mM HEPES (4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid, N-(2-Hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid), and pH adjusted to 7.3 with CsOH. Myocytes were superfused with the solution containing 10 mM CsCl, 120 mM NaCl, 10 mM TEA-Cl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM $CdCl_2$, 10 mM HEPES, 5 mM glucose, and pH adjusted to 7.3 with CsOH. Experiments were performed at room temperature (22°C). Myocytes were held at a resting potential of –100 mV, and a test potential of 25-ms duration to −20 mV was delivered at a stimulation frequency of 0.1 Hz. Cell capacitance and 60%–80% of series resistance were routinely compensated.

Immunohistochemistry of the mouse heart

Hearts of either WT or TG mice were excised, snap frozen in liquid nitrogen, and stored at −80°C. Cryosections (7-μm thickness) were prepared in 4-chamber view and mounted on 3-aminopropyltriethoxysilane–coated glass slides. A separate set of WT and TG hearts was excised, stored in 4% paraformaldehyde (PFA), and sectioned in 4-chamber view (10-μm thickness). Cryosections were permeabilized in 0.2% Triton X-100 in phosphate buffered saline for 1 hour, and PFA sections were boiled for 10 minutes in Antigen Unmasking Solution. All sections were blocked in 2% bovine serum albumin for 30 minutes and incubated with primary (overnight) and secondary (90 minutes) antibodies in 10% normal goat serum at room temperature. Cryosections were incubated with the following primary antibodies: rabbit polyclonal anti-$Na_V1.5$ (1:200, ASC-005, Alomone Laboratories, Jerusalem, Israel), mouse monoclonal anti-α-actinin (1:1000, Sigma), and rabbit polyclonal anti-Hcn4 (1:200, AB5808, Chemicon, Temecula, CA). Alexa-conjugated goat anti-mouse and goat anti-rabbit secondary antibodies were used (1:250, Molecular Probes, Invitrogen, Waltham, MA). For the PFA-embedded section, the primary antibodies used were as follows: Hcn4 goat polyclonal (1:250, Santa Cruz Biotechnology, Dallas, TX) and rabbit polyclonal anti-$Na_V1.5$ (1:200, ASC-005, Alomone Laboratories). Secondary antibodies coupled to an Alexa fluorescent tag (1:250, Invitrogen) were used for visualization. Confocal imaging of sections was performed using a confocal laser scanning microscope (BioRad MRC 1024) equipped with a 15-mV krypton/argon laser using the 568- and 488-nm excitation lines and 605DF32 and 522DF35 emission filters.

Statistical analysis

All values are expressed as mean ± SEM. The statistical significance of differences between values of ECG parameters and electrophysiological recording data from WT and TG mice was determined using a *t* test. A difference with $P < .05$ was considered to be significant.

Results

P-R interval in hearts of TG mice is 3 times shorter than that in hearts of WT mice

Typical ECG records from 1 WT mouse (panel a) and 2 TG mice (panels b and c) are shown in Figure 1A. Whereas R-R intervals were similar in all mice, the P-R interval was